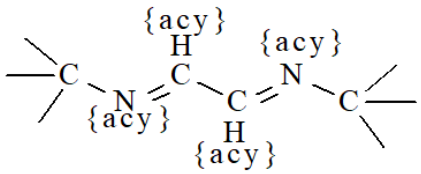
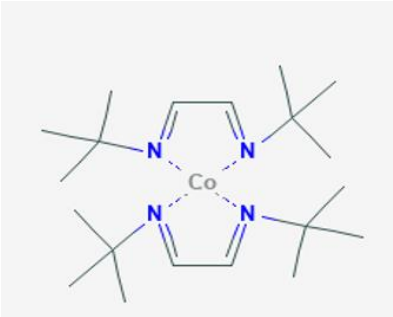
Detailed information for each alert such as structural boundaries, mechanisms, local training sets and references associated with each observed data is provided in the tables below.
DNA binding alerts:

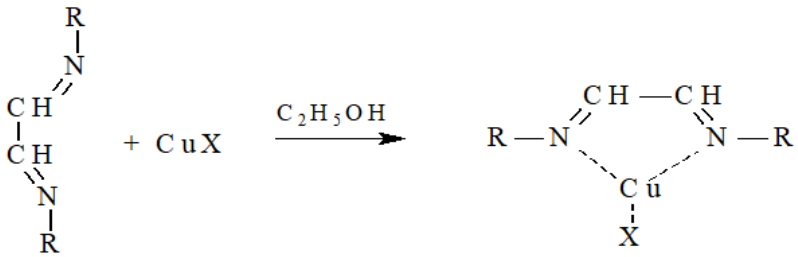
Individual profile/alert	
Name	1,4-Diazabutadiene Derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: Radical Mechanistic Alert: ROS generation

The target chemical, N,N'-Ditert-butylethane-1,2-diimine, is known to act as ligand for some transition metals such as copper and cobalt. Cobalt complex with suggested structure depicted below:



is suspected of causing genotoxicity efects [1].

Cuprous chloride was also coordinated by diazabutadiene (DAB-R) ligands to form Cu(I)-(DAB-R) complexes. The following scheme of chelate complex formation was proposed [2]:

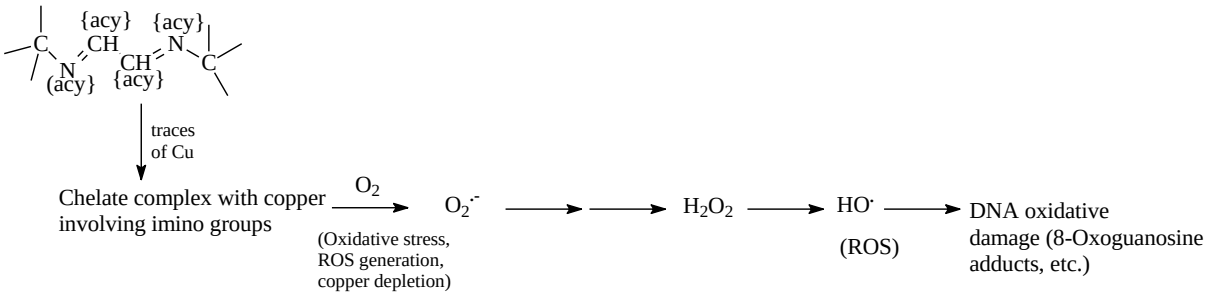


(X is Cl or I; R is C{ar}, C{sp3}

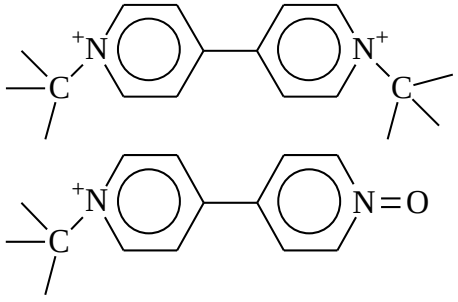
Also, novel tetrahedral copper(I) mixed-ligand complexes with other diimine-type ligands were synthesized, and genotoxicity effects of these complexes were suspected [3].

Despite of lack of definitive data, there are other publications, reporting experimentally observed mutagenicity of similar copper-chelating complexes. For instance, polyethylene polyamines showed mutagenicity in bacterial cells, indicating that in vitro genotoxic effect could be caused by oxidative stress and formation of ROS, triggered as a consequence of copper depletion [4].

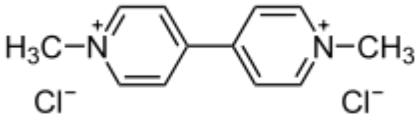
Based on the above speculations, and the presence of traces of transition metals such as coper in the incubation medium, the following, rather simplified mechanistic scheme can be expertly proposed:



Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Cobalt; N,N'-ditert-butylethane-1,2-diimine (Compound), NIH, PubChem; https://pubchem.ncbi.nlm.nih.gov/compound/131698366. Last visited: June, 2021 2. Liu, Y., L. Yang, Efficient Synthesis of Triarylamines Catalyzed by Copper (I) Diazabutadiene Complexes, Chin. J. Chem. 2015, XX, 1—6; http://dx.doi.org/10.1002/cjoc.201400787. Last visited: June, 2021. 3. Gandin, V., M. Porchia, Fr. Tisato, A. Zanella, E. Severin, A. Dolmella, Chr. Marzano, Novel Mixed-Ligand Copper(I) Complexes: Role of Diimine Ligands, J. Med. Chem. 56 (2013), 7416−7430. 4. Assessment Report, Cufence (International non-proprietary name: trientine hydrochloride), Procedure No. EMEA/H/C/004111/0000, European Medical Agency, Committee for Medicinal Products for Human Use (CHMP), 29 May 2019.

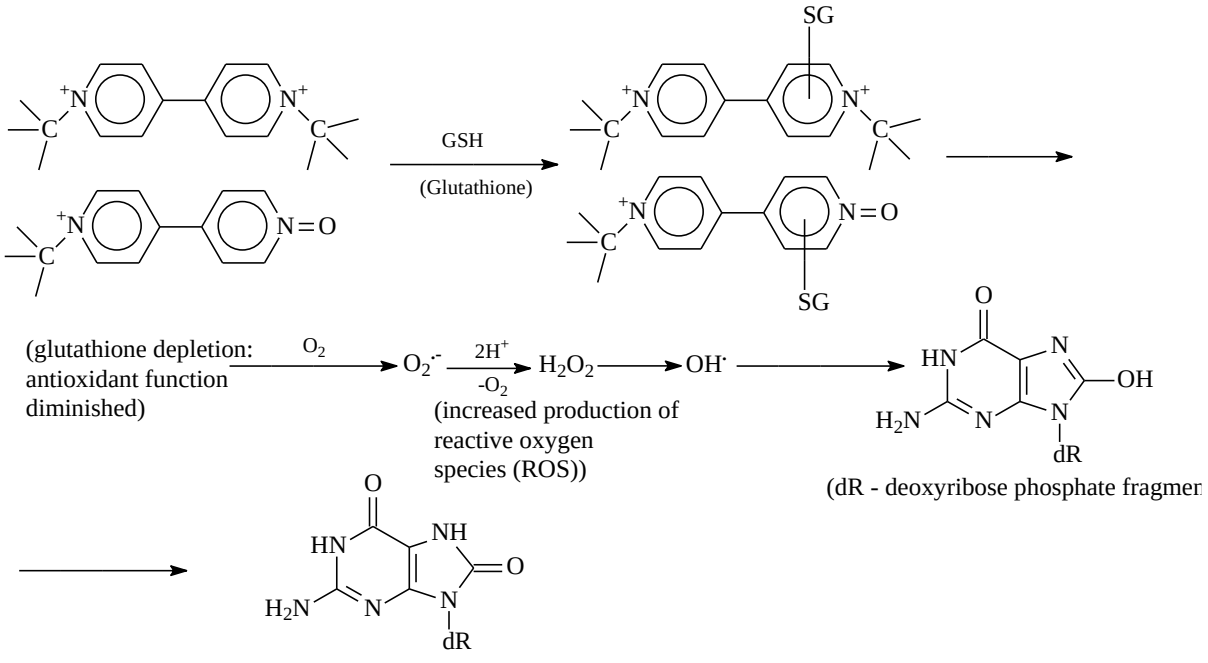
Individual profile/alert	
Name	4,4'-Bipyridinium Salts and N-Oxides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: Radical Mechanistic Alert: Radical mechanism via ROS formation

The chemical Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride):



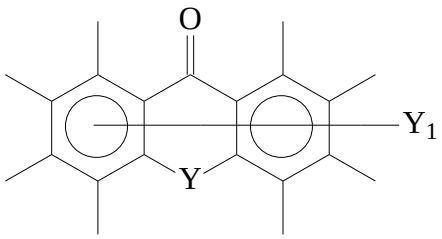
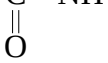
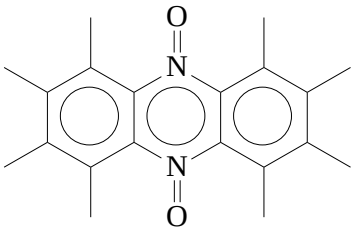
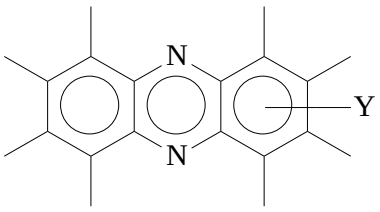
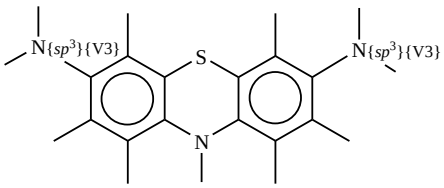
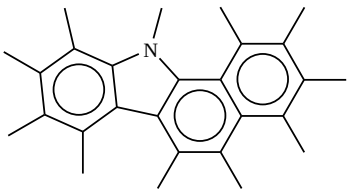
was used as an intracellular generator of oxygen free radicals, and was found to be highly mutagenic for Salmonella typhimurium. It caused both base-pair substitution and frameshift mutations. The mutagenicity of paraquat was dependent on the presence of a supply of both electrons and oxygen. The mutagenicity of paraquat thus appears to be due to its ability to exacerbate the intracellular production of superoxide radicals. Superoxide anion radicals (O.2-), hydrogen peroxide (H2O2), and hydroxyl radicals (OH.) are the intermediates (reactive oxygen species, ROS) formed during the progressive one-electron reduction of dioxygen [1].

Based on the above discussions, the following simplified mechanistic scheme involving the participation of intracellular glutathione (GSH) in generation of ROS can be expertly proposed:

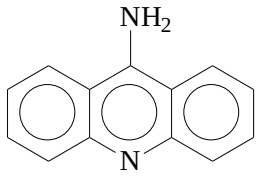


Set of chemicals used for profile development	4,4'-Bipyridinium Salts and N-Oxides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Moody, C. S., H.M. Hassan, Mutagenicity of oxygen free radicals, Proc. Natl. Acad. Sci. USA 79 (1982), 2855 – 2859.

Individual profile/alert	
Name	Acridone, Thioxanthone, Xanthone, Phenazine and Other Fused-Ring Heterocyclic DNA Intercalators

Type of profile	Structural alert
Description/applicability domain	(I): Acridone, Thioxanthone and Xanthone Derivatives  (Y is O, S{V_2}, N{V_3}) (Y_1 can be -OH, -O-CH_3, -NH{{sp3}{V3}, -CH_3, -CH_2OH,  No other substituents allowed, except for -H; total number of substituents in both benzene rings: 2 - 5) **(I)** (II), (III): Phenazine and Phenazine N,N'-Dioxide Derivatives   (Y can be combinations between -H and -NH_2 or -H, NH_2 and -OH or OCH_3) (I) (II) (IV): Phenothiazine derivatives (V): Benzocarbazole derivatives   (IV) (V)
Mechanism	Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation Mechanistic Domain: Radical Mechanistic Alert: ROS generation
A number of tricyclic acridone, thioacridone and thioxanthene derivatives are known to act as DNA intercalating agents and possess in vitro bacterial mutagenicity in a broad range of intensity. Generally, acridones showed the highest bacterial mutagenicity [1].	

All intercalating agents contain, as an important requirement, a planar electron-rich structural fragment. In such a case, binding to DNA is enhanced when there is substituent bearing, for example, an amino group, which can bind electrostatically to the phosphate groups of DNA. Thus planar tricyclic and tetracyclic ring systems can be accommodated between the successive base pairs of DNA [5]. With the frameshift mutations, base pairs relative to the original sequence are gained or lost, and the reading frame of genetic code is altered. Frameshift mutagens may stimulate the induction of mutations by covalent or non-covalent interactions. For example, acridine compounds are the most familiar frameshift mutagens, that intercalate between DNA base pairs. Intercalation is sufficient for mutagenesis, since, for example, chemicals such as 9-aminoacridine:



leads to base pairs being gained or lost when the DNA containing the intercalated planar ring system is replicated [6].

The mutagenicities of naturally occurring xanthones have been tested in Salmonella typhimurium TA100, TA98, TA97, and TA2637 by the preincubation method. Gentisein, gentisin, isogentisin, 1-hydroxy-3,7-dimethoxyxanthone, 1,3,7-trimethoxyxanthone, desmethylbellidifolin, bellidifolin and dimethylbellidifolin were found to be mutagenic, but unsubstituted xanthone was not mutagenic to TA100, TA98, TA97 and TA2637 with or without metabolic activation system. The beta-O-glucosides, norswertianolin and swertianolin, were only mutagenic when a metabolic activation system containing beta-glucosidase was used [2].

Several methylthioxanthone analogues, including lucanthone, were found to be non-mutagenic for Salmonella typhimurium but were activated to mutagens by a rat liver microsome preparation. Hydroxymethyl analogues, including hycanthone, were mutagenic in the absence of microsomes. The hydroxymethyl derivatives seemed to be the more proximal mutagens [3].

The in vitro microsomal metabolism studies of several xanthone derivatives such as 1-hydroxy-2,3,5-trimethoxy xanthone, 1-hydroxy-2,3,4,7-tetramethoxyxanthone, 1-hydroxy-2,3,4,5-tetramethoxyxanthone, 1,5-dihydroxy-2,3-dimethoxyxanthone, etc. has shown that metabolism occurs mainly at 2-, 4-, 5, and 7-positions, and the metabolites formed are also bioactive compounds [4].

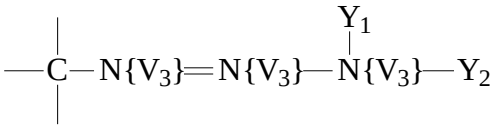
The bacterial mutagenicity of another class of chemicals, phenazine derivatives such as 2,3-diaminophenazine and 2-amino-3-hydroxyphenazine, both without and with S9 metabolic activation has been proved in the Salmonella typhimurium strain TA98. This suggests that, similarly to other major mutagenic aromatic amines, both compounds act as frameshift mutagens [7]. The presence of other electron-donating substituents such as methoxy-(OCH3) group in the aminophenazine molecule was shown to contribute to mutagenicity by enhancing the electron density on the aromatic ring [8].

The phenazine di-N-oxide derivative myxin was found to cause DNA strand cleavage under aerobic conditions which could result either from deoxygenative metabolism or from redox cycling. Redox cycling has the potential to generate reactive oxygen species (ROS), including the DNA-cleaving hydroxyl radical. Thus one-electron bioreductive activation of aromatic N-oxides can be assumed, which might cause genotoxic effects [9].

The planar structure of phenothiazine derivatives suggests DNA intercalation as a possible mechanistic explanation of their bacterial mutagenicity [10]. Such as assumption was also postulated for some benzocarbazole derivatives [11].

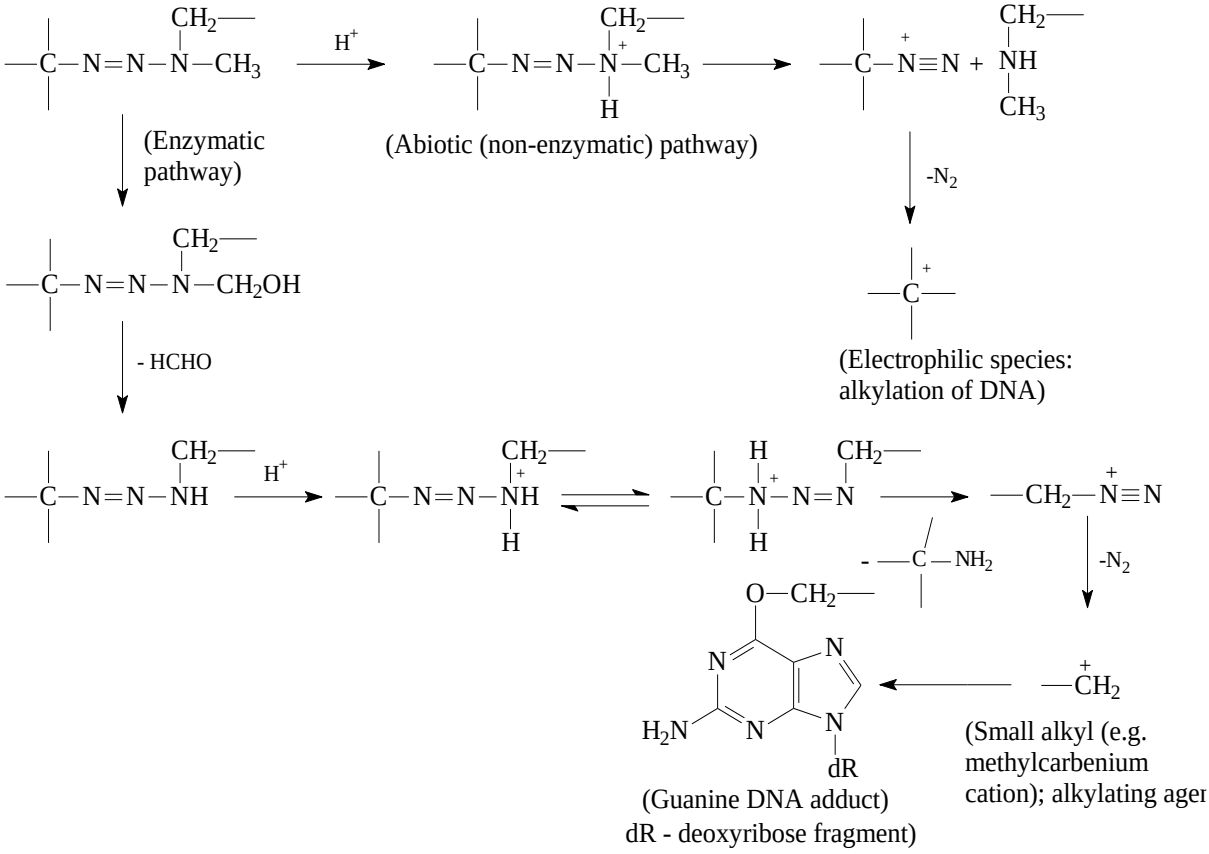
Set of chemicals used for profile development	Acridone, Thioxanthone, Xanthone, Phenazine and Other Fused-Ring Heterocyclic DNA Intercalators
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Denny, W. A., P. M. Turner, Gr. J. Atwell, G. W. Rewcastle, L. R. Ferguson, *Structure-Activity Relationship for the Mutagenic Activity*

	of Tricyclic Intercalating Agents in Salmonella typhimurium, Mutat. Res. **232** (1990), 233 – 241. 2. Matsushima, T., A. Araki, O. Yagame, M. Muramatsu, K. Koyama, K. Ohsawa, S. Natori, H. Tomimori, *Mutagenicities of Xanthone Derivatives in Salmonella typhimurium TA100, TA98, TA97, and TA2637*, Mutat. Res. **150** (1985), 141 – 146. 3. Harman, Ph. E., P. B. Hulbert, E. Bueding, D. D. Taylor, *Microsomal Activation to Mutagens of Antischistosomal Methyl Thioxanthenones and Initial Tests on a Possibly Non-Mutagenic Analogue*, Mutat. Res./Environ. Mutag. Rel. Subjects **31**(2) (1975), 87 – 95. 4. Feng, R., Y. Y. Zhang, X. Chen, Y. Wang, J. G. Shi, Ch. T. Che, J. H. K. Yeung, J. Y. Ma, X. Sh. Tan, Ch. Yang, Y. L. Deng, Y. K. Zhang, *In Vitro Study on Metabolite Profile of Bioactive Xanthones Isolated from Halenia elliptica D. Don by High Performance Liquid Chromatography Coupled to Ion Trap Time-of-Flight Mass Spectrometry*, J. Pharm. Biomed. Anal. **62** (2012), 228 – 234. 5. Double, J. C., J. R. Brown, *Evaluation of the Binding of Some Substituted Anthraquinones and Naphthacenequinones to DNA*, Communications, J. Pharm. Pharmac. **28** (1976), 166 – 169. **6.** Hoffman, G. R., R. P. P. Fuchs, *Mechanisms of Frameshift Mutations: Insight from Aromatic Amines*, Chem. Res. Toxicol. **10**(4) (1997), 347 – 359. **7.** Sarrif, A. M., G. T. Arce, D. F. Krahn, R. M. O`neil, V. L. Reynolds, *Evaluation of Carbendazim for Gene Mutations in the Salmonella/Ames Plate-Incorporation Assay: The Role of Aminophenazine Impurities*, Mutat. Res. **321** (1994), 43 – 56. 8. Watanabe, T., T. Hirayama, S. Fukui, *Phenazine Derivatives as the Mutagenic Reaction Product from o- or m-Phenylenediamine Derivatives with Hydrogen Peroxide*, Mutat. Res. **227** (1989), 135 – 145. 9. Chowdhury, G., U. Sarkar, S. Pullen, W. R. Wilson, A. Rajapakse, T. F. Knotts, K. S. Gates, *DNA Strand Cleavage by the Phenazine Di-N-Oxide Natural Product Myxin Under Both Aerobic and Anaerobic Conditions*, Chem. Res. Toxicol. **25** (2012), 197 – 206. 10. Gocke, E., *Review of the Genotoxic Properties of Chloropromazine and Related Phenothiazines*, Mutat. Res. **366** (1996), 9 – 21. 11. Ferlin, M. Gr., Chr. Marzano, V. Gandin, St. Dall, Acqua, L. D. Via, *DNA Binding Ellipticine Analogues: Synthesis, Biological Evaluation, and Structure-Activity Relationships*, ChemMedChem **4** (2009), 363 – 377.

Individual profile/alert	
Name	Acyclic Triazenes
Type of profile	Structural alert
Description/applicability domain	 (Y_1, Y_2 are $-CH_3$, or $-H_2C-C_6H_5$ or $-CH_2CH_3$ or -H or $-CH(CH_3)_2$ (number of -H can be 0 or 1))

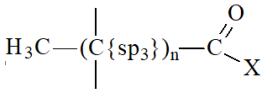
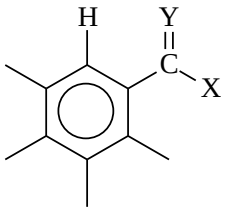
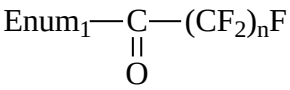
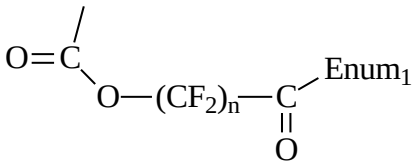
Mechanism	Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after carbenium ion formation

Acyclic triazene structural fragment has been approved as specific toxicophore, eliciting bacterial mutagenicity, due to its high reactivity [1]. According to some publications, the linear aryl-N,N-dialkyl triazenes are considered to require non-enzymatic cleavage of the diazoamino fragment or microsomal (metabolic) activation and subsequent heterolytic cleavage in order to elicit genotoxicity effects. The studies on the mutagenicity of a linear aryl-N-monoalkyl triazene in Salmonella typhimurium bacteria have also shown that it is a potent direct-acting mutagen, which predominantly causes base-substitution mutations [2]. 3-Methyl-1-phenyltriazene and a series of ring-substituted triazenes such as 4-methylphenyl, 4-chlorophenyl and 2,4,6-trichlorophenyl derivatives have also been studied for their mutagenic activity in Salmonella typhimurium strains. It has been shown that methylating agents are released after heterolysis of these monomethylphenyltriazenes, confirming that the metabolic oxidative N-dealkylation of N,N-dimethylphenyltriazenes produces monomethyl products, acting as alkylating agents on DNA bases. The heterolysis of monomethylphenyltriazenes leads to the generation of two types reactive species: methyldiazonium and phenyltriazonium cations. The mutagenic activity of the monomethylphenyltriazenes on Salmonella typhimurium strain TA1530 was attributed to the methyldiazonium cation released after heterolysis. In the assay with S9 metabolic activation, the enzyme nucleophiles present in the liver fraction protected bacteria against the toxic action of the arenediazonium cations, leading to increased number of surviving mutants [3]. The mechanism of action of some triazene compounds as alkylating agents with similar chemical, physical, antitumour and mutagenic properties has been mainly related to methylation of O6 guanine DNA bases, mediated by the methyldiazonium cation as a highly-reactive intermediate. The active structural moiety of these compounds is the triazenyl group. For some of these compounds, the following stages of their metabolic activation have been proposed: (1) formation of hydroxymethylated product catalyzed by CYP 450 isoforms; (2) conversion of the molecule to its monomethyl derivatives after elimination of formaldehyde; (3) tautomerization and generation of alkyldiazonium cation as reactive species. The oxidative delakylation step is mainly catalyzed by the microsomal CYP1A1, CYP1A2, and CYP2E1 isoenzymes [4].
Aryl-N,N-dialkyltriazenes with longer alkyl chains such as 1-phenyl-3,3-diisopropyltriazene, 1-phenyl-3,3-diisobutyltriazene, 1-phenyl-3,3-di-n-butyltriazene and 1-phenyl-3-methyl-3-sec-butyltriazene are, however, negative as parent chemical but positive after S9 metabolic activation [5].
On the basis of the literature data available, the following scheme of bioactivation of triazene derivatives can be expertly assumed:



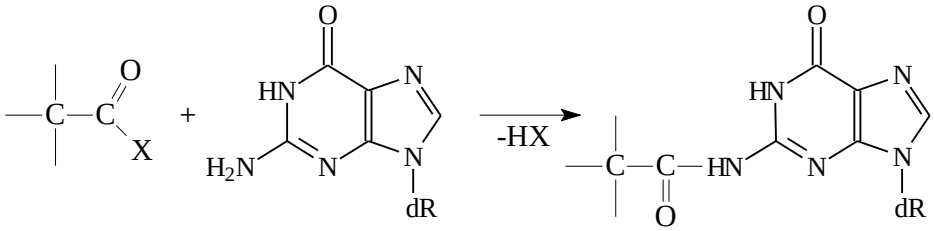
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kazius, J., R. McGuire, R. Bursi, *Derivation and Validation of Toxicophores for Mutagenicity Prediction*, J. Med. Chem. **48** (2005), 312 – 320. 2. Thomas, H. F., D. L. Brown, Ph. E. Hartman, E. H. White, Z. Hartman, *Aryl-Monoalkyl and Cyclic Triazenes: Direct-Acting Mutagens*, Mutat. Res. **60** (1979), 25 – 32. 3. Malaveille, Ch., G. Brun, G. Kolar, et al., *Mutagenic and Alkylating Activities of 3-Methyl-1-Phenyltriazenes and Their Possible Role as Carcinogenic Metabolites of the Parent Dimethyl Compounds*, Canc. Res. **42** (1982), 1446 – 1453. 4. Marchesi, Fr., M. Turriziani, Gr. Tortorelli, G. Avvisati, Fr. Torino, L. De Vecchis, *Triazene Compounds: Mechanism of Action and Related DNA Repair Systems*, Pharmacol. Res. **56** (2007), 275 – 287. 5. Sieh, D. H., A. W. Anderws, C. J. Michejda, *Mutagenicity of Trialkyltriazenes: Mutagenic Potency of Alkyldiazonium Ions, the Putative Ultimate Carcinogens from Dialkylnitrosamines*, Mutat. Res. **73** (1980), 227 – 235.

Individual profile/alert	
Name	Acyl Halides

Type of profile	Structural alert
Description/applicability domain	 (n = 1 - 3) (I)  (Y is O or N-OH); X is –NO2, -C#N, Cl or F; No other substituents) (II)  (Enum1 is F or Cl; n = 1 – 4) (III)  (Enum1 is Cl or F; n = 1 – 4) (IV)
Mechanism	Mechanistic Domain: SN2Ac Mechanistic Alert: Direct acylation involving a leaving group

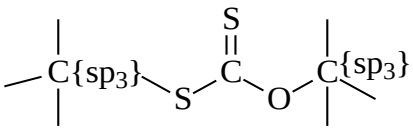
Such chemicals are believed to be direct-acting mutagens predominantly.
Mixed results for bacterial mutagenicity (Ames test) have been obtained for benzoyl chloride [1]. Generally, acyl halides are known to undergo nucleophilic substitution reactions and are mostly mutagenic, i.e., and capable of interacting with DNA [2]. The bacterial mutagenicity is mostly associated with the lower-molecular weight aliphatic acyl halides, which could be due to steric reasons and the stronger electron-donating effects of longer alkyl chains which may reduce reactivity towards DNA. As far as aromatic acyl chlorides (benzoyl chloride derivatives) are concerned, chemicals such as 3-nitro- and 4-nitrobenzoyl chloride are direct-acting mutagens [3], due, partly to the contribution of the electron-withdrawing nitro group attached to the aromatic ring. Generally, more electronegative substituents attached to benzene ring are believed to increase acylating reactivity and mutagenicity.

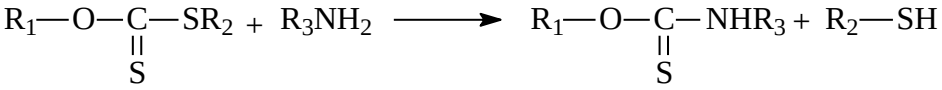
A mixture of methylglyoxal and hydrogen peroxide has been found to react with 2'-deoxyguanosine to form N2-acetyl-2'deoxyguanosine [4]. By analogy, direct DNA acylation mechanism by acyl halides such as acetyl chloride can be expertly suggested:



Deoxyguanosine fragment DNA acylated adduct

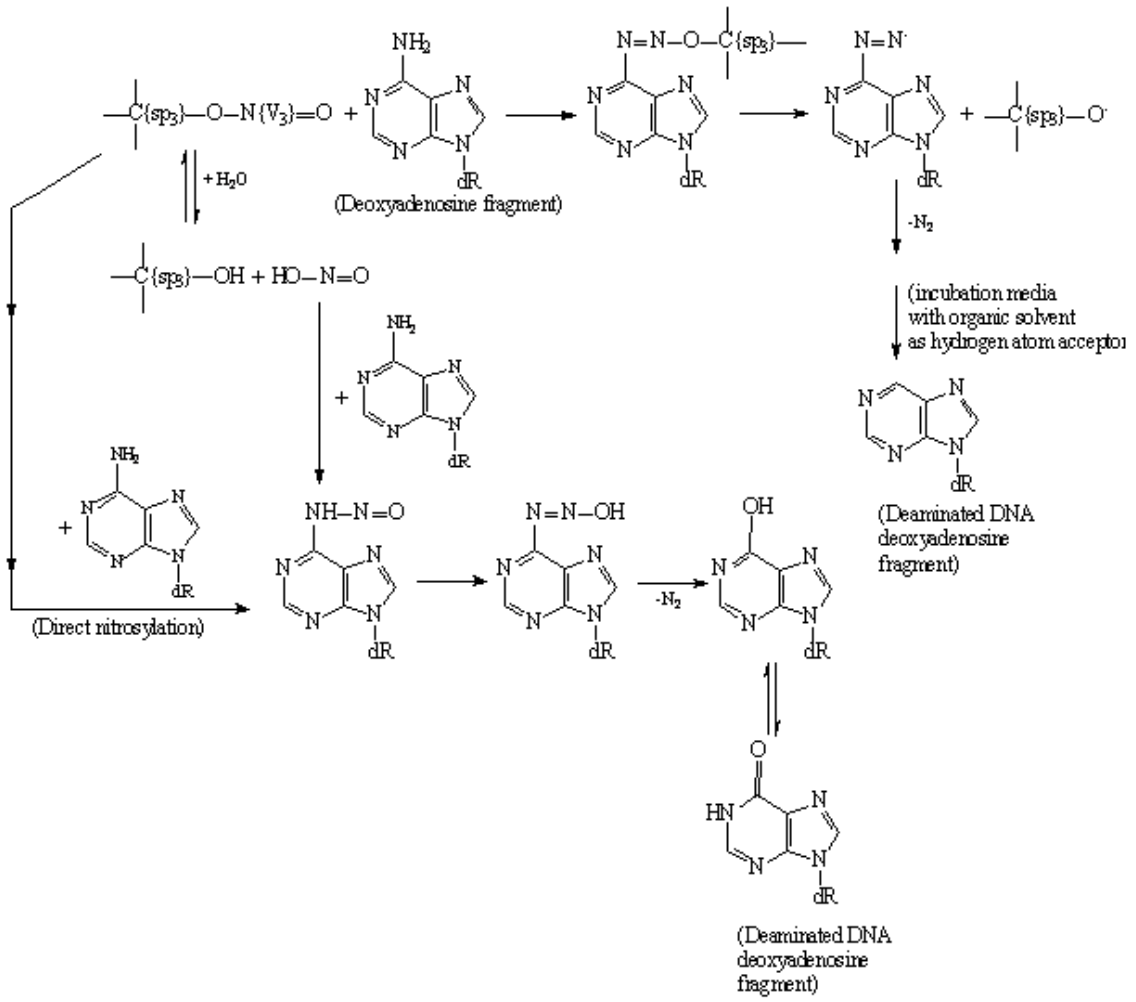
Set of chemicals used for profile development	Acyl Halides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. World Health Organization, International Agency for Research on Cancer, *α*-Chlorinated Toluenes and Benzoyl Chloride in *Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **1999**, Vol. 71, pp 453-477. http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-19.pdf Last visited: June, 2021. 2. Sawatari, K., Nakanishi, Y., Matsushima, T., Relationships between chemical structures and mutagenicity: a preliminary survey for a database of mutagenicity test results of new work place chemicals. *Ind. Health*, **2001**, 39(4), 341-345. 3. Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.*, **1987**, 9(Suppl. 9), 1-109. 4. Tada, A., Wakabayashi, K., Totsuka, Y., Sugimura, T., Tsuji, K., Nukaya, H., ^{32}P-Postlabeling analysis of a DNA adduct, an N^2-acetyl derivative of guanine, formed *in vitro* by methylglyoxal and hydrogen peroxide in combination. *Mutat. Res.*, **1996**, 351(2), 173-180.

Individual profile/alert	
Name	Alkyl Xanthate Esters
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: Nucleophilic substitution on activated primary amino group
The reactions of hydrolysis and aminolysis of a series of S-substituted O-alkylxanthate esters were studied in 20% aqueous methanol at 35°C. The pH-rate profiles of the hydrolyses were consistent with water and hydroxide-ion-catalyzed reactions. The following reaction scheme of interaction with primary alkane amine was suggested by the authors [1]:	



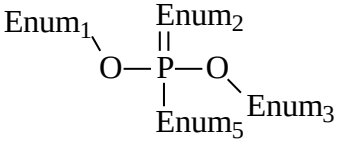
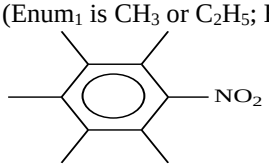
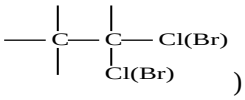
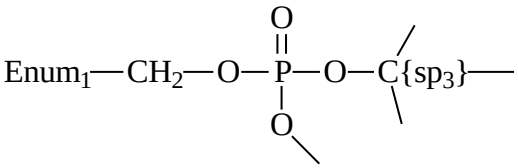
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Humeres, Ed., V. Soldi, M. Klug, M. Nunes, C. M. S. Oliveira, P. J. Barrie, Hydrolysis and aminolysis of alkyl xanthate esters and cellulose analogues, Can. J. Chem. 77 (1999), 1050 – 1056.

Individual profile/alert	
Name	Alkylnitrites
Type of profile	Structural alert
Description/applicability domain	Y—O—N{V$_3$}═O (Y is C{sp3})
Mechanism	Mechanistic Domain: SN1 or SN2 Mechanistic Alert: Nitrosation Mechanistic Domain: AN2 Mechanistic Alert: Formation of adducts similar to Shiff bases Mechanistic Domain: Radical Mechanistic Alert: DNA base deamination after radical decomposition
The following generalized scheme for the formation of mutagenic species by alkylnitrites can be suggested based on literature	

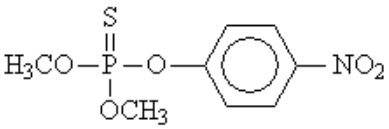


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Tornqvist, M., U. Rannug, A. Jonsson, L. Ehrenberg, *Mutagenicity of Methyl Nitrite in Salmonella typhimurium*, Mutat. Res. **117** (1983), 47 – 54. 2. Dunkel, V. C., A. M. Rogers-Back, T. E. Lawlar, J. W. Harbell, Th. P. Cameron, *Mutagenicity of Some Alkyl Nitrites Used as Recreational Drugs*, Environ. Molec. Mutag. **14** (1989), 115 – 122. 3. *Organic Functional Group Transformations, Vol. 1 Synthesis: Carbon with No Attached Heteroatoms* (Ed. By A. R. Katritzky, O. M. Cohn, Ch. W. Rees, Elsevier Science Ltd. 1995; http://www.amazon.com/Comprehensive-Organic-Functional-Group-Transformations/dp/0080423221#reader_0080423221. Last visited: June, 2021. 4. Wild, D., M. T. King, E. Gocke, K. Eckhardt, *Study of Artificial Flavouring Substances for Mutagenicity in the Salmonella/Microsome, BASC and Micronucleus Test*, Fd. Chem. Toxicol. **21**(6) (1983), 707 – 719.

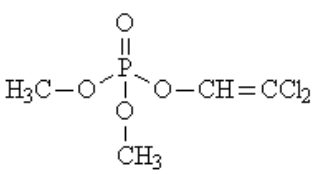
	5. Ehrenberg, L., S. Hussain, M. N. Saleh, U. Lundqvist, *Nitrous Esters – A Genetical Hazard from Nitrogen Oxides (NO_x)*, Hereditas **92**(1) (1980), 127 – 130.

Individual profile/alert	
Name	Alkylphosphates, Alkylthiophosphates and Alkylphosphonates
Type of profile	Structural alert
Description/applicability domain	 ($Enum_1$ is CH_3 or C_2H_5; $Enum_2$ is O or S; $Enum_3$ is CH_3 or C_2H_5 or  (one NO_2 only); $Enum_5$ is –OC or S{V2}C or )  (Enum1 is Cl or Br)
Mechanism	S_N2 Alkylation

The compound methylparathion:



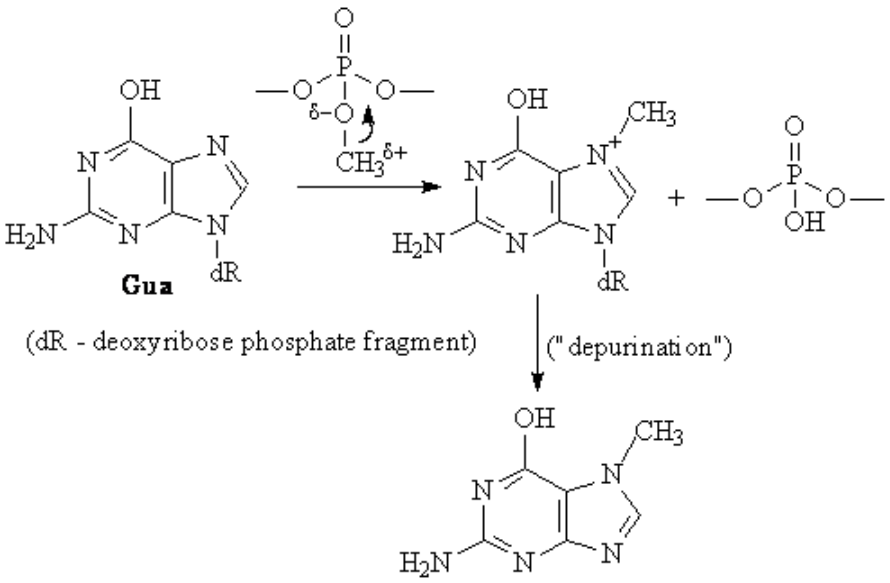
which belongs to the organothiophosphate group of insecticides also exhibits mutagenicity [2]: perhaps the aromatic nitro group strongly contributes to this effect. Alkylation of DNA has been proposed as the essential step for mutation interactions of the organophosphate insecticides dichlorovos and trichlorfon, and no evidence for a role of metabolic activation in the mutagenicity of these compounds was found [3]. Dichlorovos (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate) (agricultural pesticide):



was found to be a relatively weak methylating agent, which was mutagenic as a parent as well as after

metabolic activation. Dichlorvos was also shown to act as methylating agent of nucleophiles, and, more specifically, to induce strand breaks in isolated DNA [4, 5]. Moreover, dichlorvos (organic phosphate ester with dichlorovinyl side chain), and trichlorphon, which have similar structures were found to be mutagenic in the *Salmonella* strain TA1535 [6]. Also, the ability of other organophosphates and thiophosphates such as methylbromphenvinphos, methylparathion and malathion to elicit methylation of N7 of guanine fragment in DNA *in vitro* has been studied, and 7-methylguanine was the main methylation adduct [7]. This was confirmed by the findings that, generally, organophosphate insecticides, containing at least two methyl ester groups in their molecular structure such as *dichlorvos* and *naled* elicited *Ames* mutagenic activity [8].

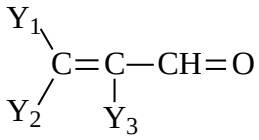
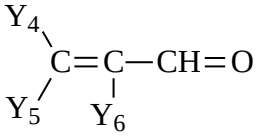
Therefore, the alkylation mechanism seems to be more plausible for this class of compounds, as expertly outlined below in Scheme 1

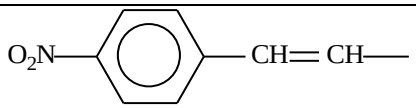


Scheme 1

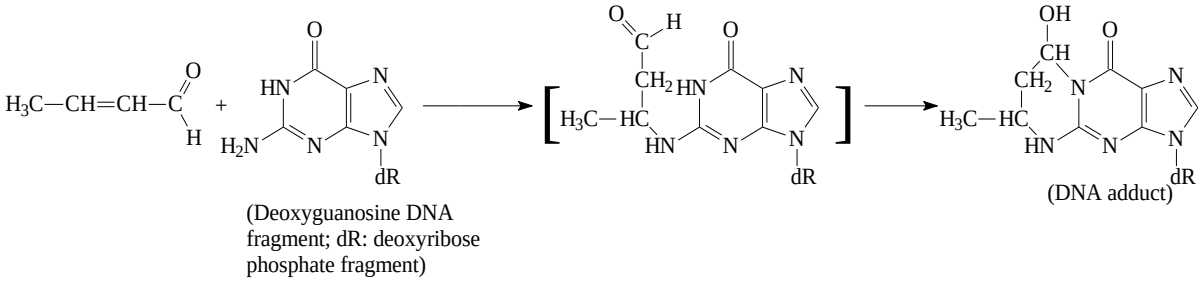
Set of chemicals used for profile development	Alkylphosphates, Alkylthiophosphates and Alkylphosphonates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Methyl Parathion*, IPCS Inchem, International Programme on Chemical Safety, Environmental Health Criteria 145; http://www.inchem.org/documents/ehc/ehc/ehc145.htm. Last visited: June, 2021. 2. Wang, T. C., Ch. M. Lin, L. W. Lo, Genotoxicity *of Methoxyphosphinyl Insecticide in Mammalian Cells*, Zool. Studies **42**(3) (2003), 462 – 469. 3. Braun, R., J. Schoneich, L. Weisslog, W. Dedek, *Activity of Organophosphorus Insecticides in bacterial tests for Mutagenicity and DNA Repair – Direct Alkylation vs. Metabolic Activation and Breakdown. I. Butonate, Vinylbutonate, Trichlorfon, Dichlorvos, Demethyl Dichlorvos and Demethyl Vinylbutonate*, Chem. Biol. Interact., **39**(3) (1982), 339 – 350. 4. *Mutagenicity of Dichlorovos*, Committee on Mutagenicity of

	Chemicals in Food, Consumer Products and the Environment, January 2002; 5. Lofroth, G., *Alkylation of DNA by Dichlorvos*, Naturwissenschaften **57**(8) (1970), 393 – 394. DOI: 10.1007/bf00599981 6. Carere, A., V. A. Ortali, G. gardamone, G. Morpurgo, *Mutagenicity pf Dichlorovos and Other Struc turally Related Pesticides in Salmonella and Streptomyces*, Chem.-Biol. Interact. **22** (1978), 297 – 308. 7. Wiaderkiewicz, R., Z. Walter, W. Reimschussel, *Sites of Methylation of DNA Bases by the Action of Organophosphorus Insecticides In Vitro*, Acta Biochim. Pol. **33**(2) (1986), 73 – 85 https://www.ncbi.nlm.nih.gov/pubmed/3766014 Last visited: June, 2021. 8. Hour, T. C., L. Chen, J. K. Lin, *Comparative Investigation on the Mutagenicities of Organophosphate, Phthalimide, Pyrethroid and Carbamate Insecticides by the Ames and Lactam tests*, Mutagen. **13**(2) (1998), 157 – 166.

Individual profile/alert	
Name	Alpha,Beta-Unsaturated Aldehydes
Type of profile	Structural alert
Description/applicability domain	A. Simple monofunctional α,β-Unsaturated aldehydes:  Y_1, Y_2 are H (both); or CH_3 (both); or combination of H and n-C_nH_{2n+1} (n = 1 – 4); or combination of H and H_3C-CH=CH- ; Y_3 is H ***(Notes: 1. If both Y_1 and Y_2 are H, Y_3 can be also n-C_nH_{2n+1} (n = 1 – 4));*** ***2. If only one of Y_1 or Y_2 is H, Y_3 can be –CH_3)*** B. α,β-Unsaturated aldehydes with additional electron-withdrawing substituents (EWG):  Y_4 and Y_5 are X (where X is Cl or Br); or combinations of X with –COOH, -CH=O, -NO_2 or -CN; or combinations of H with X or with –COOH, -CH=O, -NO_2 or -CN or combinations of H with –CH_2-O-C(O)CH_3 or with –NH-C_6H_5 or

	 ; Y_6 is –H, X or CH_3
Mechanism	Mechanistic Domain: A_N2 Mechanistic Alert: Nucleophilic addition to α,β-unsaturated carbonyl compounds Mechanistic Domain: A_N2 Mechanistic Alert: Schiff base formation

Generally, different interactions with the formation of DNA adducts, leading to genotoxic and mutagenic responses may occur with this class of compounds such as formation of cyclic DNA adducts, frameshift-type interactions, strand breaks, cross-linking, etc. Also, some metabolic activation reactions are possible such as epoxidation, formation of radicals, nitro group reduction, *etc*. The predominant interaction for α,β-unsaturated aldehydes is the formation of cyclic adducts with DNA bases [1]. For the bacterial mutagen and carcinogen crotonaldehyde, $H_3C–CH=CH–CH=O$ as well as for other α,β-unsaturated aldehydes, formation of adducts by initial Michael-type A_N2-1,4-addition has been reported [2]. Thus the formation of one of the initial adducts is suggested to take place, according to the following scheme:

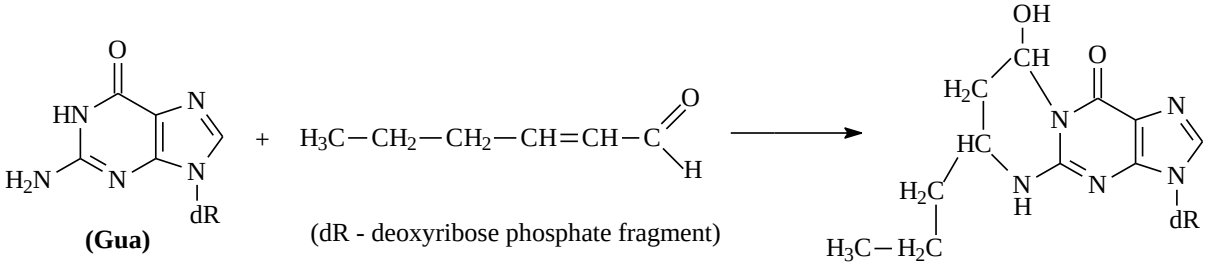


(Deoxyguanosine DNA fragment; dR: deoxyribose phosphate fragment)

(DNA adduct)

Scheme 1

Such type of cyclic 1,N^2-propanodeoxyguanosine adducts can be also formed with 2-hexenal, which, similarly to acrolein and croton aldehyde is also mutagenic and genotoxic α,β-unsaturated aldehyde. The following scheme for the formation of the DNA deoxyguanosine adduct has been proposed [3]:



(Gua)

(dR - deoxyribose phosphate fragment)

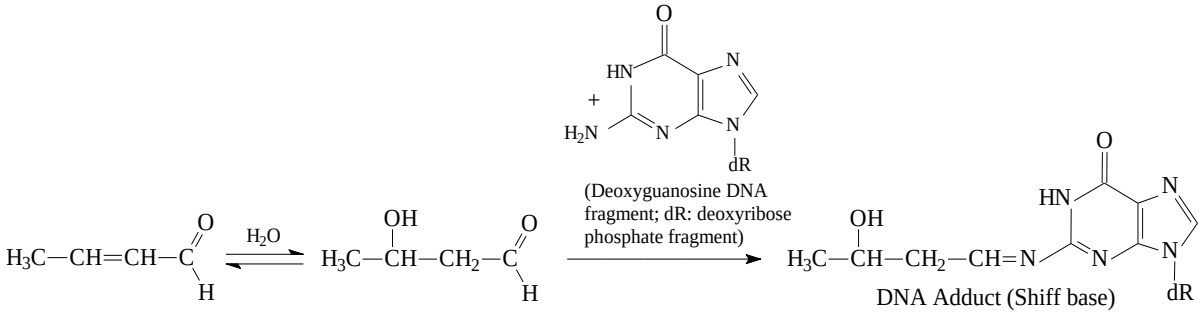
DNA adduct

Scheme 2

α,β-Unsaturated aldehydes as reactive compounds and electrophiles are a class of mutagenic and carcinogenic chemicals that form promutagenic 1,N^2-propanodeoxyguanosine adducts of the types shown above [4, 5]. Other studies were associated with the structure-mutagenicity relationships of 2- and 3-alkylsubstituted α,β-unsaturated aldehydes (α- and β-alkylacroleins). Alpha–alkylacroleins such as 2-methylacrolein, 2-ethylacrolein, 2-

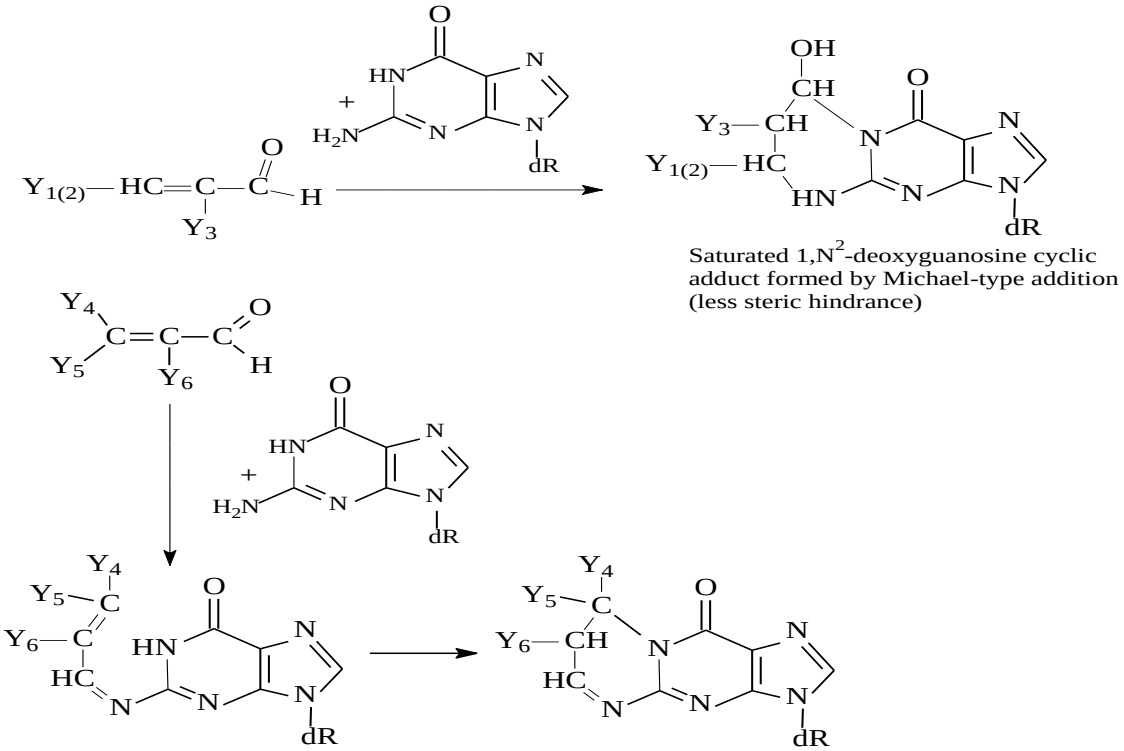
propylacrolein, and 2-butylacrolein were found to be mutagenic in *Salmonella typhimurium TA 100* without exogenous metabolic S9-activation system; however, the results were affected by the bacterial toxicity of alkylacroleins. This increasing toxicity was explained by the ability of compounds with longer alkyl chain to better penetrate into the bacterial cell, due to their higher hydrophobicity. Addition of S9 mix has led to decrease in mutagenicity because of partial detoxification of the substances by the nucleophilic components of the S9 mix such as glutathione [5]. Generally, the highest direct mutagenic activities were observed for the lowest members of the homologous series of α,β-unsaturated aldehydes with shorter, non-branched alkyl chains, more compact structures and less steric hindrance effects. Factors influencing the electrophilicity of aldehydes also cause changes in their mutagenic activities. Moreover, compounds from this class which are non-mutagenic as parent chemicals usually do not show mutagenicity after metabolic activation with S9 mix [6].

Apart from the Michael addition-type adduct, Shiff bases have been identified as major DNA adducts of α,β-unsaturated aldehydes such as crotonaldehyde. Accordingly, the following mechanistic scheme has been proposed for this case [7]:



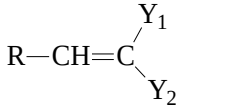
Scheme 3

Shiff base formation adducts can be usually associated with mechanisms, eliciting bacterial mutagenicity for sterically hindered α,β-unsaturated aldehydes, including those, containing electron-withdrawing substituents (EWG), which increases their electrophilicity. Some other schemes for formation of DNA adducts have been also suggested [1]. Thus the following more generalized mechanistic schemes, associated with DNA adducts formation and bacterial mutagenicity can be outlined:



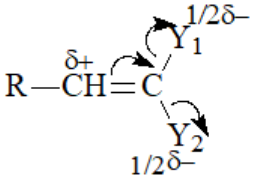
Scheme 4

Set of chemicals used for profile development	Alpha,Beta-Unsaturated Aldehydes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Eder, E., Environ. Health Persp. **88** (1990), 99 – 106. 2. Hecht, S. S., Toxicology **166** (1-2) (2001), 31 – 36. 3. Schuler, D., Carcinogenesis **20**(7) (1999), 1345 – 1350. 4. Hansen, E., Toxicol. Sci **81** (2004), 190 – 197. 5. Eder, E., Environ. Mol. Mutag. **37**(4) (2001), 324 – 328. 6. Lutz, D., Mutat. Res. **93** (1982), 305 – 315. 7. Wang, M., Chem. Res. Toxicol. **14** (2001), 423 – 430.

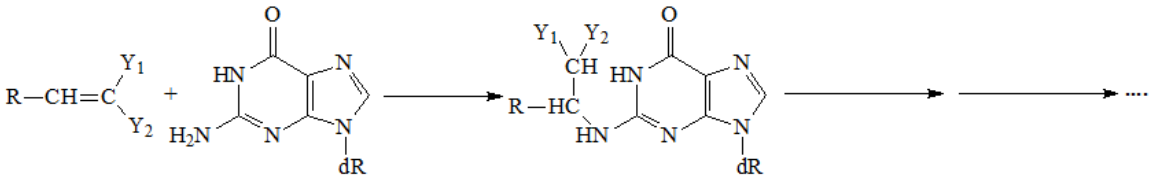
Individual profile/alert	
Name	Alpha-Beta Conjugated Alkene Derivatives with Geminal Electron-Withdrawing Groups
Type of profile	Structural alert
Description/applicability domain	 (R is C or H; Y_1, Y_2 are —C≡N or —NO_2 or —CH=O or  or  ; Y_1 and Y_2 belong to different-type functionalities))

Mechanism	A_N2 Michael-type conjugate addition to activated alkene derivatives

It is expertly assumed that the combination of geminally attached strong electron-withdrawing substituents (EWG) with double or triple bonds (Y1 and Y2, see above), capable of enhanced conjugation with the C=C bond gives rise to an electron deficiency at the β-carbon atom and strong electrophilicity:



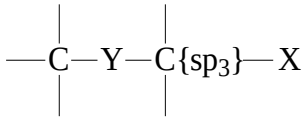
Thus some DNA alkylating capability becomes possible and it could materialize itself via mechanistic scheme, similar to Michael-type addition [4, 5], as follows:



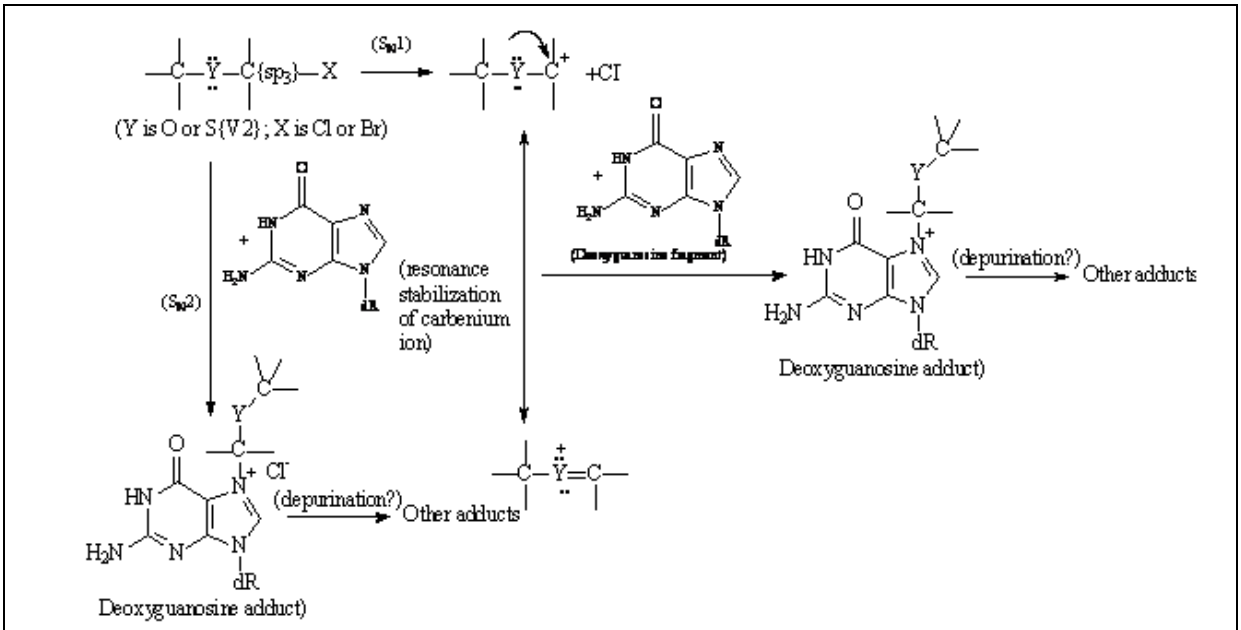
(Deoxyguanosine DNA fragment; dR: deoxyribose phosphate fragment)

(Possible initially formed DNA adduct)

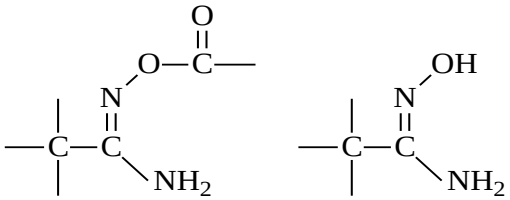
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Rietveld, Mutat. Res. **188** (1987), 97 – 104. 2. *2-Propenoic Acid, 2-Cyano-, Methyl Ester (CAS 137-05-3) MSDS*; http://www.guidechem.com/msds/137-05-3.html. Last visited: June, 2021. 3. Andersen, Mutat. Res. **102** (1982), 373 – 381. 4. Hecht, Toxicology **166** (1-2) (2001), 31 – 36. 5. Solomon, Canc. Res. **45** (1985), 3465 – 3470.

Individual profile/alert	
Name	Alpha-Haloethers
Type of profile	Structural alert
Description/applicability domain	 (Y is O or S{V2}; X is Cl or Br
Mechanism	S_N1 after carbenium ion formation and S_N2 at an sp3 carbon atom

The following mechanistic schemes can be expertly outlined:

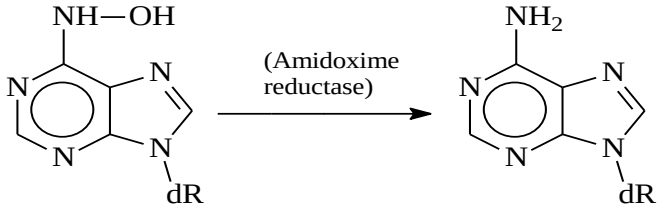


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Selected Chloroalkyl Ethers*, World Health Organization, International Programme on Chemical Safety, Environmental Health Criteria 201, (1998); http://www.inchem.org/documents/ehc/ehc/ehc201.htm, Last visited: June, 2021. 2. Van Duuren, Ann. New York Acad. Sci **163,** No. 2 (1969), 633 – 650; DOI: 10.1111/j.1749-6632.1969.tb24883.x. 3. Fishbein, Mutat. Res. **32** (1976), 267 – 308). 4. Zajdela, Canc. Res. **40** (1980), 352 – 356. 5. Enoch, ATLA **39** (2011), 131 – 145. 6. Enoch, Crit. Rev. Toxicol. **41**(9) (2011), 783 – 802. 7. Van Duuren, Ann. New York Acad. Sci **534** (1988), 620 – 634.

Individual profile/alert	
Name	Amidoxime Esters and Amidoximes
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: Radical Mechanistic Alert: N-O Bond Homolytic Cleavage Mechanistic Domain: AN2 Mechanistic Alert: Nucleophilic addition to activated C=C bond

	Mechanistic Domain: SN2 Mechanistic Alert: Nucleophilic substitution on activated primary amino group

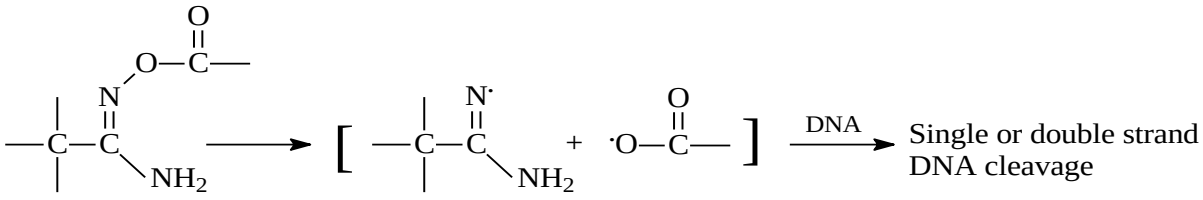
The metabolic reduction of some N-hydroxylated compounds such as amidoximes (N-hydroxylamines) as pro-drugs of amidines has been reported. Due their strong basicity, amidines, guanidines, and amidinohydrazones are protonated under physiological conditions, being very hydrophilic, and are usually not absorbed from the gastrointestinal tract. However, the N-hydroxylated derivatives of amidines (amidoximes), guanidines (N-hydroxyamidines), and amidinohydrazones (N-hydroxyamidinohydrazones) are less basic because of the introduction of oxygen atom. They are absorbed from the gastrointestinal tract and then reduced to the pharmacologically active amidines, guanidines, and amidinohydrazones [1]. Hence, the metabolic reduction of this sub-class of xenobiotic compounds is associated with their biological activity.
N-Hydroxylated nucleobases and nucleosides as N-hydroxylaminopurine (HAP) or N-hydroxyadenosine (HAPR) may be generated endogenously in the course of cell metabolism by cytochrome P450 and oxidative stress or by a deviating nucleotide biosynthesis. These compounds are regarded as toxic and mutagenic for prokaryotic and eukaryotic cells. For the DNA replication fidelity, it is important that organisms are capable of removing such damaged base analogs from DNA precursors. In vitro, some mitochondrial amidoxime-reducing enzymes were found to be capable of reducing N-hydroxylated nucleobases and nucleoside analogs to the corresponding “original” nucleobases and nucleosides, and this is regarded as metabolic detoxification process [2]:



(N-Hydroxyadenosine) (Adenosine)

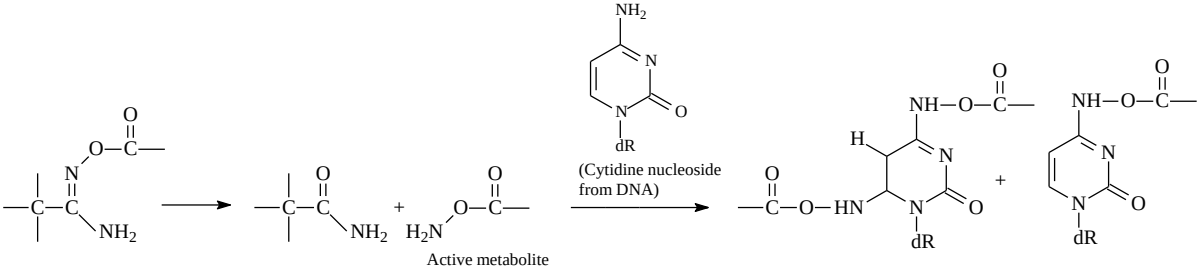
Some amidoxime ester derivatives have been subjected to UV irradiation, to undergo photo-cleavage of the N-O bond via radical mechanism. They act as metal-free DNA photo-cleavers, and the homolysis of the weak N-O bond of the oxime ester generates active aroyloxyl or heteroaroyloxyl radicals, able to attack DNA. On the other hand, aliphatic acyloxyl radicals may rapidly undergo decarboxylation, producing less active radical species [3].
Based on the above information, the following simplified mechanistic schemes can be expertly proposed:

A. Radical (homolytic) mechanism:



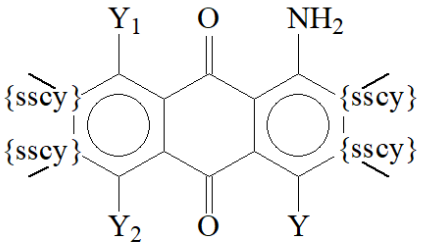
(Radical species)

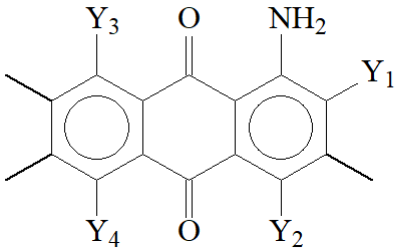
B. Heterolytic mechanism - similar to that for Non-Aromatic Hydroxylamines:



(Expertly assumed adducts)

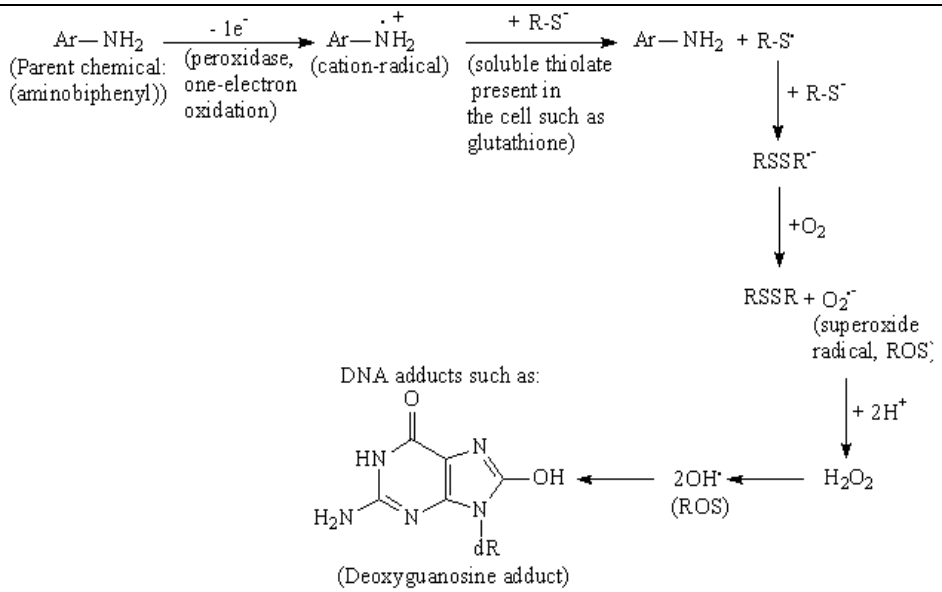
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Clement, B., Reduction of N-Hydroxylated Compounds: Amidoximes (N-Hydroxylamines) as Pro-Drugs of Amidines, Drug Metabol. Rev. 32(3) (2002), 565 – 579. 2. Plitzko, Br., A. Havemeyer, Th. Kunze, B. Clement, The Pivotal Role of the Mitochondrial Amidoxime ReducingComponent 2 in Protecting Human Cells against Apoptotic Effects of the Base Analog N6-Hydroxylaminopurine, J. Biol. Chem. 290(16) (2015), 10126 – 10135; DOI 10.1074/jbc.M115.640052. 3. Pasoli, M., K. Dafnopoulos, N. P. Andreou, P. S. Gritzapis, M. Koffa, Al. E. Koumbus, G. Psomas, K. C. Fylaktakidou, Pyridine and p-Nitrophenyl Oxime Esters with Possible Photochemotherapeutic Activity: Synthesis, DNA Photocleavage and DNA Binding Studies, Molecules 21 (2016), 864; doi:10.3390/molecules21070864.

Individual profile/alert	
Name	Amino Anthraquinones
Type of profile	Structural alert
Description/applicability domain	 (Y can be -OH or -NH_2; Y_1, Y_2 can be -OH, -NH_2 or -H) {sscy}: non-fused sites **(I)**

	 (Y_1 can be -Cl, -Br, -COOH, -OH or -NH_2); Y_2 can be Cl or Br or -H; Y_3, Y_4 can be -OH, -NH_2 or -H) **(II)**
Mechanism	S_N1 Nucleophilic attack after metabolic nitrenium ion formation, Non-covalent interaction DNA intercalation & Radical ROS formation (indirect)

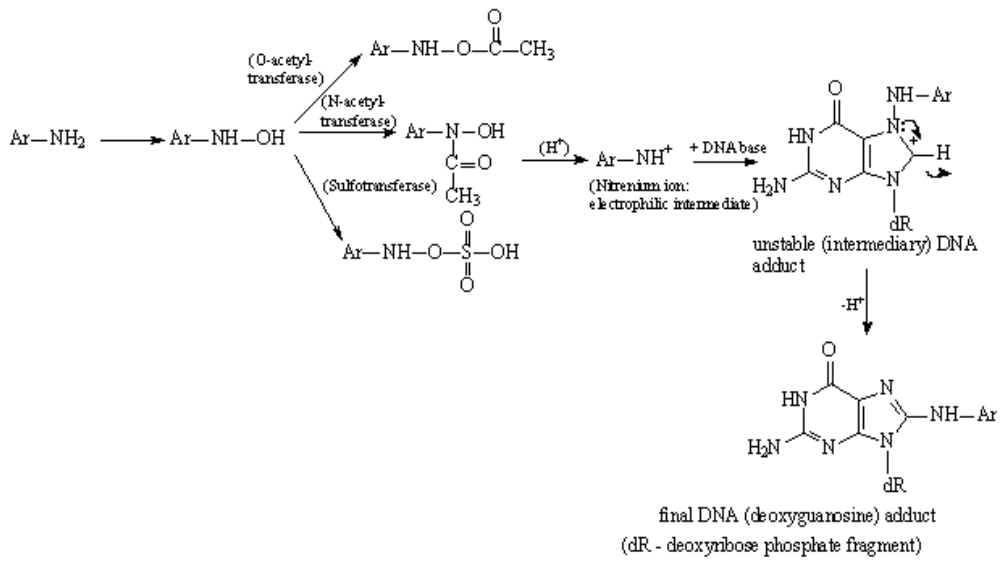
DNA intercalation: The presence of some electron-donating substituents with +M-effect can contribute to the direct mutagenicity of such chemicals, since the benzene rings become more electron-rich and this enhances the non-covalent interaction of the parent chemicals with DNA. Particularly important in this respect are substituents such as –NH_2 and –OH located at *o*- or *p*-postions towards each other. Conjugation effects, planarity and the location of at least one of the primary amino groups at position 1 are also contributing factors

Endogenous generation of reactive oxygen species (ROS). Peroxidase enzymes might be present in *Salmonella typhimurium* bacterial strains, which are associated with endogenous generation of oxygen intermediates [7]. Generally, genotoxicity by oxygen intermediates may be caused by oxidative stress as a result of intracellular species, which can undergo one-electron oxidation-reduction reactions catalyzed by peroxidases to radical species. The latter interact with oxygen to form reactive oxygen species (ROS), which can attack the biological macromolecules such as DNA causing genotoxicity. Such processes can be mediated by thiols and/or glutathione present in the cells shown below in Scheme 1 [8, 9]:



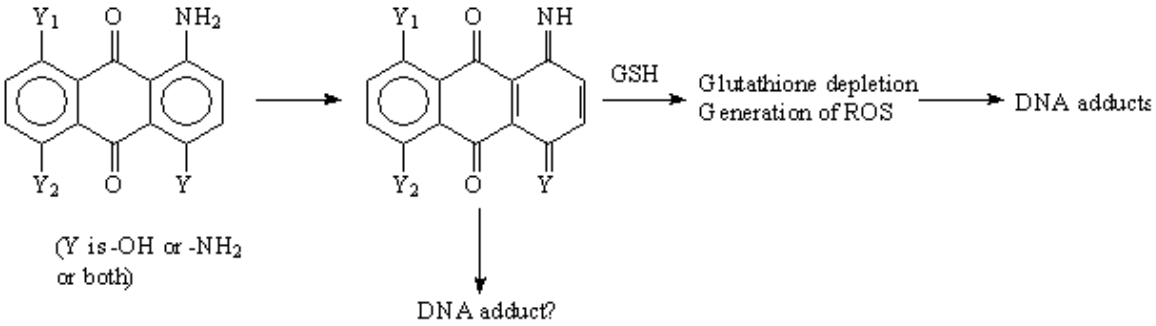
Scheme 1

Mutagenicity after metabolic activation with S9 mix. There is strong evidence that aromatic amines, including aminoanthraquinone derivatives in many cases require metabolic activation with the external microsomal S9 system for eliciting mutagenicity and carcinogenicity. According to an excellent review on the bioactivation pathways of organic functional groups, the obligatory step in the bioactivation of all aniline derivatives involves enzymatic N-hydroxylation on the primary amine nitrogen, leading to the formation of *N-hydroxylamine* intermediate. These reactive N-hydroxylamine derivatives (metabolites) can undergo phase II conjugation, to generate the more reactive N-O sulfate and/or N-O acetyl conjugates. The excellent leaving group capability of sulfonyloxy- and acetoxy-functionalities in these conjugates is believed to lead to a highly reactive *nitrenium ion*. The nitrenium ion electrophilic species may readily bind covalently to cellular DNA and RNA [10]. The principal *in vitro* metabolic pathway causing mutagenicity of aromatic amines is therefore associated with metabolic activation induced by interactions with the CYP450 isoenzyme CYP1A2, and can be outlined as follows shown below in Scheme 2[11]:



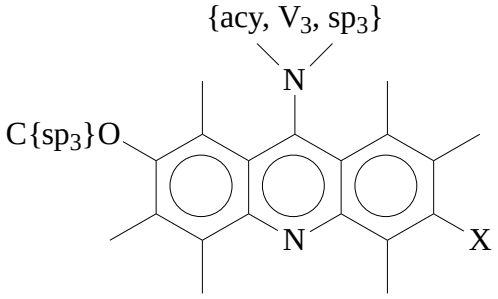
Scheme 2

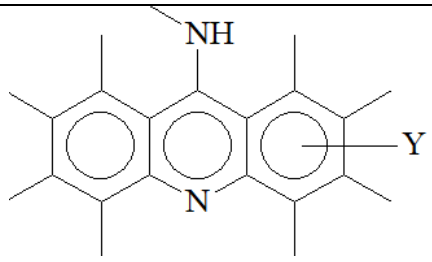
Not only is nitrenium ion chemistry implicated in the DNA damage. For some specific anthraquinone derivatives with electron-donating substituents mutually located at *p*- or *o*-positions, reactions associated with the formation of quinones, quinone imines or other quinoid structures could be involved in the elucidation of the overall mechanistic scheme of bioactivation shown below in Scheme 3 [12]:



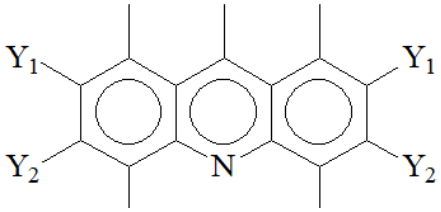
Scheme 3

Set of chemicals used for profile development	Amino Anthraquinones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Zeiger, E., Canc. Res. **47** (1987), 1287 – 1296. 2.Venturini, S., Mutat. Res. **68** (1979), 307 – 312. 3. Double, J. Pharm. Pharmac. **28** (1976), 166 – 169. 4. Gouda, Turk. J. Chem. **34** (2010), 651 – 709. 5. Brock, Mutagen. **6**(1) (1991), 35 – 46. 6. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/. 7. Lang, Mutat. Res. **191** (1987), 139 – 143. 8. Subrahmany, Chem.-Biol. Interactions **56** (1985), 185 – 199. 9. Makena, Environ. Molec. Mutagenesis **48** (2007), 404 – 413. 10. Kalgutkar, Curr. Drug Metabol. **6**(3), 2005, 161 – 225. 11. Shamovsky, JACS **133** (2011), 16168 – 16185. 12. Skipper, Carcinog. **31**(10) (2010), 50 – 58.

Individual profile/alert	
Name	Aminoacridine DNA Intercalators
Type of profile	Structural alert
Description/applicability domain	 (X can be Cl, Br)

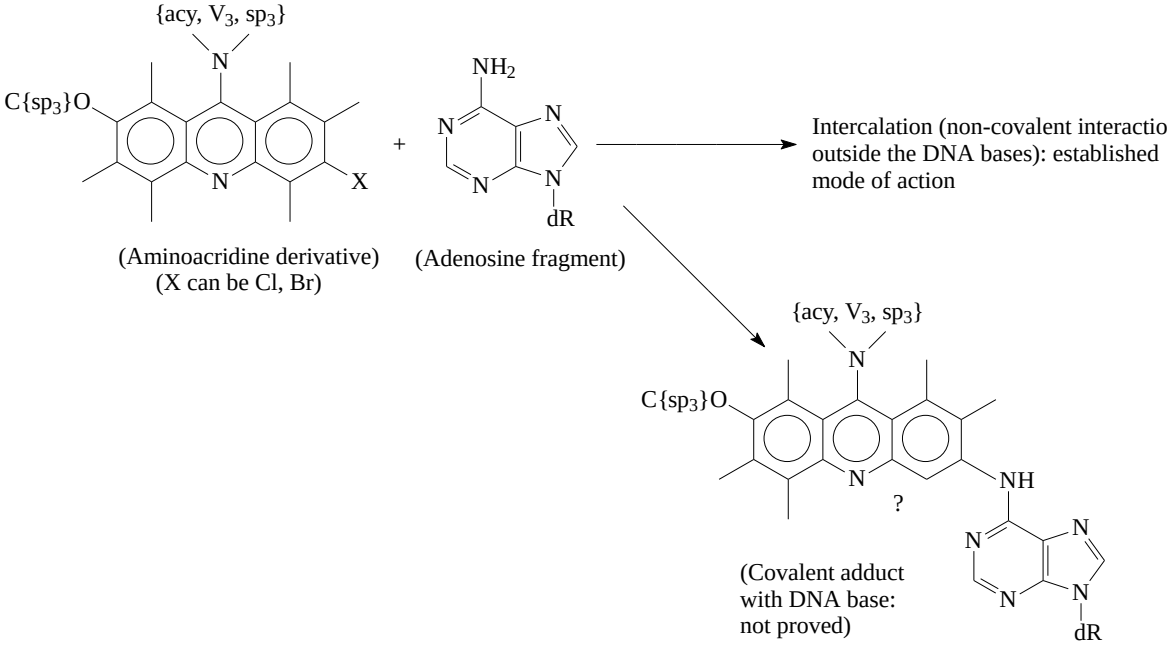


Y = -H, -NH_2

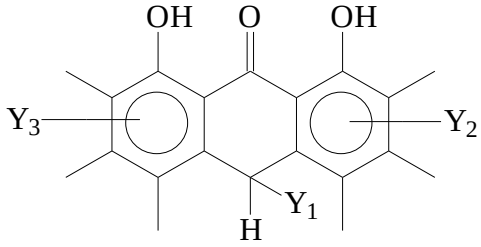
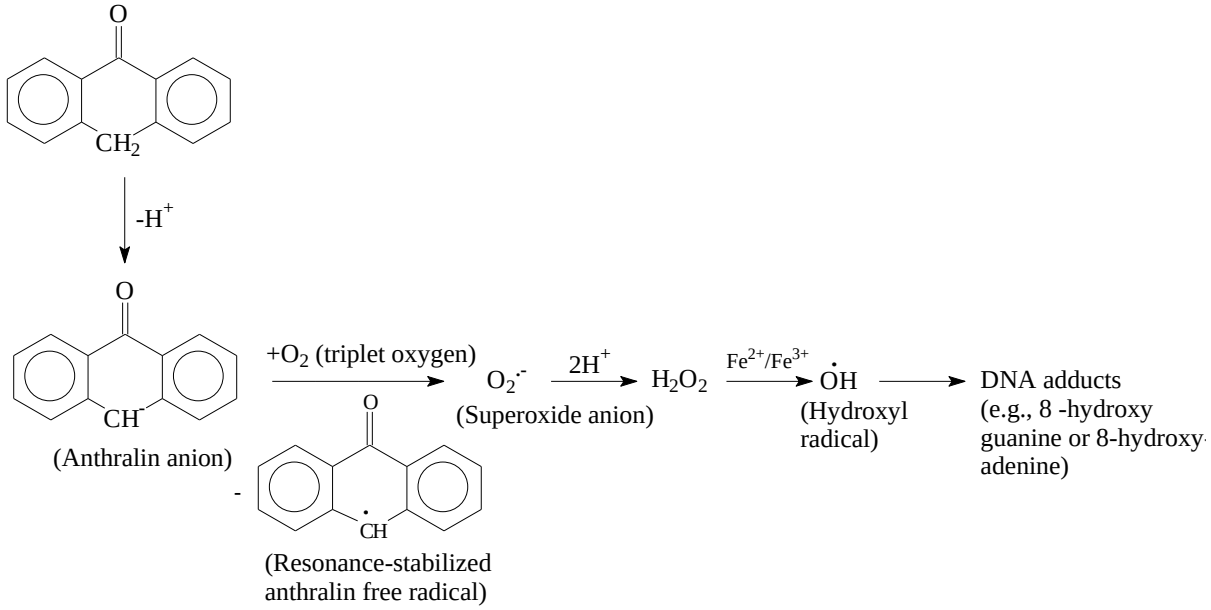


Y_1 = -H, -C{sp3}; Y_2 = -NH_2, -NHC{sp3}, -N(Csp3)$_2$

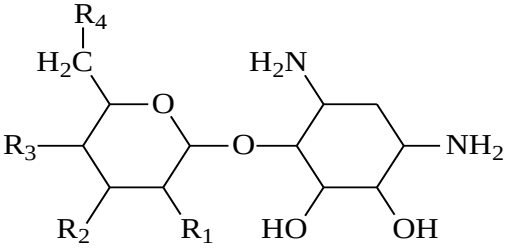
Mechanism	**Non-covalent interactions DNA intercalation**



Set of chemicals used for profile development	Aminoacridine DNA Intercalators
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kalinowska, Mutat. Res. **78** (1980), 7 – 15. 2. Yan, J. Med. Chem. **50** (2007), 4096 – 4104. 3. Wainwright, J. Antimicr. Chemother. **47** (2001), 1 – 13. 4. Hoffmann, Chem. Res. Toxicol. **10**(4) (1997), 347 – 359. 5. Fukui, Nucl. Acids Res. **24**(20) (1996), 3962 – 3967. 6. Asseline, Biocon. Chem. **7** (1996), 369 – 379. 7. Huang, Drug Metabol. Dispos. **34**(7) (2006), 1136 – 1144. 8. Denny, Mutat. Res. **232** (1990), 233 – 241. 9. Ferguson, Eur. J. Canc. **26**(6) (1990), 700 – 714.

Individual profile/alert	
Name	Anthrones
Type of profile	Structural alert
Description/applicability domain	 (Y_1 can be —H or —C(=O)—$(CH_2)_n$H (n = 1 - 3) Y_2, Y_3 can be -H or -CH_3 or -OCH_3 or their combinations)
Mechanism	Radical mechanism by ROS formation (indirect)
	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Muller, Gen. Pharmac. **27**(8) (1996), 1325 – 1335. 2. Mannisto, Arch. Toxicol. **59** (1986), 180 – 185).

Individual profile/alert	
Name	Antibiotic Aminoglycoside Derivatives
Type of profile	Structural alert

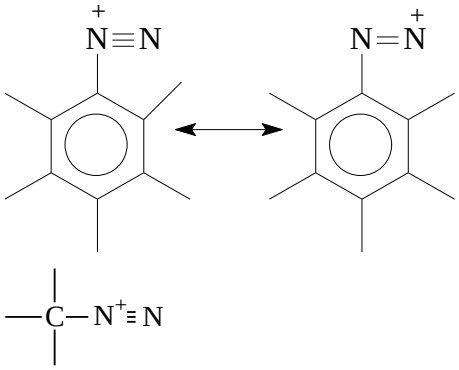
Description/applicability domain	 (R_1 and R_4 are NH_2, R_2 and R_3 are OH; R_1 is NH_2, R_2 - R_4 are OH; R_1 is OH, R_2 is NH_2, R_3, R_4 are OH; R_1, R_2 are OH, R_3 is NH_2, R_4 is OH; R_1 – R_3 are OH, R_4 is NH_2 or CH=O)
Mechanism	Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation

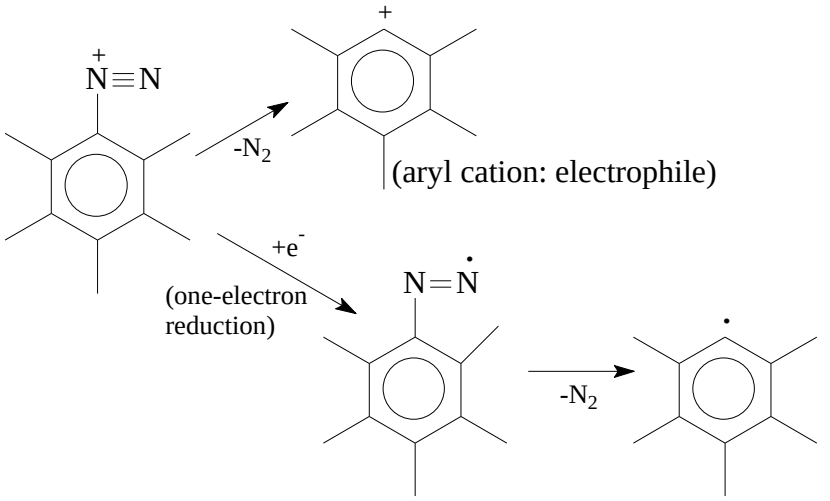
The structure/activity relationships of the pseudodisaccharide core found in aminoglycoside antibiotics were qualitatively studied with a series of synthetic analogues, in which the position of amino groups was varied around the glucopyranose ring. The naturally occurring compound Neamine was the most efficient in the series, according to assays for in vitro RNA binding and antibiotic activity. Therefore, neamine was used as a common core structure for the synthesis of new antibiotics, which were evaluated for binding to models of the Escherichia coli 16S ribosomal RNA, in vitro protein synthesis inhibition, and antibiotic activity. Analysis of RNA binding revealed some correlation between the relative affinity and specificity of RNA binding, and antibacterial efficacy. A linear correlation between in vitro translation inhibition and antibiotic activity was observed [1]. This also suggests in vitro genotoxicity impact.

Abasic sites are probably the most common lesions in DNA, resulting from the hydrolytic cleavage of glycosidic bonds that can occur spontaneously and through DNA alkylation by anticancer agents, by radiotherapy and during the repair processes of damaged nucleic bases. If not repaired, the abasic sites can be mutagenic or lethal. Thus, compounds able to specifically bind and react at abasic sites of DNA have attracted much attention for therapeutic and diagnostic purposes. Therefore, mutagenicity can be elicited by the efficient cleavage activity of characteristic antibiotic drugs of the major aminoglycosides (AG) family at abasic sites introduced either by depurination in DNA or site-specifically in a synthetic oligonucleotide. Among the antibiotic AG drugs selected for this study, neomycin B (neamine derivative) was regarded as the most efficient (a 0.1 μM concentration induces 50% cleavage of an abasic site containing DNA). This cleavage activity could be related to the aminoglycoside toxicity, including genotoxicity but can also find medicinal applications through potentiation of cancer radiotherapy and chemotherapy with alkylating drugs. DNA intercalation processes were proposed to be involved in the DNA cleavage affinity [2].

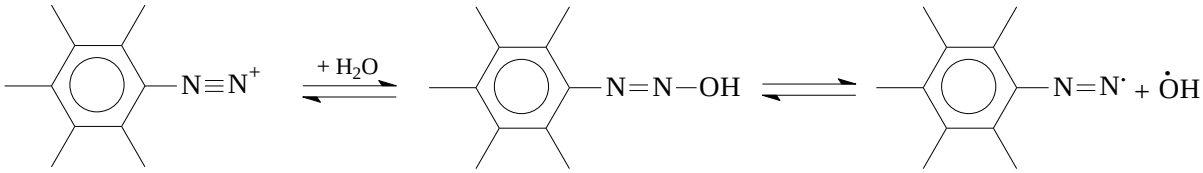
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Greenberg, W. A., E. Sc. Priestley, P. S. Sears, Ph. B. Alper, Chr. Rosenbohm, M. Hendrix, Shang-Cheng Hung, Chi-Huey Wong, Design and Synthesis of New Aminoglycoside Antibiotics Containing Neamine as an Optimal Core Structure: Correlation of Antibiotic Activity with in Vitro Inhibition of Translation, J. Am. Chem. Soc. 121 (1999), 6527 – 6541. 2. De Oliveira, P. M., J. F. Constant, M. Peuchmar, I. Pitta, J. L. Decout, Antibiotic drugs aminoglycosides cleave DNA at abasic sites: shedding new light on their toxicity? Chem. Res. Toxicol.

	26(11) (2013), 1710 – 1719.

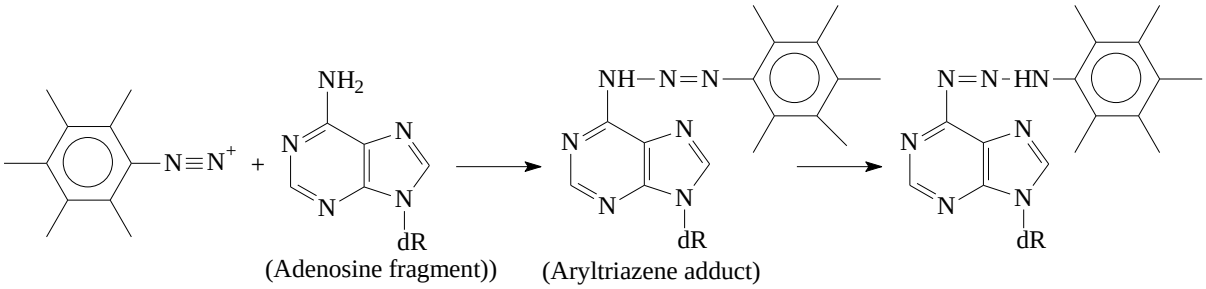
Individual profile/alert	
Name	Arenediazonium and Diazonium Salts
Type of profile	Structural alert
Description/applicability domain	 (C: any carbon atom) (Note: If C is C{ar}, then the two resonance forms should remain as active alerts, since in some (rare) cases, the 2D structure comes as the resonance form on the right (see above))
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: Direct nucleophilic attack on diazonium cation Mechanistic Domain: Radical Mechanistic Alert: Radical attack after one-electron reduction of diazonium cation
It has been suggested that the mechanism of genotoxicity of arenediazonium salts is governed by the arenediazonium salt itself or by the aryl radicals generated. The reactions of several arenediazonium salts with purine bases, their mutagenicity, and the ability to cause DNA damage and generate free radicals have been studied. It has been suggested that the arenediazonium cation or the aryl radical could act as ultimate genotoxins. Arenediazonium cations can react directly with DNA macromolecules or can be converted by reduction or decomposition to other reactive and genotoxic species. For example, arenediazonium ions can also undergo one-electron reduction to diazenyl radicals which, by losing nitrogen, can give rise to aryl radicals. Aryl and diazenyl radicals form adducts with DNA at the C8 carbon of adenine or guanine. The decomposition pathways for arenediazonium ions can be expressed as follows:	

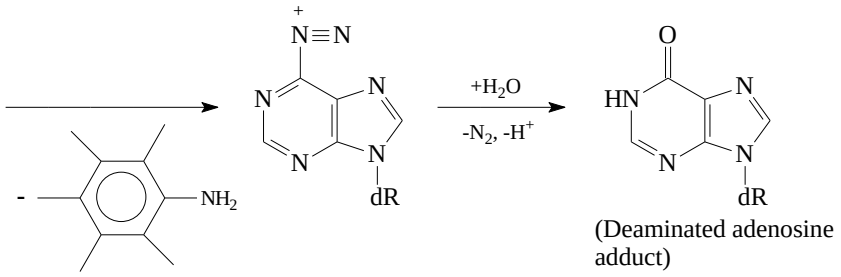


Also, hydroxyl radicals can be formed under these conditions:

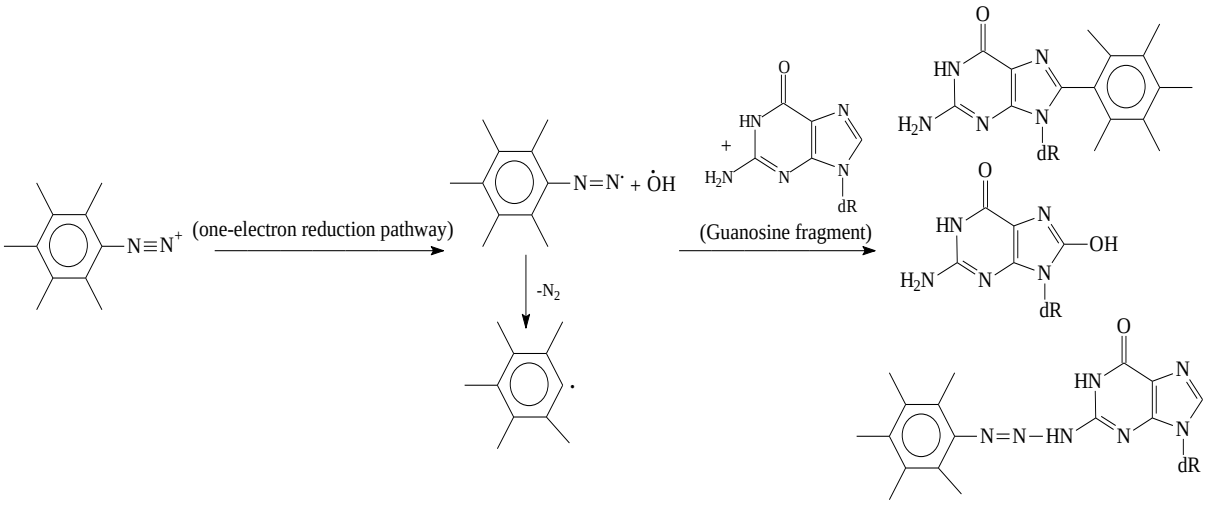


When the arenediazonium ion is the reactive intermediate, the following scheme for the formation of adduct seems to be operative:



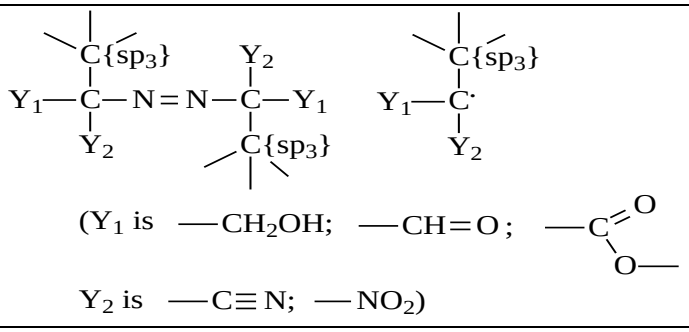


Alternatively, radical mechanism is also possible:



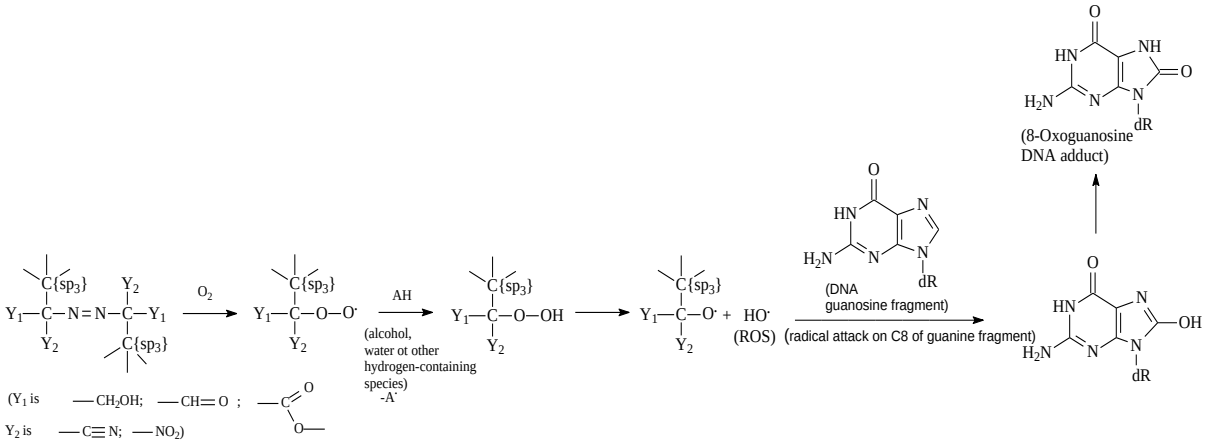
The C8 position of guanine is sufficiently nucleophilic to react directly with arenediazinium cations but it is more reactive to radicals, as shown in the scheme above. The nature of the reactive species depends mainly on the kind of substituent on the phenyl ring of the arenediazonium salt [1]. The mutagenic activities of arenediazonium salts such as arenediazonium fluoroborates have also been confirmed in other publications [2].

Set of chemicals used for profile development	Arenediazonium and Diazonium Salts
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Lawson, T., P. M. Gannett, W. M. Yau, N. S. Dalal, B. Toth, Different Patterns of Mutagenicity of Arenediazonium Ions in V79 Cells and Salmonella typhimurium TA102: Evidence for Different Mechanisms of Action, J. Agric. Food Chem. 43 (1995), 2627 – 2635. 2. Malaveille, Chr., G. Brun, G. Kolar, H. Bartsch, Mutagenic and Alkylating Activities of 3-Methyl-1-Phenyltriazenes and Their Possible Role as Carcinogenic metabolites of the Parent Dimethyl Compounds, Canc. Res. 42 (1982), 1446 – 1453.

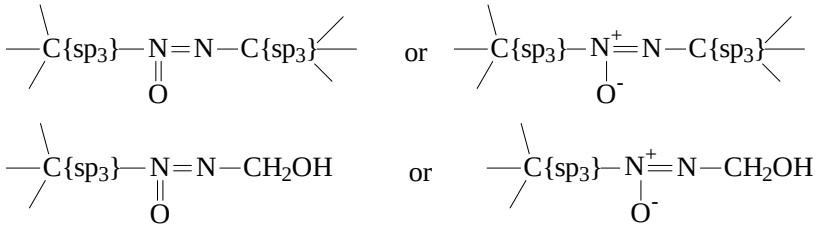
Individual profile/alert	
Name	Azoalkanes with Activating Electron-Withdrawing Groups (EWG)
Type of profile	Structural alert
Description/applicability domain	

Mechanism	Mechanistic Domain: Radical Mechanistic Alert: Radical mechanism via ROS formation

It is now accepted that free radicals, especially active oxygen-centered radicals such as hydroxyl, alkoxyl and peroxyl radicals attack lipids, carbohydrates, proteins and DNA to induce membrane damage, protein modification, enzyme inactivation and strand breaks, and base modification of DNA. This eventually causes a variety of toxicity events such as genotoxicity, mutagenicity and cancer. It is also well known that azo compounds generate free radicals by decomposition, and give peroxyl radicals in the presence of oxygen [1]. Hydrophilic azo compounds such as the target chemical (Table 1) with two or more strongly polar electron-withdrawing substituents (EWG) are capable of decomposing to stabilized carbon-centered radicals and nitrogen molecules. The carbon-centered radicals, in turn, react rapidly with molecular oxygen to give peroxyl radicals, and, eventually, reactive oxygen species (ROS) which elicit genotoxic and mutagenic effects.
Based on the above discussions, the following simplified mechanistic scheme, associated with generation of reactive oxygen species (ROS) can be expertly proposed:

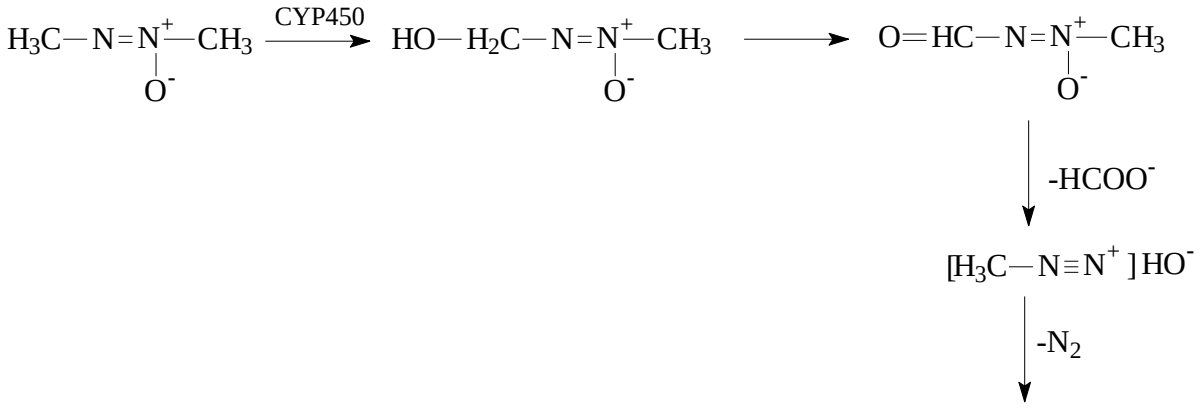


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Yasudaa, H., N. Noguchi,M. Mikib, W. Morinbub, K. Hiranob, Th. Ogihara, T. Tanabeb, M. Minob, K. Terao, E. Niki, Hepatic damage induced by perfusion of radical generating azo compound and its inhibition by vitamin E, Chemico-Biological Interactions 97 (1995), 11 - 23

Individual profile/alert	
Name	Azoxyalkanes
Type of profile	Structural alert
Description/applicability domain	

Mechanism	Mechanistic Domain: SN1 Mechanistic Alert: Direct nucleophilic attack on diazonium cation (DNA alkylation)

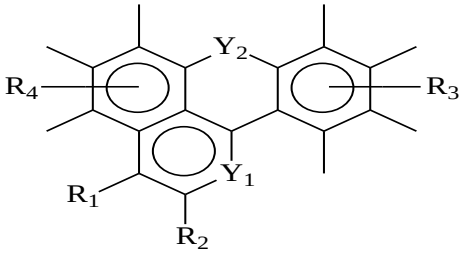
The metabolism of both the azoxymethane and methylazoxymethanol acetate is associated with an ester hydrolysis (for methylazoxymethanol acetate only), and microsomal oxidative N-dealkylation. The mutagenicity and DNA reactivity effects could be mainly due to generation of diazene and alkyl radicals or carbenium and alkanediazonium ions. The following mechanistic scheme of generation of reactive species has been suggested [3, 4]:



The mechanistic scheme is consistent with the assumption that the active end-products of chemicals such as 1,2-dimethylhydrazine and azoxymethane which cause mutagenic DNA lesions such as O6-methylated guanosine and O4-methylated thymidine [5].

Set of chemicals used for profile development	Azoxyalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. CCRIS: Methylazoxymethanol Acetate, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=592-62-1 Last visited: June, 2021. 2. CCRIS: Azoxymethane, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=25843-45-2 last visited: June, 2021 3. Sohn, O. S., H. Ishizaki, Ch. S. Yang, E. S. Fiala, Metabolism of Azoxymethane, Methylazoxymethanol and N-Nitrosodimethylamine by Cytochrome P450IIE1, Carcinog. 12(1) (1991), 127 – 131. 4. Campbell, R. L., J. D. Suppnick, J. M. Hettrick, N. D. Nigro, Rat Liver Microsome-Mediated N-Demethylation and Mutagenicity of Azoxymethane, Canc. Res. 38 (1978), 4585 – 4590. 5. Xiao, W., M. Nowak, S. Laferte, T. Fontanie, Mutagenicity and Toxicity of the DNA Alkylation Carcinogens 1,2-Dimethylhydrazine and Azoxymethane in Escherichia coli and Salmonella Typhimurium, Mutagen. 11(3) (1996), 241 – 245.

Individual profile/alert	
Name	Benzanthrone Derivatives

Type of profile	Structural alert
Description/applicability domain	 (No more than 4 fused rings and no more than one C=O group in the molecular structure; (Y1 can be: C{ar}-H or N{ar}; Y2 is C=O or –CH-OH; R1 is –H or –OH or –OCH3 or -S{V2}CH2- or N{sp3}{V3} or –O-C6H4-; R2 can be –H or –OCH3 or –OH; If Y1 is N{ar}, both R1 and R2 are –H; R3 and R4 are –H (all) or –OH or –OCH3 or combinations; No more than two R3 and R4 other than –H on each ring)
Mechanism	Mechanistic Domain: Radical Mechanistic Alert: ROS generation Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation

Polycyclic aromatic hydrocarbons (PAHs), containing ketone (or quinone) functionality are regarded as environmental contaminants. Their formation during mammalian metabolism can lead to genotoxicity and carcinogenicity through a number of pathways including direct DNA attack, lipid or protein binding and redox cycling. The ketone (or quinone)-enriched fractions of diesel particulates and ultrafine particulate matter have been characterized as more potent toxicants than the PAH fraction for a number of toxic cellular endpoints [1].

According to one publication, a number of benzanthrone derivatives were frameshift mutagens. However, very little data, regarding the in vitro genotoxicity mechanisms of action for such chemicals has been published so far [2].

It has been proposed that reactive and redox-active polycyclic aromatic hydrocarbon (PAH) o-quinones formed by the metabolizing action of aldo-keto reductase enzymes have the potential to cause depurinating adducts leading to the formation of abasic sites and oxidative base lesions. Such lesions are caused by the formation of either singlet oxygen or hydroxyl radicals in the presence of traces of transition metals, and contribute to the experimentally observed mutagenicity and carcinogenicity [3].

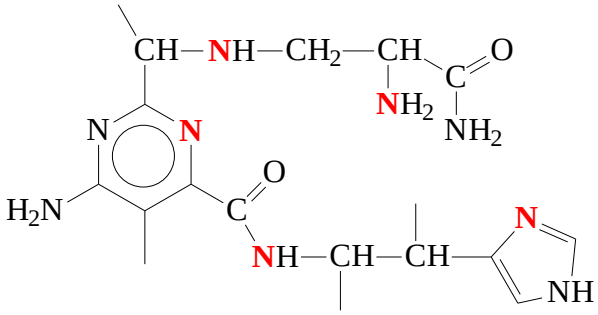
Therefore, it could be expertly assumed that a radical mechanism of generating reactive oxygen species (ROS) such as HO. and subsequent attack on DNA nucleoside bases may explain the genotoxicity of some benzanthrone derivatives. Generation of ROS is probably greatly facilitated by the abundance of phase I metabolic reactions such as Aromatic C-hydroxylation, Oxidative O-dealkylation, and Ketone reduction suggested as predominating transformations for this sub-class of chemicals.

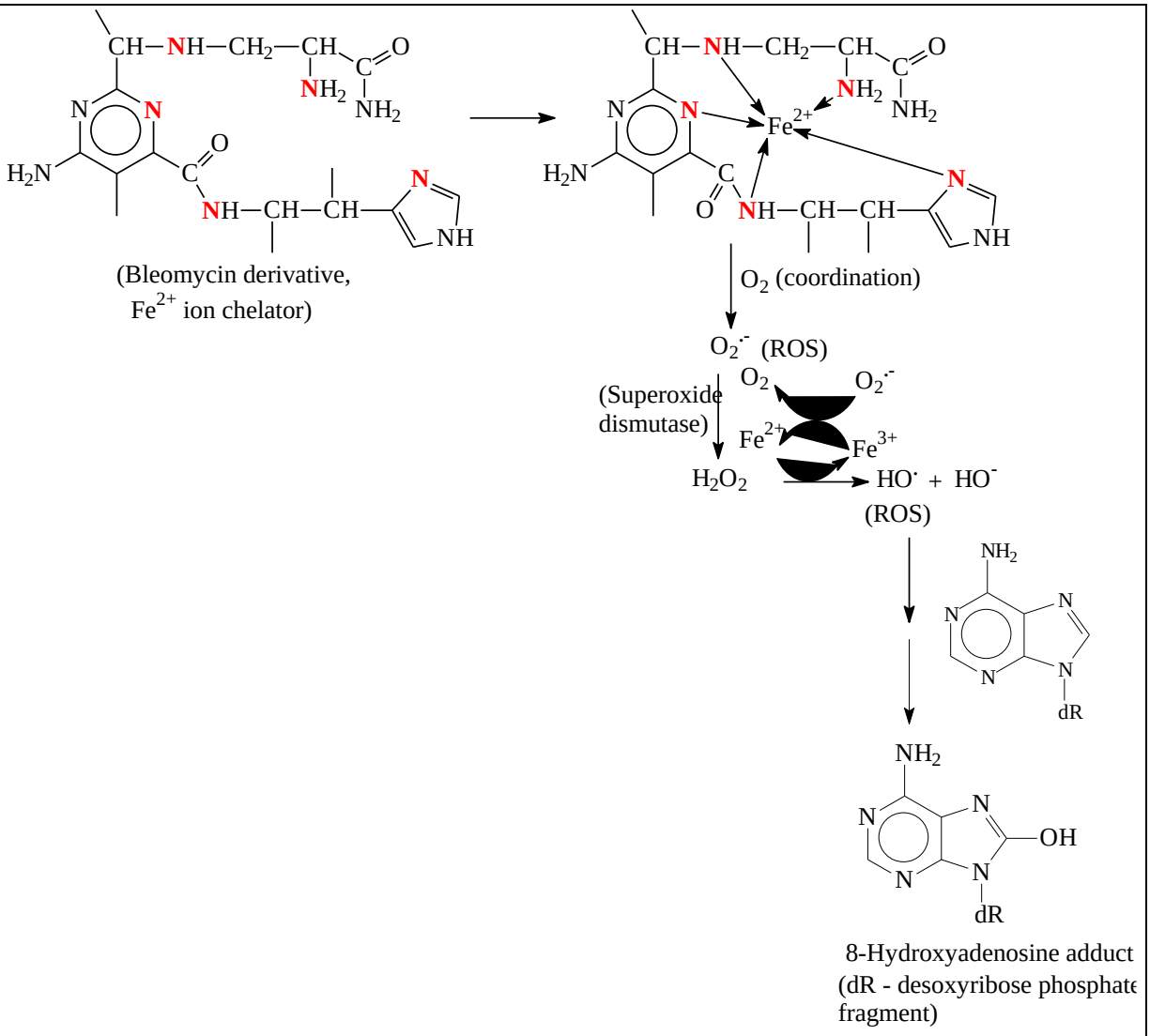
On the other hand, DNA intercalation mechanism cannot be excluded for the benzanthrone derivatives [4], similarly to a number of anthraquinones [5]. Based on the observed experimental data on mutagenicity (Table 1), some important points, regarding the structural boundaries of Ames-positive chemicals can be emphasized on:

- The presence of more than one keto (oxo) group and more than four fused rings in the molecular structure could distort the planarity and reduce intercalation and mutagenicity;
- The presence of electron-donating substituents such as –OH, -OCH3, -OC6H4-, N{sp3}{V3}, etc. may facilitate an intercalative interaction with DNA and mutagenic effects, due to delocalization (+M) effects with aromatic rings.

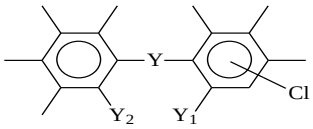
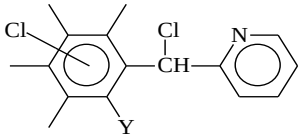
Set of chemicals used for	No chemicals in the local training set

profile development	
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Layshock, J. A., G. Wilson, K. A. Anderson, Ketone and Quinone-Substituted Polyscyclic Aromatic Hydrocarbons in Mussel Tissue, Sediment, Urban Dust, and Diesel Particulate Matrices, Environ Toxicol Chem. 2010, 29(11): 2450–2460. 2. Brown, J. P., P. S. Dietrich, Mutagenicity of Anthraquinone and Benzanthrone Derivatives in the Salmonella/Microsome Test: Activation of Anthraquinone Glycosides by Enzymic Extracts of Fat Cecal Bacteria, Mutat. Res. 1979, 66, 9 – 24. 3. Park, J. H., A. B. Troxel, R. G. Harvey, TR. M. Pennin Polycyclic Aromatic Hydrocarbon (PAH) o-Quinones Produced by the Aldo-Keto-Reductases (AKRs) Generate Abasic Sites, Oxidized Pyrimidines, and 8-Oxo-dGuo via Reactive Oxygen Species, Chem. Res. Toxicol. 2006, 19, 719 – 728. 4. Politica, D. A., Ch. K. Malik, A. K. basu, M. P. Stone, Base-Displaced Intercalated Structure of the N-(2′-Deoxyguanosin-8-yl)-3-Aminobenzanthrone DNA Adduct, Chem. Res. Toxicol. 2015, 28, 2253 – 2266. 5. Double, J. C., J. R. Brown, Evaluation of the Binding of Some Substituted Anthraquinones and Naphthacenequinones to DNA, Communications, J. Pharm. Pharmac., 1976, 28, 166 – 169.

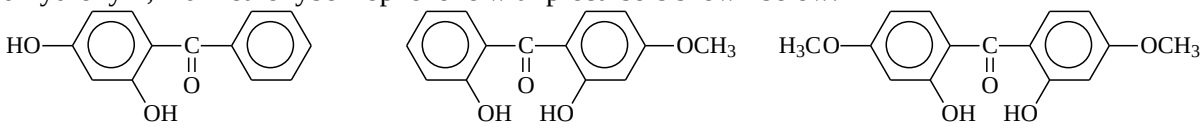
Individual profile/alert	
Name	Bleomycin and Structurally Related Compounds
Type of profile	Structural alert
Description/applicability domain	 (Nitrogen atoms, capable of coordination with Fe2+ are marked in bold and red)
Mechanism	Radical ROS generation & Non-covalent interactions DNA intercalation



Set of chemicals used for profile development	Bleomycin and Structurally Related Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Anderson, D., Mutat. Res. **329**(1) (1995**)**, 37 - 47. 2. Tom, W. M., Biochem. Pharmacol. **29** (1980), 3239 – 3244. 3. Lazo, J. St., Proc. Natl. Acad. Sci. USA **80** (1983), 3064 – 3068. 4. Yamanaka, N., Canc. Res. **38** (1978), 3900 – 3903. 5. Tuimala, J., Carcinog. **23**(6) (2002), 1003 – 1008. 6. Oppenheimer, N. J., Proc. Natl. Acad. Sci. USA **76**(11) (1979), 5616 – 5620. 7. Chapter 2, Literature Review I. Bleomycin 2.1. Chemistry of Bleomycin, University of Pretoria; http://repository.up.ac.za/bitstream/handle/2263/24472/02chapter2.pdf?sequence=3). Last visited: June, 2021. 8. Podger, D. M., Mutat. Res. **117** (1983), 9 – 19. 9. Dixon, Sc. J., Nature Chemical Biology **10** (2014), 9 – 17.

Individual profile/alert	
Name	Chlorinated Diphenylmethane and Benzophenone Derivatives
Type of profile	Structural alert
Description/applicability domain	Alert 1  (Y is CH or C ; Cl is attached anywhere to the ring(s), Y_1, Y_2 is H or OHor combinations) Other possible substituents: NH2, N{V3{sp3}, -OCH3 Alert 2  (Y is H or OH)
Mechanism	A. Chlorinated Benzophenone Derivatives (Y is C=O, Alert 1): Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation B. Chlorinated Diphenylmethane Derivatives (Y is CH-Cl, Alert 2): Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation Mechanistic Domain: SN2 Mechanistic Alert: Alkylation, nucleophilic substitution at sp3-carbon atom

A. Chlorinated Benzophenone Derivatives. Several benzophenone derivatives were suspended in water and chlorinated with sodium hypochlorite at pH 4, 7, and 10. Mutagenicity of chlorinated products was tested by Ames assay using Salmonella typhimurium TA98 and TA100. Chlorination products of 2,4-dihydroxybenzophenone, 2,2'-dihydroxy-4-methoxybenzophenone, and 2,2'-dihydroxy-4,4'-dimethoxybenzophenone with precursors shown below:



showed relatively high mutagenicity. However, the exact structures of the chlorinated products were not specified by the authors [1].

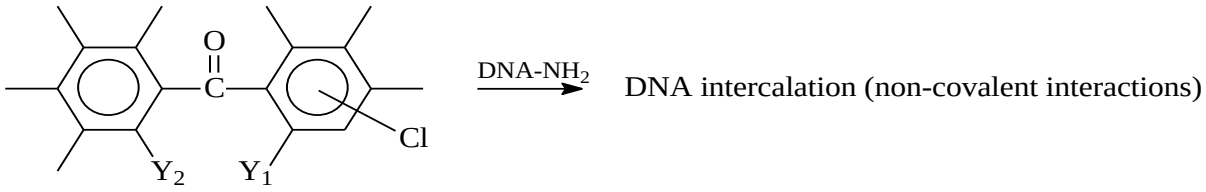
According to other publications, some hydroxylated benzophenone derivatives with structures shown above such as Benzophenone-1 (2,4-Dihydroxybenzophenone), Benzophenone-8 (2,2'-Dihydroxy-4-methoxybenzophenone), and particularly, their chlorination products have shown bacterial mutagenicity in some Salmonella typhimurium strains such as TA98 and TA100 [2, 3].This confirms the experimental observations [1] reported above.

No mechanistic studies, regarding the formation of DNA adducts of chlorinated benzophenone derivatives have been reported. According to other publications, benzophenone and some of its derivatives are capable of intercalating DNA macromolecule [4, 5] by non-covalent interactions. This is mainly due to the specific core structure of benzophenone. It could be assumed that the presence of some polar electron-donating substituents such as -OH, -OCH3, -N{V3}{sp3} enhance this process, causing bacterial mutagenicity in a number of cases. The role of chlorine atoms attached to the phenyl ring(s) of chlorinated benzophenone derivatives would increase genotoxicity via hydrophobicity

effects, by facilitating interaction with DNA, etc. The presence of attached –OH group(s) in positions 2,2' or both (i.e., in o-position with respect to the carbonyl group "bridge") is essential but this specific structural feature is hard to be explained.

B. Chlorinated Diphenylmethane Derivatives. Here DNA intercalation mechanism is also possible, especially when electron-donating substituents such as -OH are attached to the aromatic ring(s). However, the presence of labile halogen in the "bridge" between the two phenyl rings in combination with the additional electron-withdrawing chlorine atom(s) attached to the aromatic rings could be more important. Additionally, if one of the benzenoid-type aromatic ring is pyridinyl one, its electron-withdrawing effect would facilitate the SN2 substitution of the "bridge" chlorine atom by the DNA nucleophilic fragment.

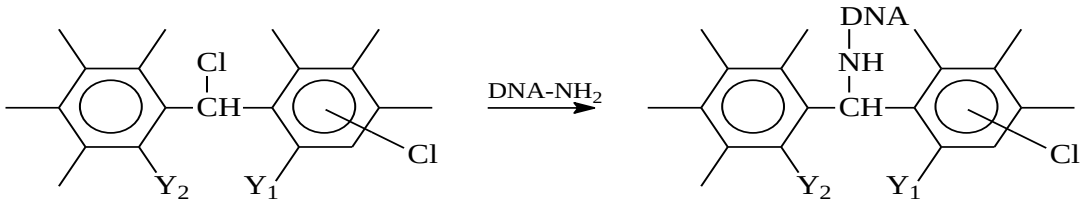
Therefore the following mechanistic schemes, contributing to bacterial mutagenicity of this sub-class of compounds can be expertly proposed:



Cl is attached anywhere to the ring(s), Y_1, Y_2 is H or OH or combinations)

DNA-NH_2: DNA purine/pyrimidine base with -NH_2 group

Scheme 1

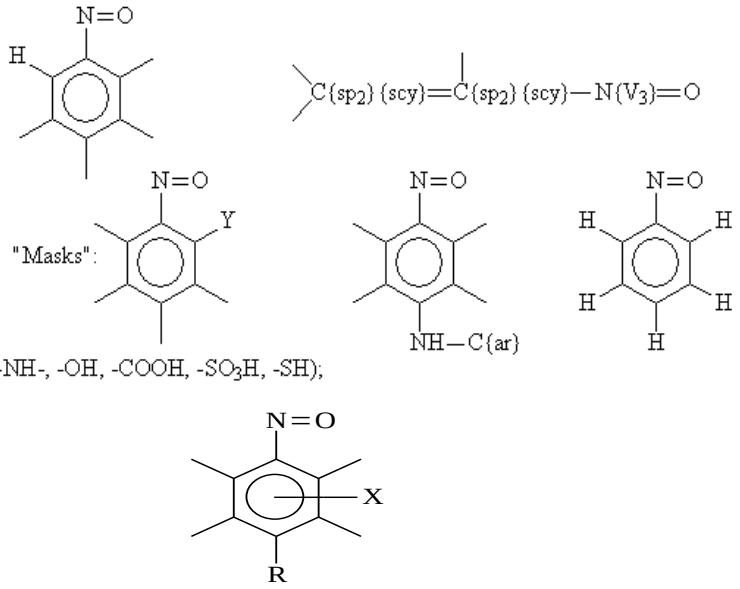


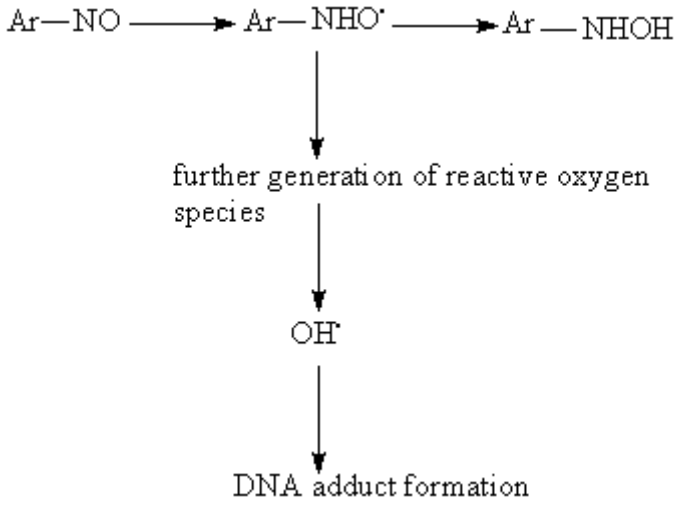
(DNA adduct)

Scheme 2 (SN2 mechanism)

Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Yamamoto, T., D. Nakajima, S. Goto, S. Onodera, A. Yasuhara, Shin-ichi Sakai M. Soma, Mutagenicity of Chlorination Products of Benzophenone and Its Derivatives, J. Environ. Chem. (Published by Japan Society of Environmental Chemistry), 14(2), (2004), 335 – 342 (Abstract). 2. Wang, W.Q., H.-Xin Duan, Zh. T. pei, R. R. Xu, Z.T. Quin, Evaluation by the Ames Assay of the Mutagenicity of UV Filters Using Benzophenone and Benzophenone-1, Int. J. Environ. Res. Public Health, 15 (2018), 1907; doi:10.3390/ijerph15091907. 3. Tarek Manasfi, Michel de Méo, Bruno Coulomb, Carole Giorgio, Sylvain Ravier, et al. Development of mutagenic activity following the chlorination of the sunscreen UV filter benzophenone-8 (dioxyben- zone) in bromide-rich water. International Journal of Hygiene and Environmental Health, Elsevier, 222(4) (2019), 663 – 669; 10.1016/j.ijheh.2019.04.003. hal-02144752. 4. Dumont, E., A. Monari, Benzophenone and DNA: Evidence for a

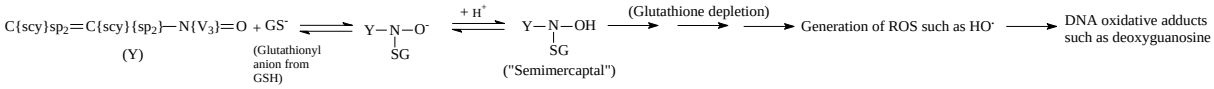
	Double Insertion Mode and Its Spectral Signature, J. Phys. Chem. Lett. 4 (2013), 4119 −4124. 5. Snyder, R. D., J. McNulty, G. Zairov, D. E. Ewing, L. B. Henry, The influence of N-dialkyl and other cationic substituents on DNA intercalation and genotoxicity, Mutat. Res. 578 (2005) 88–99.

Individual profile/alert	
Name	C-Nitroso Compounds
Type of profile	Structural alert
Description/applicability domain	 X is Cl or Br (no more than two), no other substituents; R is H or OH or OCH_3 (only one of either OH or OCH_3); no other substituents
Mechanism	S_N1 Nucleophilic substitution after glutathione-induced nitrenium ion formation and Radical ROS generation (indirect) Radical ROS generation by glutathione depletion
Radical mechanism - the formation of reactive entities such as $ArNHO^{\cdot}$ is known to be implicated in the oxidative DNA damage. Nitrosoarene functionality has superior ability in electron uptake and, for example, nitrosopyrene *in vivo* has significant contribution to the DNA adduct formation. The following mechanistic Scheme 1 is assumed to operate in such cases [6]:	



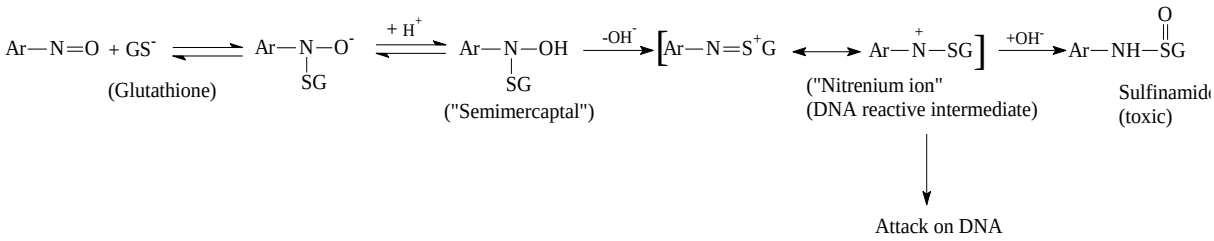
Scheme 1a

As a result from the generation of reactive oxygen species (ROS) such as O2.- and/or HO.. and other radicals such as ArNHO., an additional formation of DNA adducts occurs. For example, hydroxyl radical is DNA-reactive and 8-hydroxyguanine DNA adducts can be formed [7, 8].
Generation of ROS may also occur as a result of glutathione depletion, due to the reactivity of the nitroso group towards GSH as shown in Scheme 2 below. This could be related to the mutagenicity of, e.g., some conjugated 1-nitroso-1-cycloalkenes, containing the structural fragment (II) indicated above. In such a case the proposed mechanistic scheme could be depicted as follows (Scheme 1b):



Scheme 1b

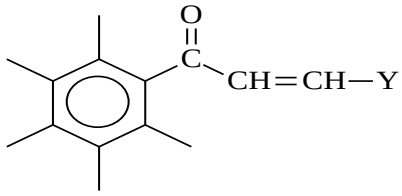
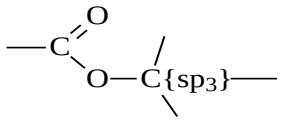
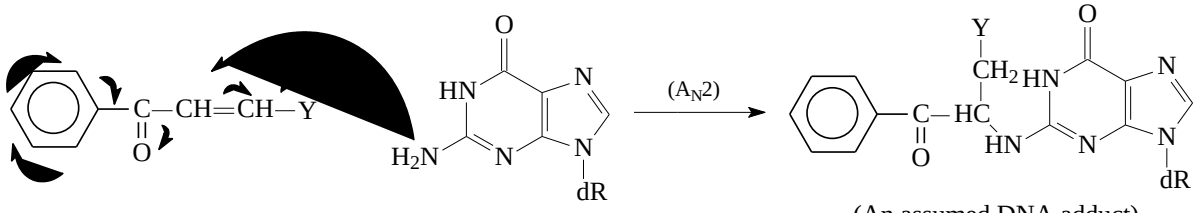
-Non-Radical Mechanism: pseudo-nitrenium ion formation with glutathione (or other thiols) Scheme 2 [4]:



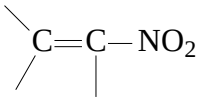
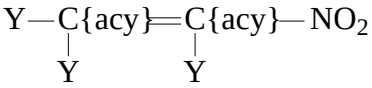
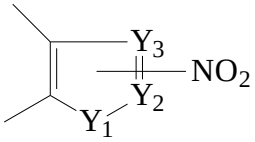
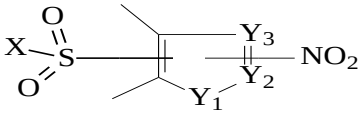
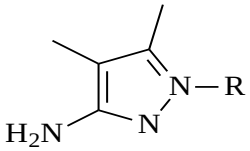
Scheme 2

Set of chemicals used for profile development	C-Nitroso Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. McCoy, Mutat. Res. **173** (1986), 245 – 250. 2. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/. 3. Kranendonk, Mutag. **12**(4) (1997), 245 – 254. 4. Eyer, Environ. Health Persp. **102**, Suppl. 6 (1994), 123 – 132. 5. Galleman, Environ. Health Persp. **102** (Suppl. 6) (1994), 137 – 142.

	6. Kovacic, PCurrent Med. Chem. **8** (2001), 773 – 796. 7. Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 8. Wiseman, Biochem. J. **313** (1996), 17 – 29.

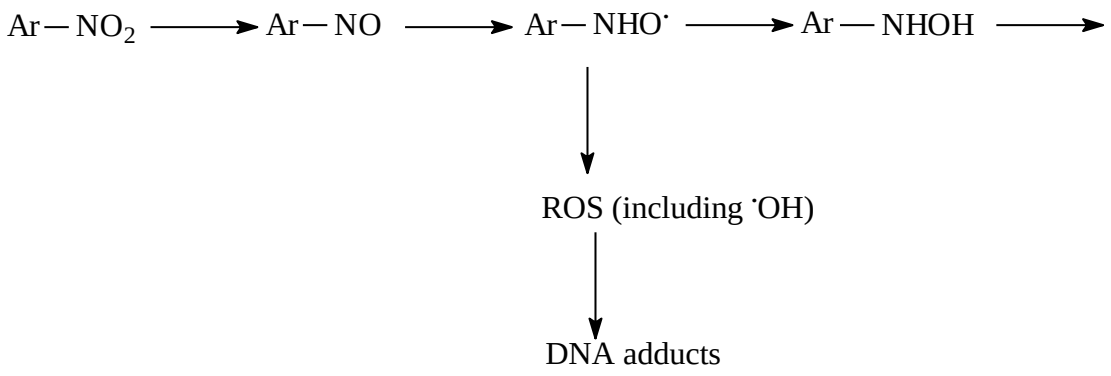
Individual profile/alert	
Name	Conjugated Benzoylethene Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is —C≡N; —NO_2; )
Mechanism	Mechanistic Domain: AN2 Mechanistic Alert: Michael-type nucleophilic addition to α,β-unsaturated compounds conjugated with EWG
There are very few data, regarding the in vitro bacterial mutagenicity of this class of chemicals. The target compound (Table 1) has been reported to be a strong bacterial mutagen even without microsomal/S9 metabolic activation [1]. It can be expertly assumed that chemicals with similar structure, containing strong electron-withdrawing functionalities (EWG) conjugated with the rest of the molecule would also elicit bacterial mutagenicity. The presence of benzoyl moiety with conjugated structure would additionally facilitate the electrophilicity of the carbon in beta-position with respect to the EWG, and the formation of DNA adduct as shown in Scheme 1 below:  (Deoxyguanosine DNA fragment; dR: deoxyribose phosphate fragment) (An assumed DNA adduct)	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Japan ISHA: Strong Mutagenic Chemical Substances; https://www.ajcsd.org/chrip_search/cmpInfDsp?cid=C007-511-82A&bcPtn=5. Last visited: June, 2021.

Individual profile/alert	
Name	Conjugated Nitroalkenes and Five-Membered Aromatic Nitro- and Amino Heterocycles

Type of profile	Structural alert
Description/applicability domain	A. Conjugated nitroalkenes  *Note*: The fragment:  where Y is acyclic C and/or H should be excluded (a "mask") B. Five membered aromatic nitroheterocycles:  Y_1 is N{V3}{sp3}or S{V2} or O Y_2 is N{V3}{sp2} or C{sp2}; Y_3 is N{V3}{sp2} or C{sp2} C. Five-membered aromatic nitroheterocyclic sylfonyl halides:  Y_1 is N{V3}{sp3}or S{V2} or O Y_2 is N{V3}{sp2} or C{sp2}; Y_3 is N{V3}{sp2} or C{sp2} X is Cl, F or Br D. Five-membered aromatic amino heterocycles  (R is –H or -CH3 or –C6H5 (phenyl); at least one hydrogen atom attached to the ring)
Mechanism	Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after nitro group reduction and nitrenium ion formation Mechanistic Domain: Radical Mechanistic Alert: ROS generation Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after metabolic N-hydroxylation and nitrenium ion formation

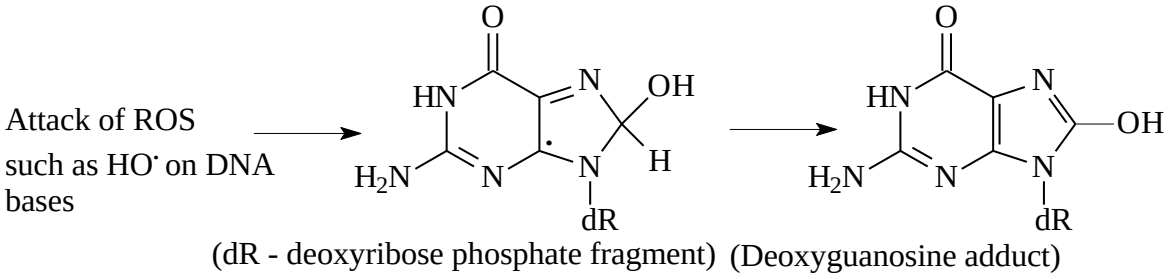
	Mechanistic Domain: SN2 Mechanistic Alert: SN2 attack on sulfur atom

Radical (homolytic) mechanism. This is one of the possible mechanisms for eliciting bacterial mutagenicity of nitro compounds. For instance, certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis [5]. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks):



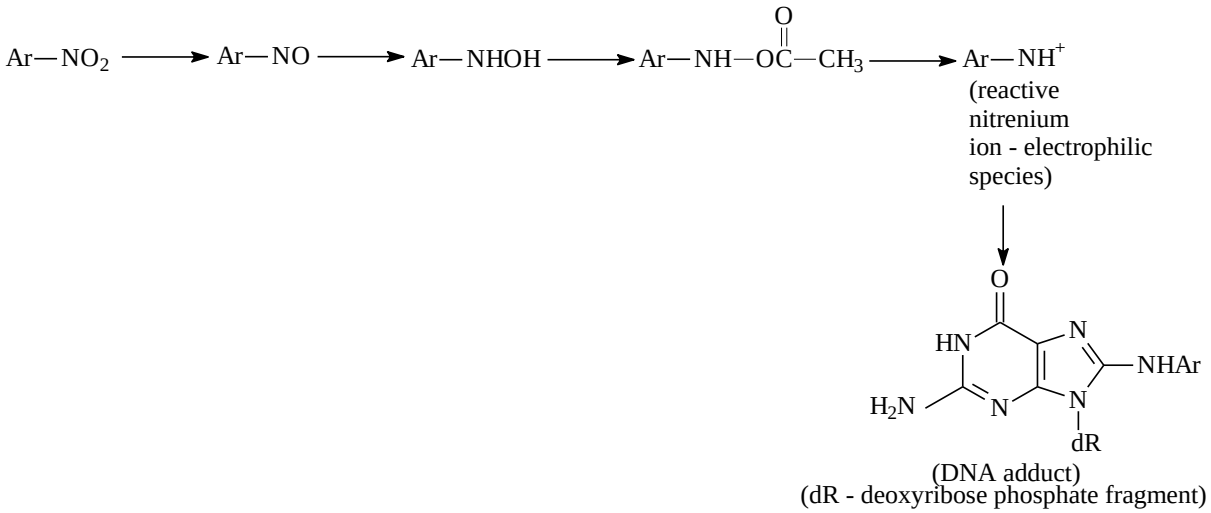
Scheme 1a

As a result, from the generation of reactive radical species such as ArNHO., an additional formation of ROS such as O2.- and/or HO. occurs. The hydroxyl radical, for example, is DNA-reactive and adducts, involving pyrimidine and purine nucleoside bases can be formed. The 8-hydroxyguanine adduct is one of the most mutagenic lesions so far discovered, which can induce DNA strands breaks, etc. [6, 7]:

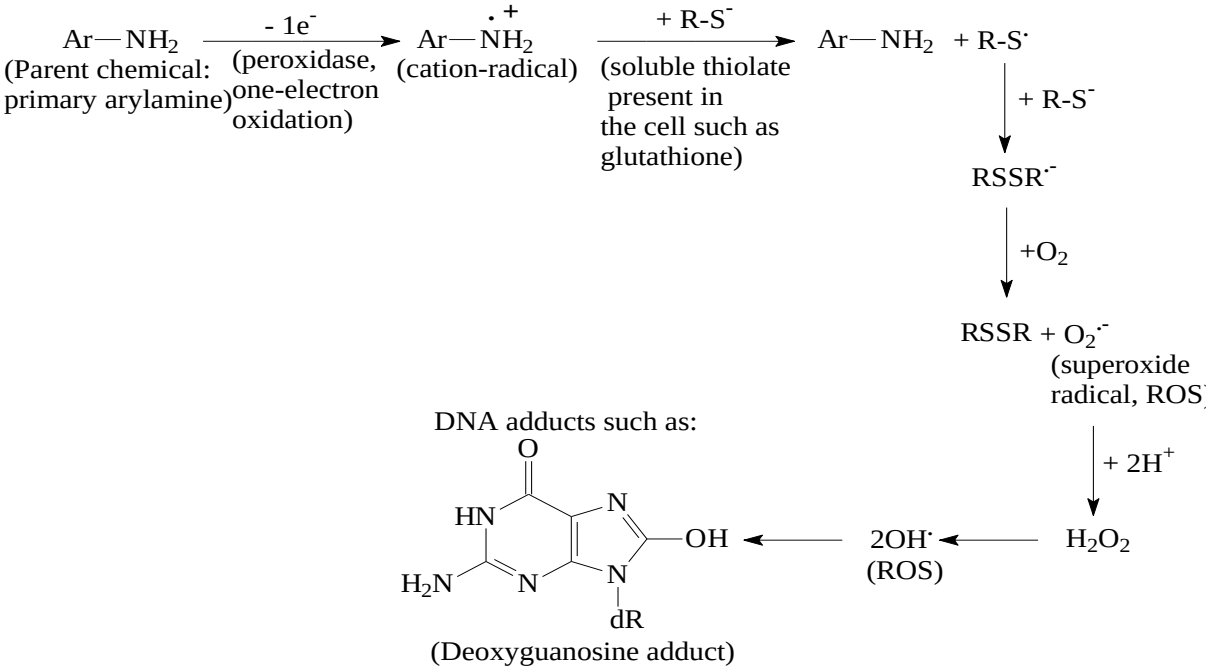


Scheme 1b

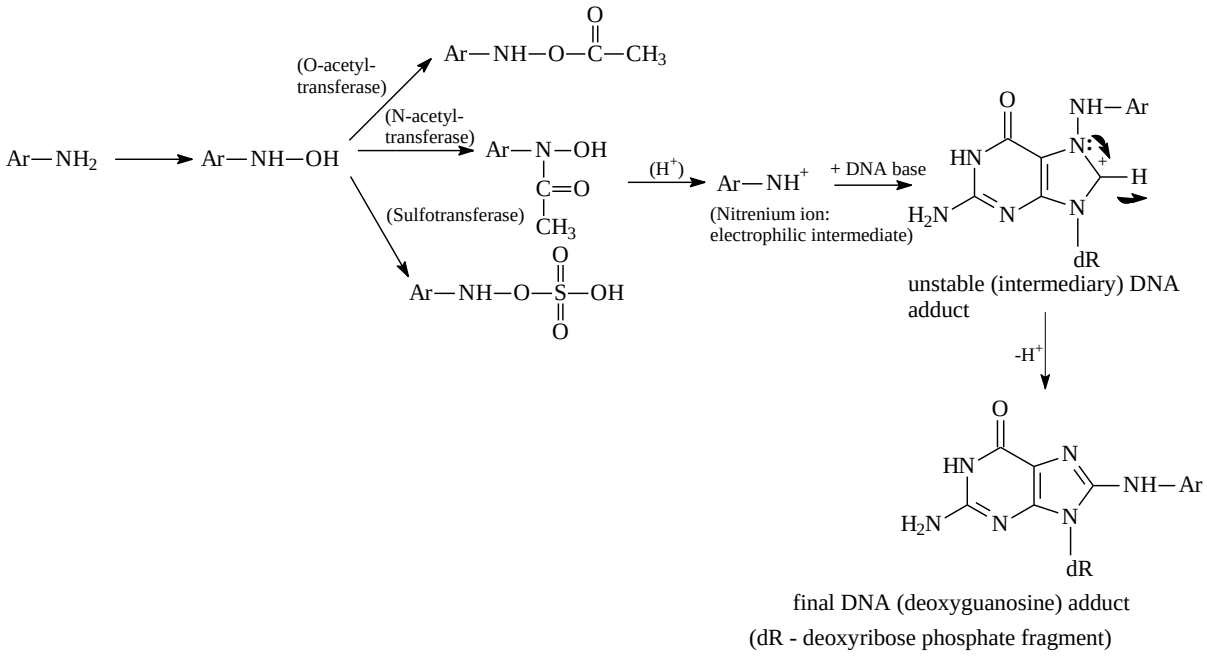
Heterolytic Mechanism. This is also an important mechanism, associated with the bacterial mutagenicity of nitroarenes and related compounds, and, more specifically, the sub-classes discussed here. It also operates in the presence of metabolic S9 activation system. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reactions with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases [1, 2, 8]:



For aromatic aminoheterocycles with structural fragment (IV) (see above), the mechanisms eliciting mutagenicity are similar to those associated with single-ring primary aromatic amines (Schemes 3 and 4)



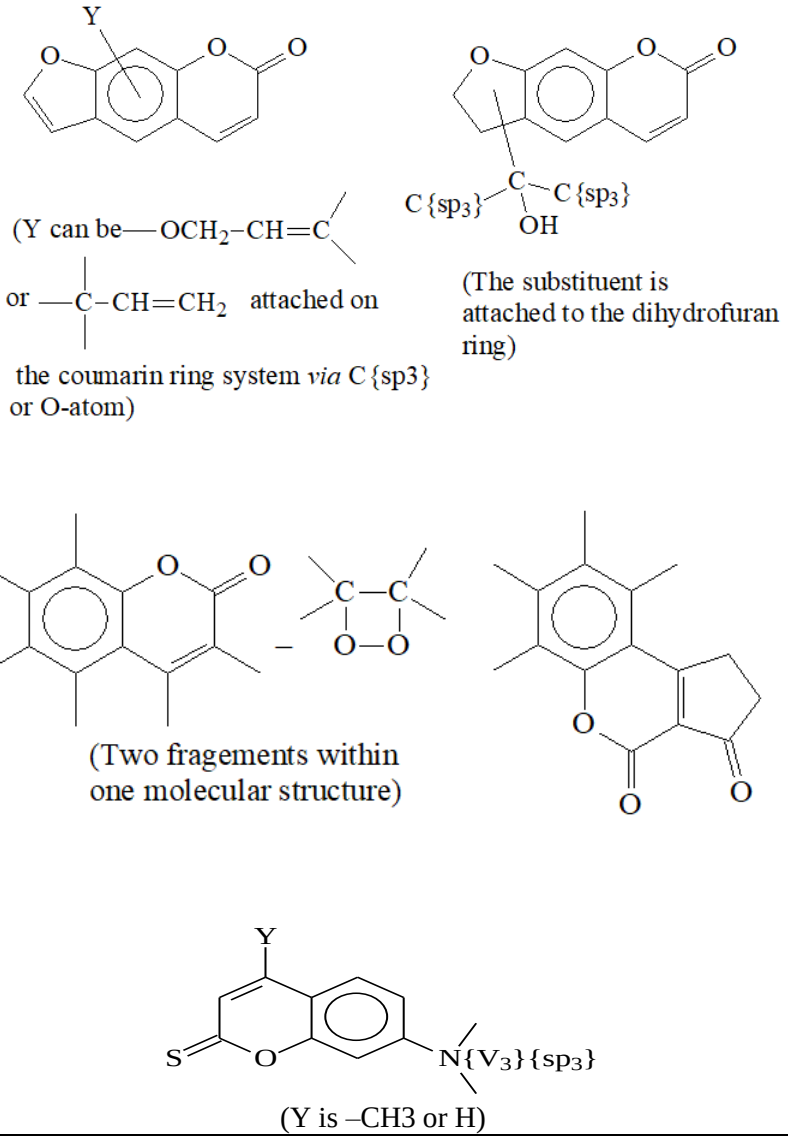
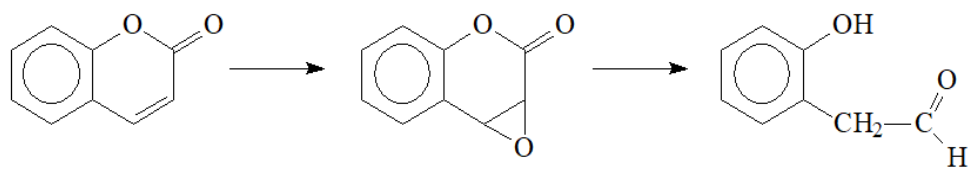
Scheme 3

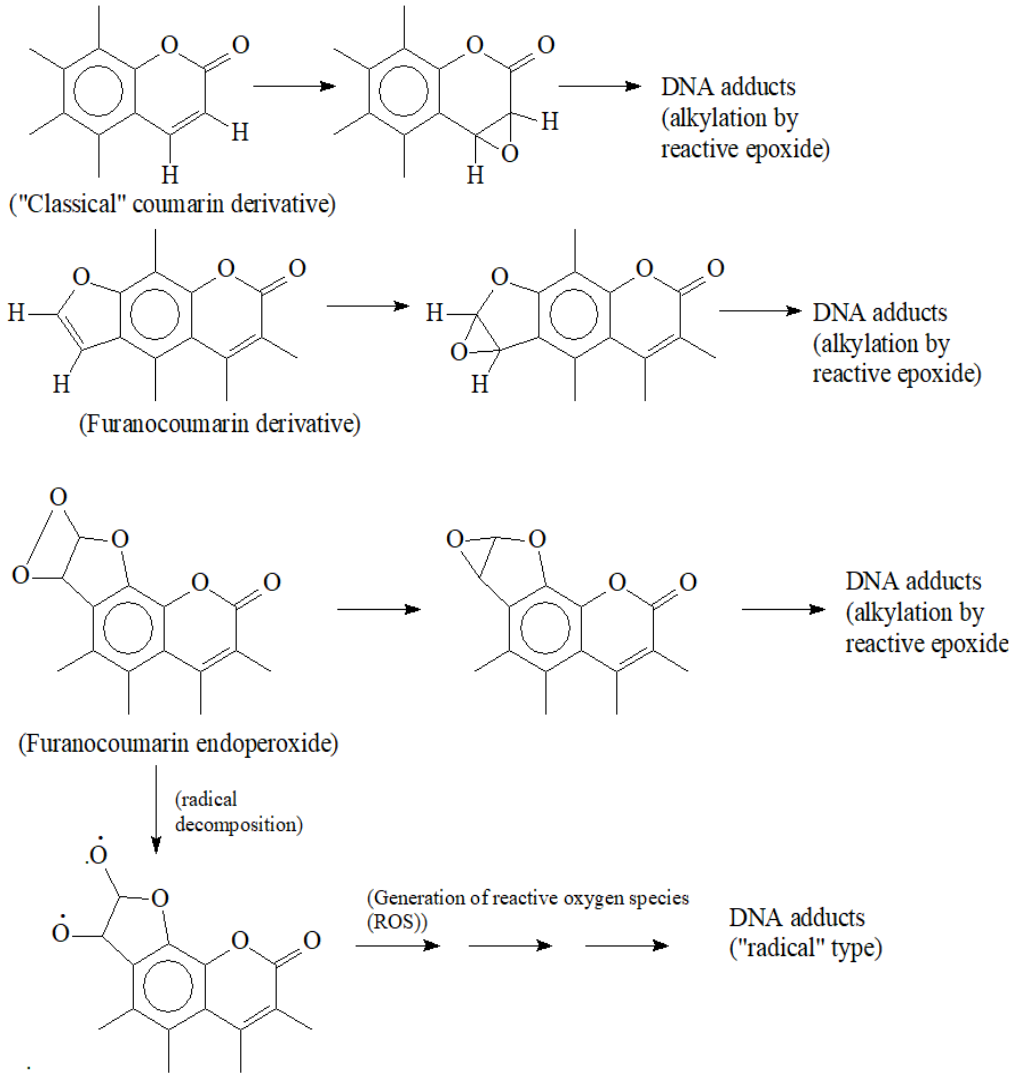


Scheme 4

Set of chemicals used for profile development	Conjugated Nitroalkenes and Five-Membered Aromatic Nitro- and Amino Heterocycles
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2.Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3.Aiub, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4.Einisto, Mutat. Res. **259** (1991), 95 – 102. 5.Kovacic, Current Med. Chem. **8**, (2001), 773 – 796. 6.Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7.Wiseman, Biochem. J. **313** (1996), 17 – 29. 8.Purohit, Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9. Ebringer, Folia Microbiol. **25** (1996), 388 – 396. 10. *Metronidazole*, Puv Chem; https://pubchem.ncbi.nlm.nih.gov/bioassay/1259407#sid=363898360&section=Test-Results. Last visited: June, 2021. 11. Wang, Canc. Res. **35** (1975), 3611 – 3617. 12. Ramos, Mutat. Res. **390** (1997), 233 – 238. 13. Benznidazole CASRN: 22994-85-0, Pub Chem**;** https://pubchem.ncbi.nlm.nih.gov/bioassay/1259407#sid=363900150&section=Test-Results. 14. Misonidazole CASRN: 13551-87-6, Puv Chem; https://pubchem.ncbi.nlm.nih.gov/bioassay/1259407#sid=363899110&section=Test-Results. Last visited: June, 2021. 15. Buschini, A., L. Ferrarini, S. Franzoni, S. Galati, M. Lazzaretti, Fr. Mussi, Cr. N. Albuquerque, T. M. A. D. Zucchi, P. Poli, Genotoxicity Revaluation of Three Commercial Nitroheterocyclic Drugs: Nifurtimox, Benznidazole, and Metronidazole, J. Parasitolog. Res. 2009; doi:10.1155/2009/463575. 16. McMahon, R. E., J. C. Cline, Chr. Z. Thompson, Assay of 855 Test

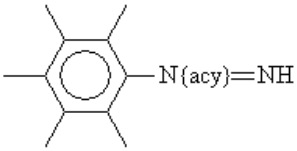
	Chemicals in Ten Tester Strains Using a New Modification of the Ames Test for Bacterial Mutagens, Canc. Res. 39 (1979), 682 – 693.

Individual profile/alert	
Name	Coumarins and Thiocoumarins
Type of profile	Structural alert
Description/applicability domain	
Mechanism	S_N2 Direct acting epoxides formed after metabolic activation, Radical ROS generation, Non-covalent interactions DNA intercalation & S_N1 DNA alkylation
	



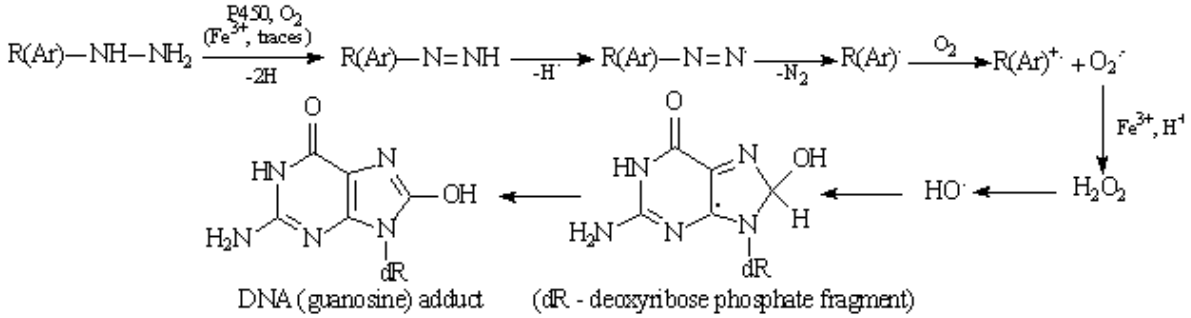
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kostova, I., Curr. Med. Chem. – Anti-Cancer Agents **5** (2005), 29 – 46. 2. *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contacts with Food (AFC) on a Request from the Commission Related to Coumarin*, Question Number EFSA-Q-2003-118 (6 October 2004), The EFSA Journal **104** (2004), 1 – 36; https://www.efsa.europa.eu/en/efsajournal/pub/104, last visited June, 2021. 3. Born, S. D., Drug Metab. Dispos. **30**(5) (2002), 483 – 487. 4. Lacy, A., Curr. Pharmac. Design **10** (2004), 3797 – 3811. 5. Zhou, S., Life Sci **74** (2004), 935 – 968. 6. *Function and Biotechnology of Plant Secondary Metabolites* (Ed. By M. Wink), Annual Plant Reviews, Vol 39, Willey-Blackwell 2010; https://onlinelibrary.wiley.com/doi/book/10.1002/9781444318876. last

	visited June, 2021. 7. Quinto, I., Mutat. Res. **136** (1984), 49 – 54. 8. Uwalfo, A. O., J. Toxicol. Environ. Health: Current Issues **13**(4 – 6) (1984), 521 – 530. 9. Adam, W., Quimica Nova **16**(4) (1993), 316 – 320. 10. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/. 11. Raney, V. M., Chem. Res. Toxicol. **6** (1993), 64 – 68. 12. Loarca-Pina, G., Mutat. Res./Fundam. Molec. Mechanisms of Mutagenesis, **398** (1 – 2) (1998), 183 – 187.

Individual profile/alert	
Name	Diazenes
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS generation (indirect)

Diazenes are intermediate products of the oxidative transformation of hydrazine derivatives, which are formed by oxidative bioactivation (see also Hydrazine Derivatives). All aryldiazenes react rapidly with oxygen, however, the corresponding reaction of aryldiazenes, containing electron-withdrawing substituents such as nitro group is slow. The major product of the bimolecular decomposition of aryldiazenes is the corresponding aromatic hydrocarbon [1].

On the basis of the available literature data, the following generalized scheme, similar to those suggested for Hydrazine Derivatives and Arenediazonium Salts can be assumed to operate via radical mechanism by reactive oxygen species (ROS) formation [2 – 6]:

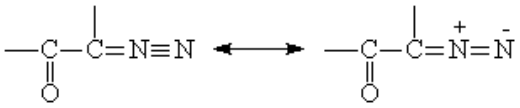
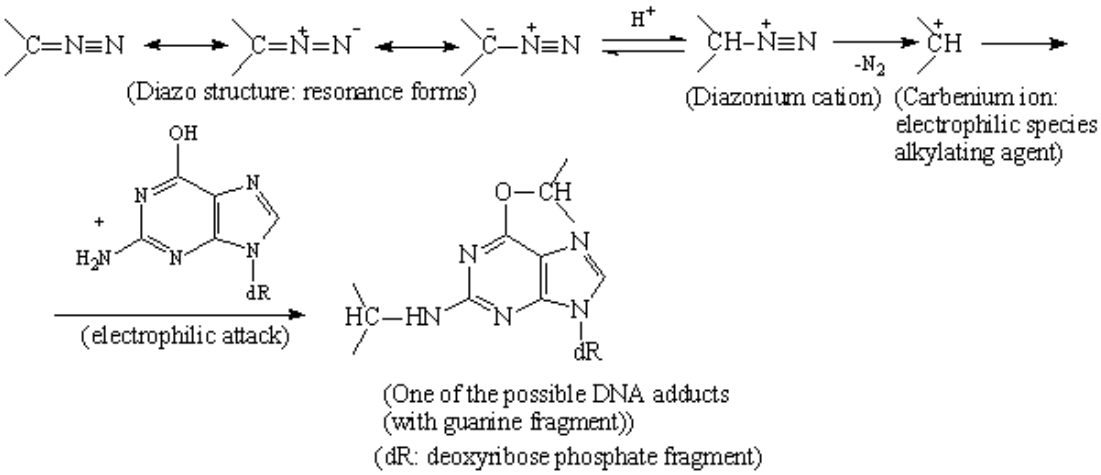


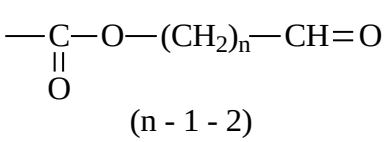
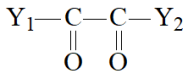
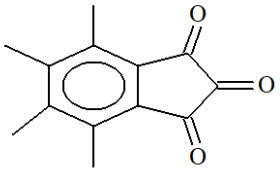
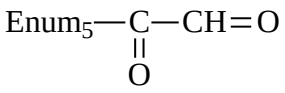
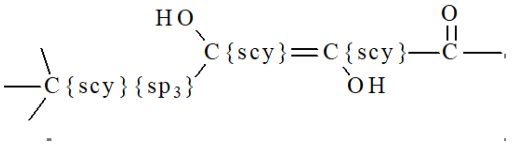
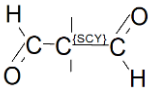
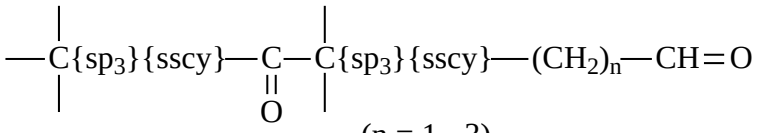
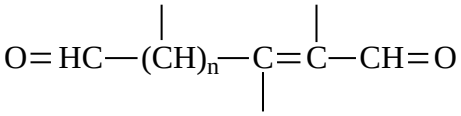
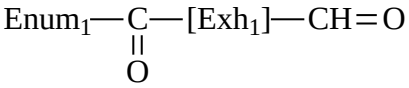
Scheme 1

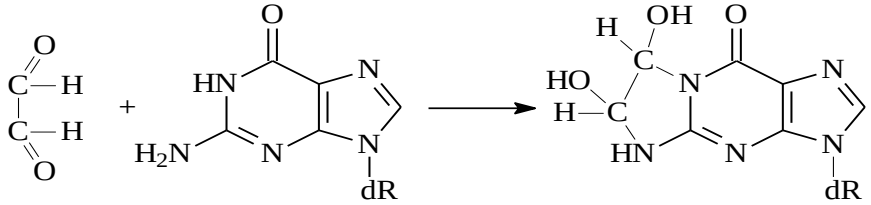
ROS can be also generated as a result from oxidation/reduction processes in bacteria without addition of exogenous S9 system. In such a case, the radical mechanism discussed above is likely to operate.

Set of chemicals used for profile development	Diazenes
Data/Knowledge used	An extensive review of the literature was performed enabling the

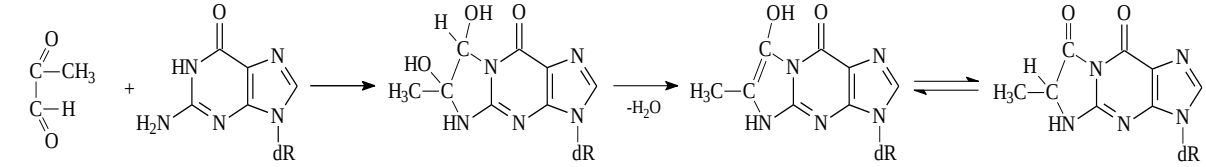
for profile development	chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kosower, J. Am. Chem. Soc. **91**(9) (1969), 2325 – 2329. 2. Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3. Kovacic, Current Med. Chem. **8** (2001), 773 – 796. 4. Rumyantseva, J. Biol. Chem. **266**(32) (1991), 21422 – 21427. 5. Quintero, Ars Pharmaceutica **41**(1) (2000), 27 – 46. 6. Gannet, Chem. Biol. Interact. **80**(1) (1991), 57 – 72.

Individual profile/alert	
Name	Diazoalkanes
Type of profile	Structural alert
Description/applicability domain	
Mechanism	S_N1 Alkylation by carbenium ion formed
The following mechanistic scheme for DNA alkylation by this class of compounds can be assumed based on literature:  	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.L. Fishbein, Studies in Environmental Science, Vol. 4, Elsevier 1979, p. 118 - 134); http://www.sciencedirect.com/science/article/pii/S0166111608713177. https://doi.org/10.1016/S0166-1116(08)71317-7 Last visited: June, 2021. 2. Pezacki, J. P., *Rate Constants and Mechanisms for Reactions of Carbenes and Cations from Oxadiazolines and Other Precursors*, Thesis for PhD degree, 1998, McMaster University. 3. Kusmierek, Nucl. Acids Res. **3**(4) (1976), 989 – 1000. 4. Farmer, Biochem. J. **135** (1973), 203 – 213.

Individual profile/alert	
Name	Dicarbonyl Compounds
Type of profile	Structural alert
Description/applicability domain	 (n - 1 - 2)  (Y_1, Y_2 can be H and/or C)   ($Enum_5$ is N{V3}{sp3} or $-OCH_3$)    (n = 1 - 3)  (n - 0 - 2)  ($Enum_1$ is H or C{sp3}; Exh_1 is $-(CH_2)_n-$ (n = 1 - 3)
Mechanism	Mechanistic Domain: AN2 Mechanistic Alert: Shiff base formation
The mutagenic activities in the *Ames* test against *Salmonella typhimurium TA100* for a series of α-dicarbonyl compounds were associated with the chemical reactivity of these compounds towards purine bases in DNA, more particularly, with the extent of stability of adducts formed. The molecular basis of mutagenic action of glyoxal derivatives, for example, was suggested to be the interaction between the dicarbonyl compound and the guanine fragments of nucleic acid with the formation of geminal carbinolamines and Shiff bases [3]. The reaction product of glyoxal and guanosine was isolated and structurally characterized. Concerning its structure, a new ring was formed involving specific positions in the guanine ring and both carbonyl functionalities of glyoxal, according to the following scheme [4]:	

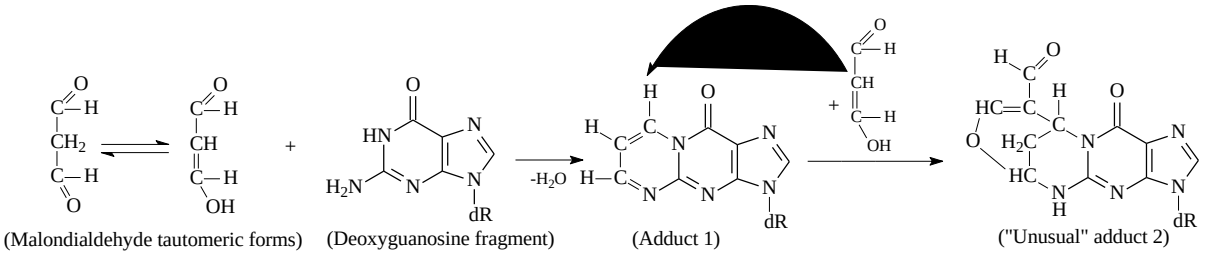


Methylglyoxal is a sugar degradation product, which is endogenously formed by fragmentation of triose phosphates during the metabolic glycolysis. It has shown bacterial mutagenicity in Salmonella typhimurium. The prolonged exposure of DNA to high concentrations of methylglyoxal under physiological conditions resulted in the sequential formation of adducts, according to the following scheme [5]:

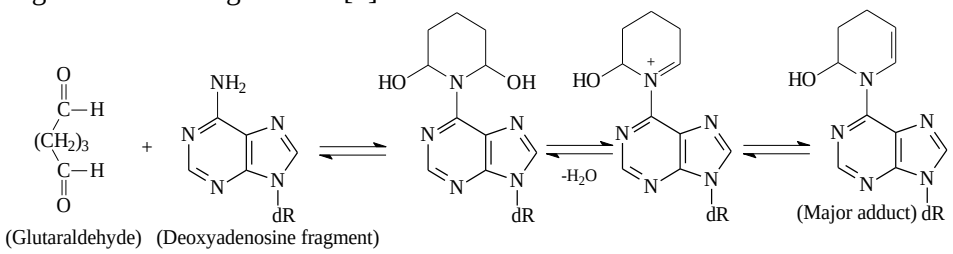


Reaction scheme, similar to those outlined above was suggested for the formation of DNA covalent adducts with the butter flavorant, diacetyl [6].

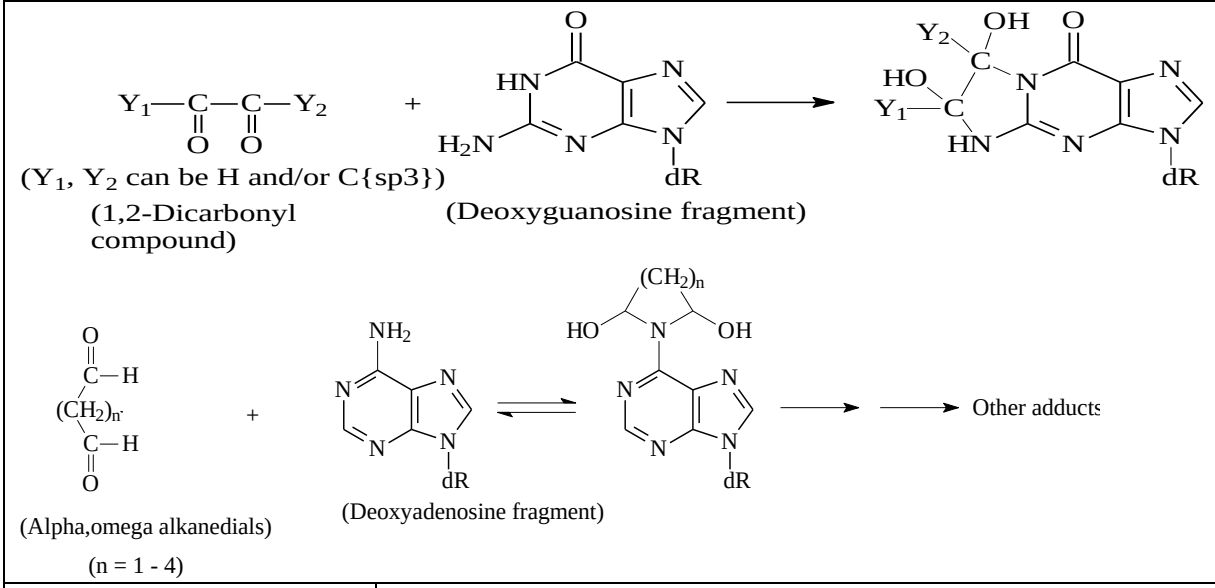
The other class of mutagenic dicarbonyl compounds such as α,ω-alkanedials acted, according to different mechanistic scheme. For instance, malondialdehyde was found to be reactive towards proteins and DNA, and, also, mutagenic. In Salmonella typhimurium it induced frameshift mutations, and structure-activity studies indicated that both carbonyl moieties were required for the formation of adducts, according to the following scheme [7]:



Finally, several unstable adducts of 2'-deoxyadenosine from the calf thymus DNA with 1,5-pentanedial (glutaraldehyde), which is also bacterial mutagen, were reported, and the major adduct was formed, according to the following scheme [8]:

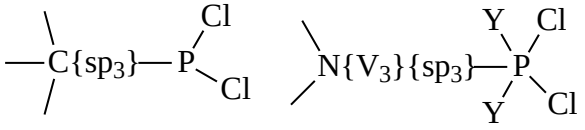
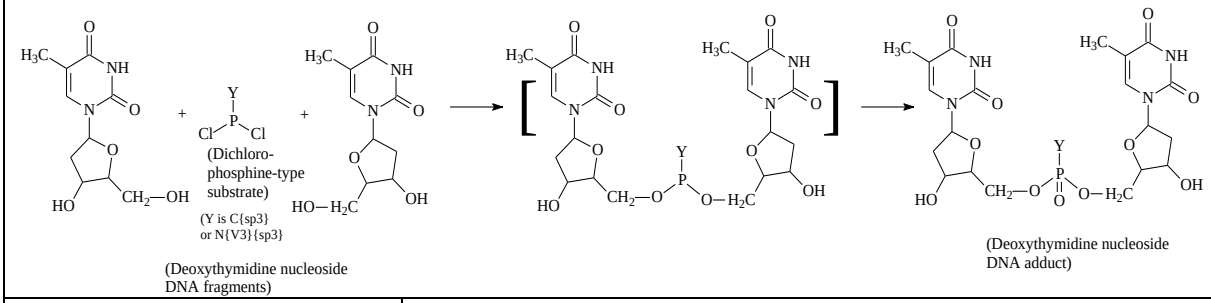


Based on the above considerations, the following generalized mechanistic schemes for this class of compounds can be outlined:

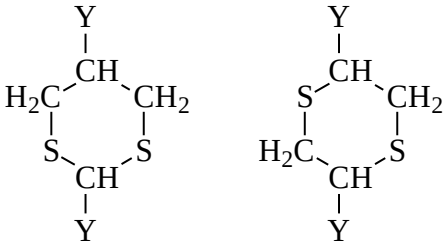


Set of chemicals used for profile development	Dicarbonyl Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Bjeldanes, L. F., H. Chew, *Mutagenicity of 1,2-Dicarbonyl Compounds: Maltol, Kojic Acid, Diacetyl, and Telated Substances*, Mutat. Res. **67** (1979), 367 – 371. 2. Dorado, L., M. R. Montoya, J. M. R. Mellado, *A Contribution to the Study of the Structure – Mutagenicity Relationship for α-Dicarbonyl Compounds Using the Ames Test*, Mutat. Res. **269** (1992), 301 – 306. 3. Mellado, J. M. R., M. R. Montoya, *Correlations between Chemical reactivity and Mutagenic Activity Against* S. typhimurium TA100 for *α-Dicarbonyl Compounds as a Proof of the Mutagenic Mechanism*, Mutat. Res. **304** (1994), 261 – 264. 4. Shapiro, R., J. Hachmann, *The Reaction of Guanine Derivatives with 1,2-Dicarbonyl Compounds*, Biochem. **5**(9) (1966), 2799 – 2807). 5. Frishmann, M., Cl. Bidmon, J. Angerer, M. Pischetsrieder, *Identification of DNA Adducts of Methylglyoxal*, Chem. Res. Toxicol. **18** (2005), 1586 – 1592). 6. More, S. S., A. Raza, R. Vince, *The Butter Flavorant, Diacetyl, Forms a Covalent Adduct with 2-Deoxyguanosine, Uncoils DNA, and Leads to Cell Death*, J. Agric. Food Chem. **60** (2012), 3311 – 3317. 7. Marnett, L. J., A. K. Basu, S. M. O, Hara, P. E. Weller, A. F. M. M. Rahman, J. P. Oliver, *Reaction of Malondialdehyde with Guanine Nucleosides: Formation of Adducts Containing OxadazabicyclononeneResidues in the Base-pairing Region*, J. Am. Chem. Soc. **108** (1986), 1348 – 1350). 8. Olsen, R., J. Backman, P. Molander, St. Ovrebo, S. Thorud,

	E. Lundanes, T. Grebrokk, L. Kronberg, *Characterization of Adducts Formed in the Reaction of Glutaraldehyde with 2'-deoxyguanosine*, Chem. Res. Toxicol. **20** (2007), 965 – 974.

Individual profile/alert	
Name	Dichlorophosphine and Dichlorophosphonium Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is N{V_3}{sp_3}, O or C or combinations))
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: Alcohol Phosphorylation
No published data on bacterial mutagenicity of the chemicals shown in Table 1 above has been found. However, according to some publications [1, 2], the following principal mechanistic scheme for formation of DNA adducts, which may elicit mutagenicity can be proposed (Scheme 1 below):  (Dichloro-phosphine-type substrate) (Y is C{sp3} or N{V3}{sp3} (Deoxythymidine nucleoside DNA fragments) (Deoxythymidine nucleoside DNA adduct)	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Loeschner, T., J Engels, One-Pot R$_P$-Diasetereoselective Synthesis of Dinucleoside Methylphosphonates Using Methyldichlorophosphine, Tetrahedron Lett. 30(41) (1989), 5587 – 5590. 2. Nawrot, B., M. Sobszak, Sl. Antoszczyk, Synthesis of Dinucleoside (N3'→ MeP') Methanephosphonamidates, Org. Lett. 4(10), (2002), 1799 – 1802.

Individual profile/alert	
Name	Dithianes
Type of profile	Structural alert

Description/applicability domain	 (Y is H or –CH=O or –CH_2-N=C=O or combinations)
Mechanism	Mechanistic Domain: AN2 Mechanistic Alert: Schiff base formation Mechanistic Domain: AN2 Mechanistic Alert: Acyl transfer via nucleophilic addition reaction (DNA carbamoylation)

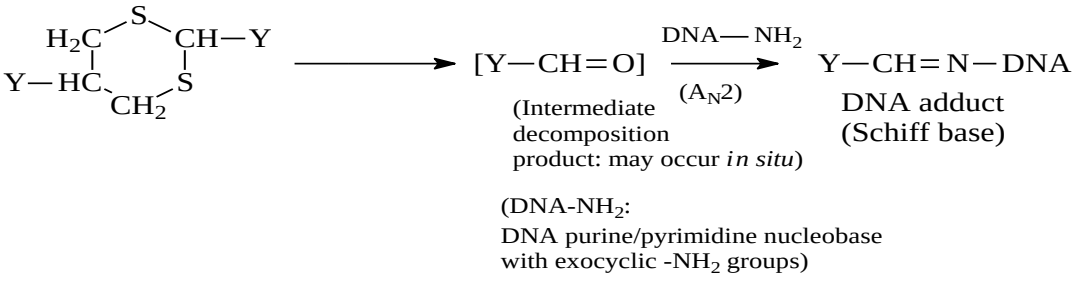
According to some authors, the decomposition of 1,3-dithiane may result in the release of low-molecular aldehyde derivative. The aldehyde can then undergo a Schiff-base reaction with DNA bases, causing mutagenicity [1].

The reactivity of methyl isocyanate (MIC) and phenyl isocyanate (PIC) with DNA, and the genotoxicity of MIC were investigated. MIC and PIC reacted with the exocyclic amino group of deoxycytidine, deoxyadenosine and deoxyguanosine nucleosides in DNA to produce carbamoylated adducts. The reactions of both isocyanates with deoxycytidine were 2 and 4 orders of magnitude higher than with deoxyadenosine and deoxyguanosine, respectively. The degree of mutagenicity effects of methylisocyanate could not be firmly established [2].

It is assumed that the bacterial mutagenicity of the target chemical (Table 1) is mainly determined by the combination of dithiane and isocyanate functionalities.

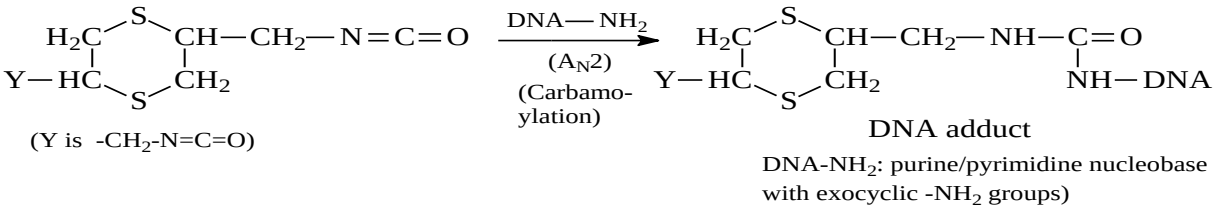
Based on the above information, the following simplified mechanistic schemes can be expertly proposed:

Mechanistic scheme A:

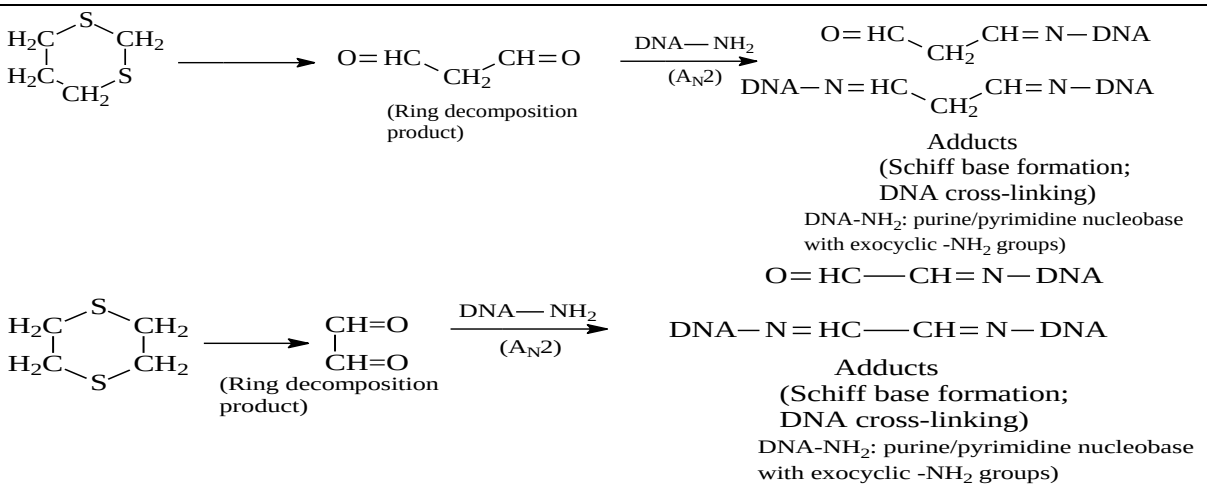


Note: If both Y are –CH=O DNA cross-linking may also occur.

Mechanistic scheme B:

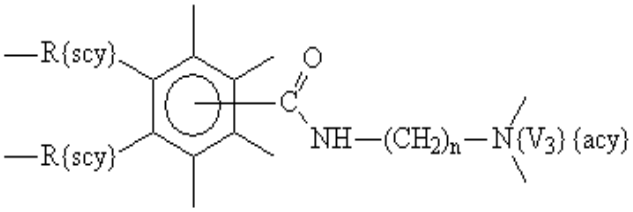
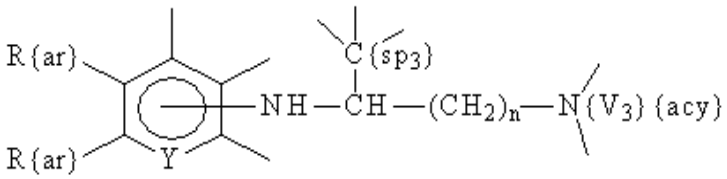


Mechanistic schemes C: Decomposition of 1,3- and 1,4-dithiane rings - more likely to be implemented if both Y are H:



Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Enoch, S. J., M. T. D. Cronin, Development of new structural alerts suitable for chemical category formation for assigning covalent and non-covalent mechanisms relevant to DNA binding, Mutat. Research 743 (2012), 10– 19. 2. Tamura, N., K. Aoki, M. S. Lee, Selective reactivities of isocyanates towards DNA bases and genotoxicity of methylcarbamoylation of DNA, Mutat. Res., 283 (1992), 97 – 106.

Individual profile/alert	
Name	DNA Intercalators with Carboxamide and Aminoalkylamine Side Chain
Type of profile	Structural alert

Description/applicability domain	 (n = 1 - 3; R{scy}: any atom in a cyclic (including aroma fragment condensed also with the aromatic ring)  (Y is N or C)
Mechanism	Non-covalent interactions DNA intercalation

Although most chemicals, capable of causing damaging genetic changes possess the ability to react chemically, more exactly, with formation of covalent bonds with DNA, acridines typically interact "physically", forming drug-DNA complexes by reversible binding. Thus the term "frameshift" or "acridine" mutagenesis can be restricted to genotoxic events that do not require covalent DNA binding. Linkage of an acridine chromophore to a basic side chain increases DNA binding affinity under physiological conditions. This is the case with the series of 9-aminoacridine carboxamide derivatives with a basic side chain, for which mutagenicity is strongly related to DNA intercalation of the acridine chromophore. The multi-cyclic planar structure and conjugation effects contribute to the positive mutagenicity effect [1, 5].

According to another publication, being less basic than aminoacridines, acridine carboxamides are weaker DNA binders [2].

The principal *in vitro* and *in vivo* metabolism of this class of chemicals is associated with the formation of acridones, and oxidative N-dealkylation and N-oxidation of the carboxamide side chain [3, 4]. This also contributes to the intercalating capability, genotoxic and carcinogenic properties of these chemicals [3, 4].

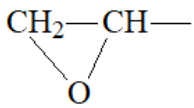
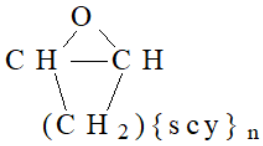
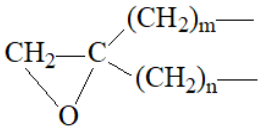
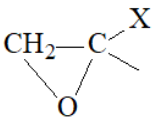
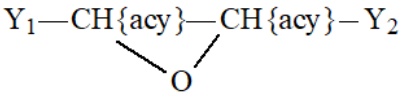
As far as some alkylaminoacridines are concerned, the results of the bacterial mutagenicity assays showed a very weak mutagenic effect of three drugs from this sub-category (chloroquine, primaquine and amodiaquine) in *Salmonella* strains TA97a and TA100, both with and without S9 mix [6].

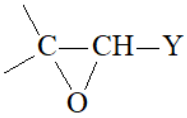
Chloroquine is both the DNA intercalating agent and topoisomerase II inhibitor, which is positive in both the Ames and CA tests [7 - 10].

The size of the 8-aminoquinoline ring system suggests that, similarly to chloroquine, primaquine is able to intercalate into DNA and may act as a weak topoisomerase II inhibitor[11, 12].

Set of chemicals used for profile development	DNA Intercalators with Carboxamide and Aminoalkylamine Side Chain
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Ferguson, L. R., Mutag. **5**(6) (1990), 529 – 540.

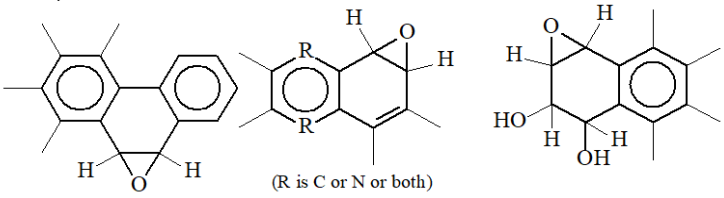
	2. Hicks, K. O., J. Phrarmacol. Exper. Ther. **297** (2001), 1088 – 1098. 3. Schlemper, B., Xenobiotica **23**(4) (1993), 361 – 371. 4. Schofield, Ph. C., Canc. Chemother. Pharmacol. **44** (1999), 51 – 58. 5. Ferguson, L. R., Eur. J. Canc. **26**(6) (1990), 700 – 714. 6. Chatterjee, T., *Mutagenesis*, **1998**, 13(6), 619 – 624. 7. Ferguson, L. R., Mutat. Res. **623** (2007), 14 – 23. 8. Snyder, R. D., Environ. Molec. Mutag. **51** (2010), 800 – 814. 9. Snyder, R. D., Mutat. Res. 609 (2006), 47 – 59. 10. Shubber, E. K., Cell Biol. Toxicol. **2**(3) (1986), 379 – 399. 11. Allison, R. G., Agents Chemother. **11**(12) (1977), 251 – 257. 12. Langer, S. W., Clin. Canc. Res. **5** (1999), 2899 – 2907.

Individual profile/alert	
Name	Epoxides and Aziridines
Type of profile	Structural alert
Description/applicability domain	Monosubstituted epoxides:  Simple cycloaliphatic epoxides:  (n = 2 - 6) ,1-Disubstituted epoxides and spiro-epoxides:  (m = 1 - 3; n = 1 - 3) (If (CH_2) is acyclic, the terminal group is $-CH_3$; CH_2 can be also cyclic)  (X is Cl or Br or CCl_3 or CBr_3) 1,2-Disubstituted epoxides (including cycloaliphatic epoxides):  Y_1 and Y_2 can be the following structural moeties: (a) $(-CH_2)_nH$ (n = 1 - 2) (b) CH_2{scy} and -CH{scy}=CH{scy}- (c) -CH{sp3}{scy} and O{scy} or -NH{scy} (d) Y_1 is Cl or Br; Y_2 is C Other terminal polarized epoxides:

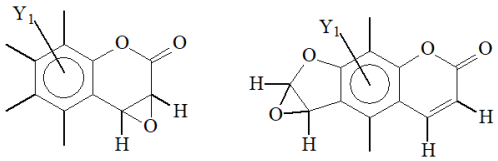


(Y can be Cl, Br or -CHO)

Metabolically derived epoxides from polycyclic aromatic hydrocarbons (PAH) and coumarin derivatives:

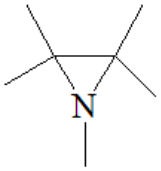


(R is C or N or both)

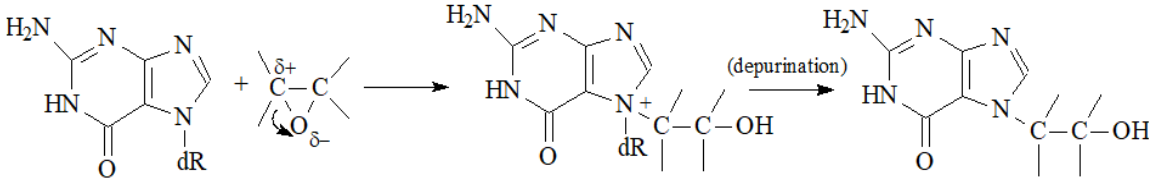


(Y_1 is –H (all) or combinations of H and $–OCH_3$, $-NH_2$, $-NO_2$, -NHOH, $-CH_3$, $-CH_2X$ (X is Cl, Br); no more than one substituent

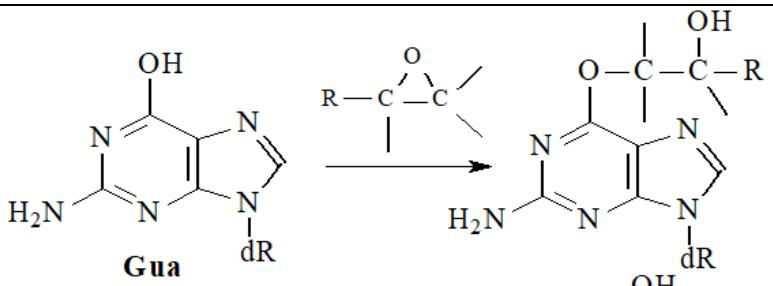
Aziridines



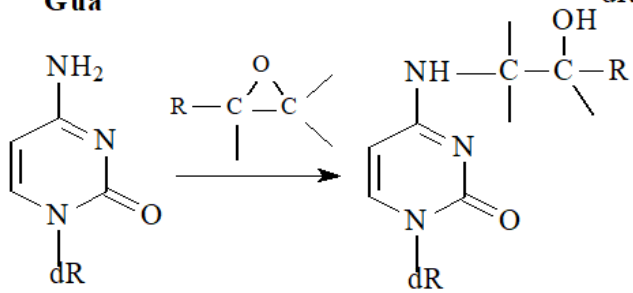
Mechanism	**S_N2 Alkylation, direct acting epoxides and related**



(DNA fragment)
(dR - deoxyribose phosphate fragment)

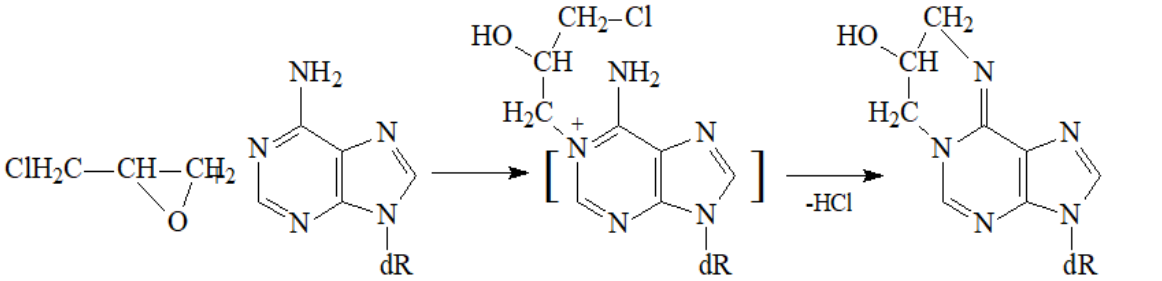


Gua

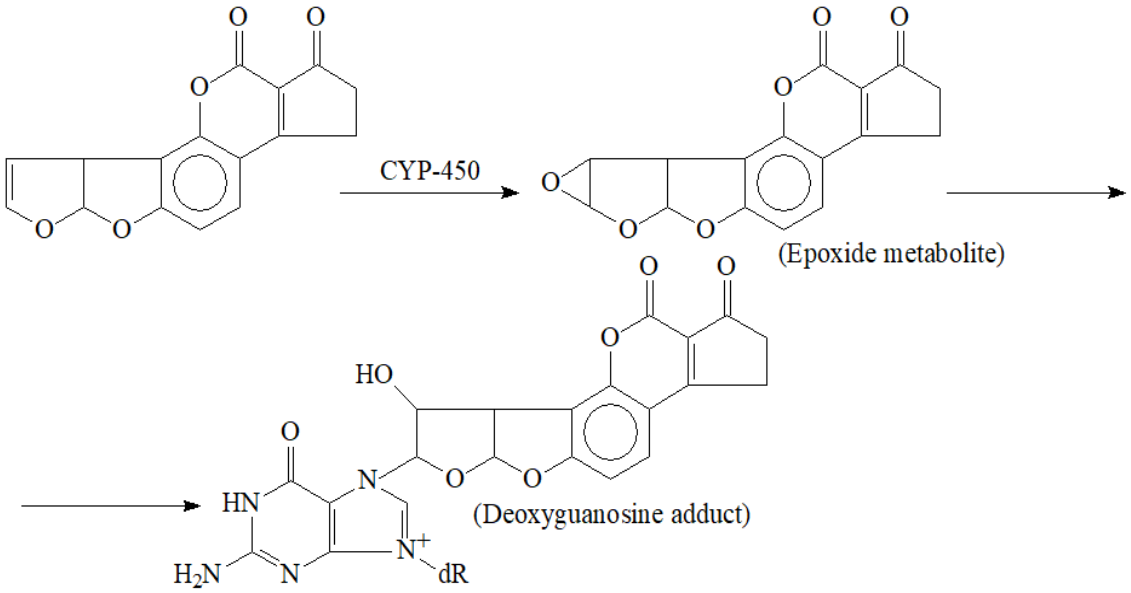


Cyt

(dR - deoxyribose phosphate fragment)

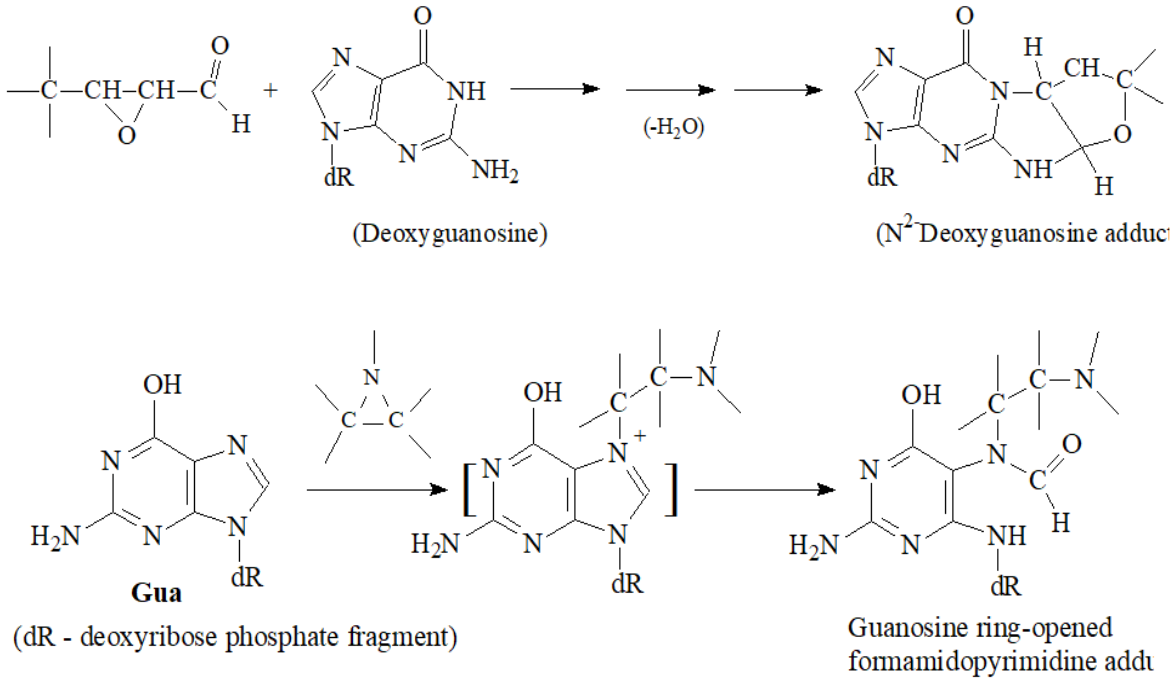


1,N6-2-hydroxypropanoadenine adduct



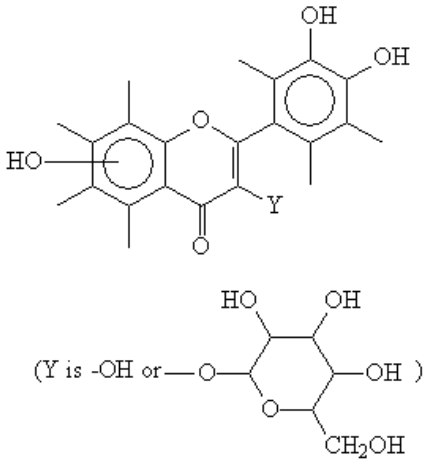
(Epoxide metabolite)

(Deoxyguanosine adduct)

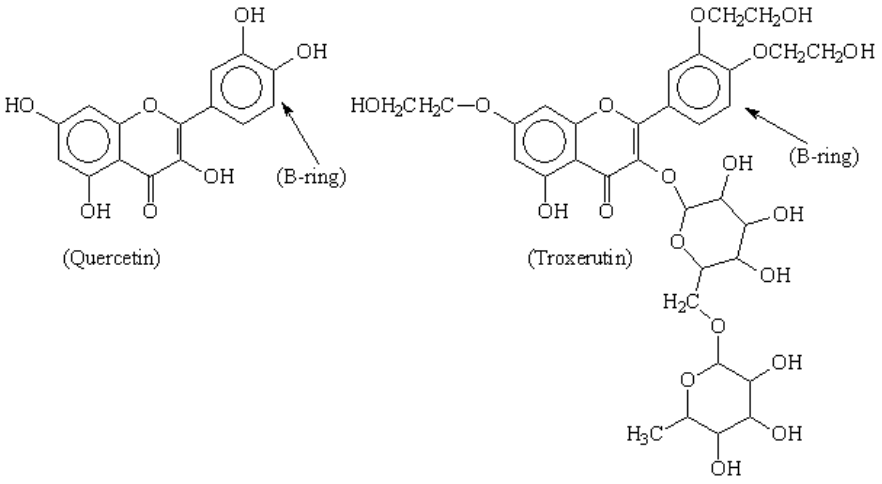


Set of chemicals used for profile development	Epoxides, Aziridines, Thiiranes and Oxetanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Koskinen, M., Chem.-Biol. Interact. **129** (2000), 209 – 229. 2. Singh, U. S., Chem. Biol. Interact. **99** (1996), 109 – 128. 3. Sawatari, K., Industrial Health **39** (2001), 341 – 345. 4. Raney, V. M., Chem. Res. Toxicol. **6** (1993), 64 – 68. 5. Wade, M. J., Mutat. Res. **66** (1979), 367 – 371. 6. Voogd, C. E., Mutat. Res. **89** (1981), 269 – 282. 7. Hemminki, K., Arch. Toxicol. **46** (1980), 277 – 285. 8. Von der Hude, Mutat. Res. **231** (1990), 205 – 218. 9. Frantz, S. W., Mutat. Res. **90** (1981), 67 – 78. 10. Meester, C. De, Toxicol. Lett. **224** (1984), 255 – 262. 11. Sinsheimer, J. E., Mutat. Res. **224** (1989), 171 – 175. 12. Glatt, H., Mutat. Res. **11** (1983), 99 – 118. 13. *Vinylidene Chloride*, Pub Chem https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=75-35-4. Last visited: June, 2021. 14. Neudecker, T., Biochem. Pharmacol. **35**(2) (1986), 195 – 200. 15. Petrova, K. V., Chem. Res. Toxicol. **20** (2007), 1685 – 1692. 16. *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contacts with Food (AFC) on a Request from the Commission Related to Coumarin*, Question Number EFSA-Q-2003-118 (6 October 2004), The EFSA Journal **104** (2004), 1 – 36; DOI: 10.2903/j.efsa.2004.104. http://www.efsa.europa.eu/en/efsajournal/doc/104.pdf. Last visited: June, 2021. 17. Born, S. D., Drug Metab. Dispos. **30**(5) (2002), 483 – 487 18. Zhou, S., Life Sci **74** (2004), 935 – 968. 19. Cussac, C., Nucleic Acids Res. **24**(9) (1996), 1742 -1746. 20. Tudek, B., J. Biochem. Molec. Biol. **36**(1) (2003), 12 – 19.

	21. Glatt, H., Canc. Res. **45** (1985), 2600 – 2607. 22. *Divinylbenzene, CAS No. 1321-74-0*, Chemical Carcinogenesis Research Information System; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=1321-74-0. Last visited: June, 2021.

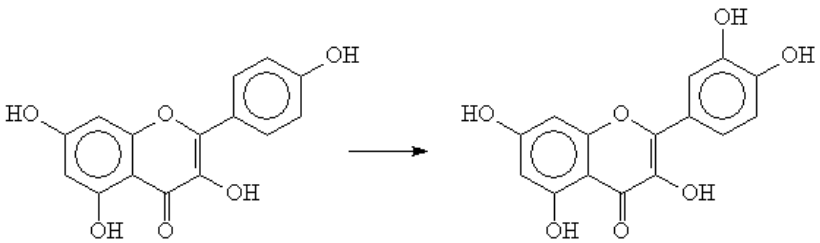
Individual profile/alert	
Name	Flavonoids
Type of profile	Structural alert
Description/applicability domain	
Mechanism	A_N2 Michael-type addition, quinoid structures and Radical ROS generation (indirect)

Certain structural requirements should be fulfilled for direct bacterial mutagenicity. For example, the flavonoid derivative, troxerutin, was not mutagenic, since the substitution of the two catechol hydroxyl group in quercetin with hydroxyethyl groups abolished mutagenicity [3]. According to another study, only those flavonols either lacking or possessing one B-ring hydroxyl group have an absolute requirement for microsomal (S9) activation. This requirement can be illustrated by the two flavonoids, quercetin (strong mutagen as parent chemical and, even more, mutagenic after metabolic activation), and troxerutin (non-mutagenic) [4]:



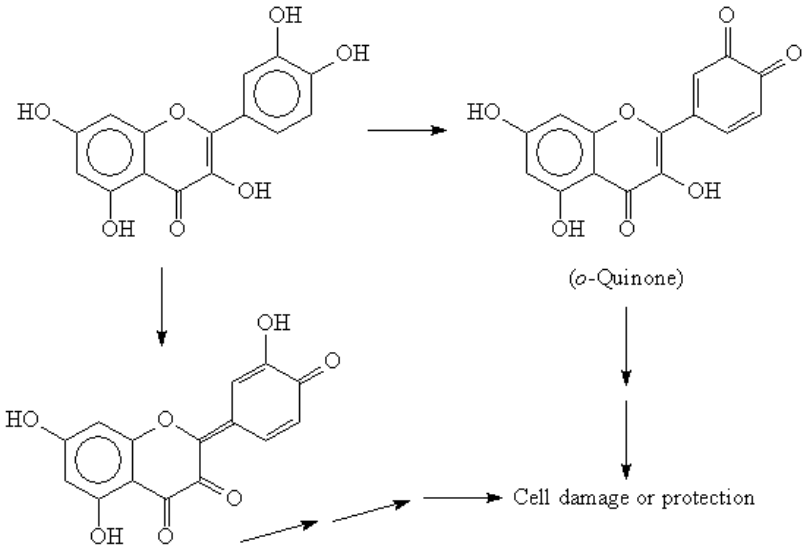
Thus the two most mutagenic chemicals from this class were quercetin (see above, mutagenic as parent chemical) and kaemferol [4]. These compounds are also the most commonly occurring

flavonoids in plants. Kaempferol, however, requires metabolic activation in order to form the active catechol-type metabolite which may, consequently, generate genotoxic *o*-quinone intermediate:

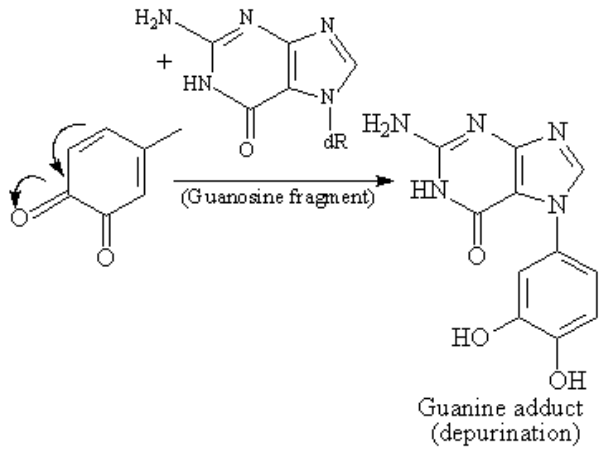


(Kaempferol)

For example, quercetin can generate active *o*-quinone/quinone methide metabolites by the following pathways [7]:

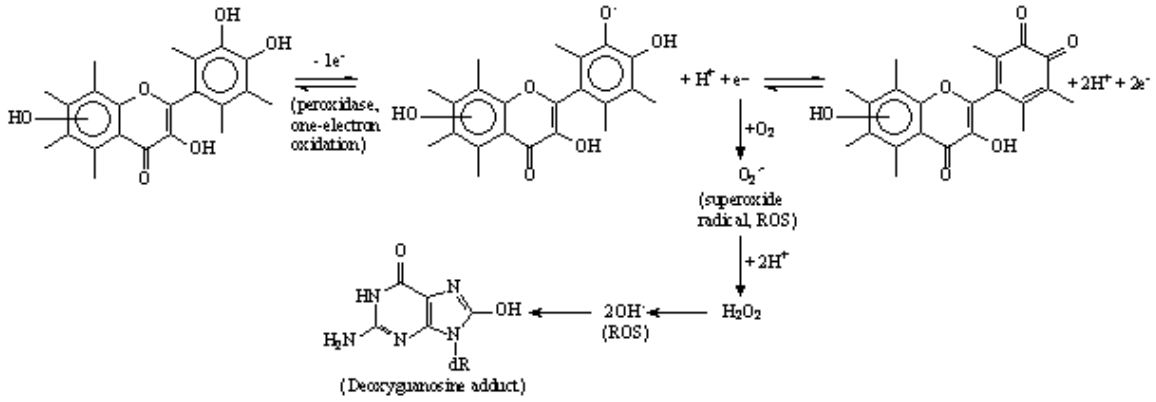


The mutagenicity of quercetin is assumed to be partly due to the generation of such active metabolites. One possible mechanism for the formation of DNA adducts from *o*-quinones could involve depurination, due to Michael addition, according to the following scheme [8]:

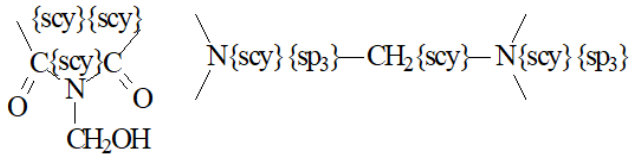


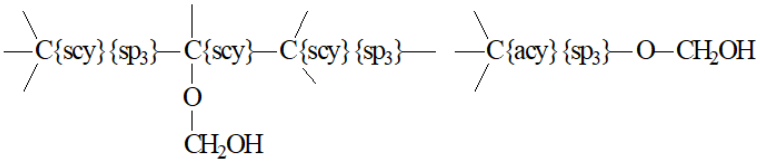
dR - desoxyribose phosphate fragme:

If the presence of endogenous peroxidase enzymes in the "classical" *Salmonella typhimurium* strains is assumed, the following mechanistic scheme involving the formation of ROS could explain the observed positive *in vitro* bacterial mutagenicity results for a few flavonoids such as quercetine as parent chemicals:



Set of chemicals used for profile development	Flavonoids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Resende, Molecules **17** (2012), 5255 – 5268. 2. Yamashita, Mutat. Res. **425** (1999), 107 – 115. 3. Marzin, Toxicol. Lett. **35** (1987), 297 – 305. 4. Brown, Mutat. Res. **66** (1979), 223 – 240. 5. Appleton, Natural Medicine J. **2**(1) (2010), 1 – 6. 6. *Chemical Carcinogenesis Research Information System (CCRIS)*; 7. Spencer, J. P. E., G. G. C. Kunhle, R. J. Williams, C. R. Evans, *Intracellular Metabolism and Bioactivity of Quercetin and Its In Vivo Metabolites*, Biochem. J. **372** (2003), 173 – 181. 8. Li, Carcinogeneis **25**(2) (2004), 289 – 297. 9. Schweigert, Environ. Microbiol. **3**(2) (2001), 81 – 91. 10. Lang, Mutat. Res. **191** (1987), 139 – 143. 11. Subrahmany, Chem.-Biol. Interactions **56** (1985), 185 – 199.

Individual profile/alert	
Name	Formaldehyde Releasers
Type of profile	Structural alert
Description/applicability domain	

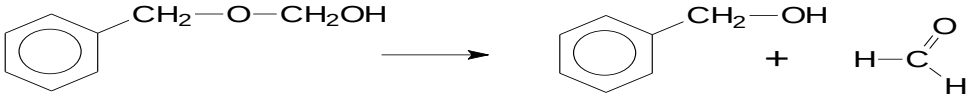
	
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: Alkylation, nucleophilic substitution on activated hydroxymethyl or methylene group, attached to N{sp3}- or O-atoms

According to one definition, formaldehyde releasers are chemicals which, in the presence of water, release formaldehyde by abiotic hydrolysis. Most have applications as preservatives and biocides in products such as cosmetics and metalworking fluids; others are used as durable press chemical finishes in textiles. A number of such chemicals are skin sensitizers, and, depending on their structure, some may be also mutagens, due to the well-known genotoxicity of formaldehyde [1]. As seen from Table 1 above, mutagenic formaldehyde releasers contain mostly cyclic structural fragments, to which hydroxymethyl (-CH2OH), or (in more specific cases), methylene (-CH2-) functionality is attached via N{sp3}- or O-atoms.

Some examples of spontaneous (abiotic) formaldehyde release are given below:

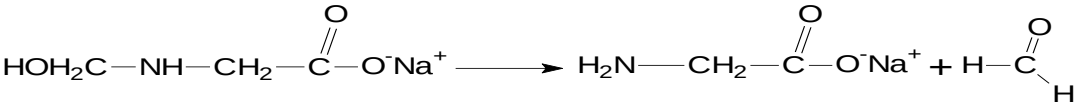
A. Benzylhemiformal:

In 1 % aqueous concentration, benzylhemiformal is known to completely decompose into formaldehyde and benzyl alcohol [1]:



B. Sodium Hydroxymethyl Glycinate

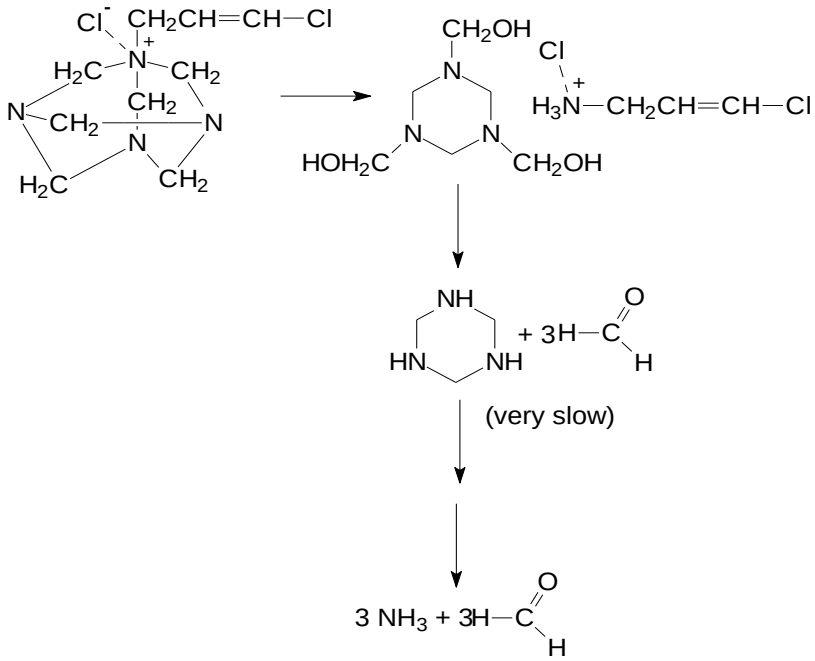
In an aqueous solution, sodium hydroxymethyl glycinate is decomposed to release formaldehyde. One molecule of formaldehyde is formed by the decomposition of each molecule of sodium hydroxymethyl glycinate, as described below:



In aqueous solutions, sodium hydroxymethyl glycinate releases some or all of the formaldehyde it contains, and thus, it may not be available for analysis as parent compounds in cosmetic products [2].

C. Quaternium-15

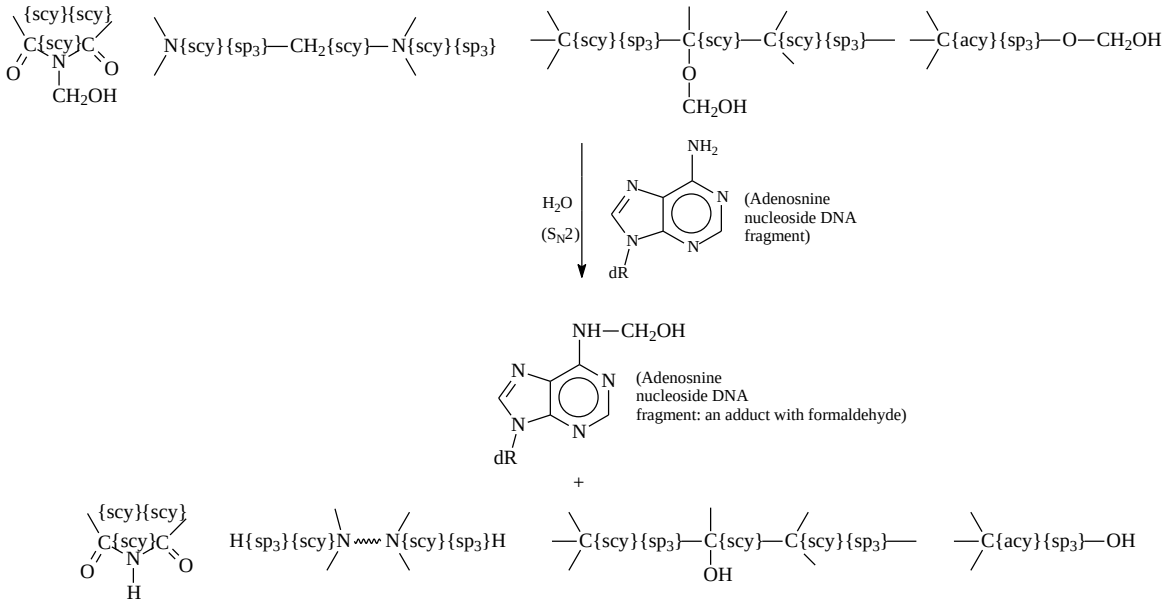
Such compounds may also release formaldehyde above pH6; hydrolysis can be slow, non-stoichiometric, and less complete at higher pH. The compound in 24 hours only released one-third of the potential formaldehyde [3]. The following stepwise abiotic (non-enzymatic) hydrolysis pathway can be assumed:



Formaldehyde induces mainly N-hydroxymethyl mono-adducts on guanine, adenine and cytosine, and, also, N-methylene crosslinks between adjacent purine nucleobases in DNA. These crosslinks are associated with types of DNA damage, which are potentially fatal for the cell survival if they are not removed by the nucleotide excision repair pathway. Formaldehyde was found to induce tandem base substitutions, mainly at 5'-GG (guanosine-guanosine) and 5'-GA (guanosine-adenine) sequences, which could arise from intra-strand DNA crosslinks [4].

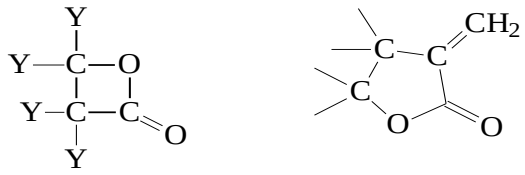
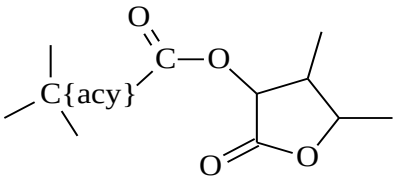
According to another publication, N-hydroxymethyl lesions have been instrumentally proved for cytosine, guanine and adenine nucleobases. The most persistent adducts resulting in pronounced mutagenicity were reported for adenine [5].

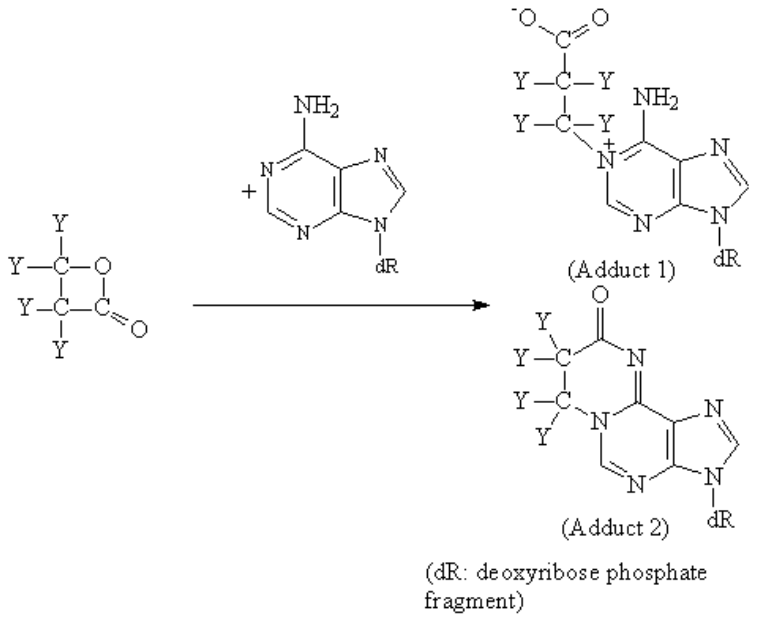
In these cases, formaldehyde release may occur "in situ", i.e. the nucleobase acts as stronger nuclophile, which replaces water and attaches the hydroxymethyl fragment directly to the amino group by SN2-type reaction. Based on the above discussions, the following, rather simplified mechanistic scheme, involving all previously defined Ames-positive structural alerting fragments can be expertly proposed:



Set of chemicals used for profile	Formaldehyde Releasers

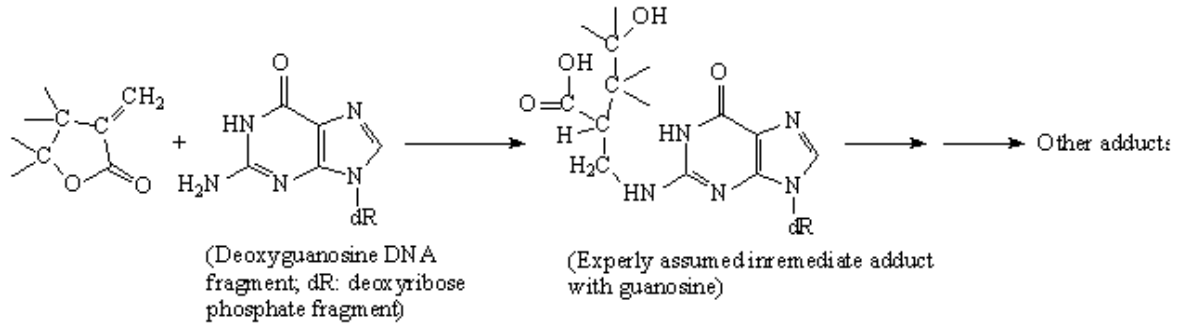
development	
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Emeis, D., A. C. De Groot, J. Brinkmann, Determination of Formaldehyde in Formaldehyde-Releaser Patch Test Preparations, Contact Dermatitis 63 (2010), 57 – 62. 2. Opinion Concerning the Determination of Certain Formaldehyde Releasers in Cosmetic Products (Adopted by the SCCNFP During the 22th Plenary Meeting of 17 December 2002), The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers; https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out188_en.pdf. Last visited: June, 2021. 3. Rossmoore, H. W., M. Sondossi, Applications and Mode of Action of Formaldehyde Condensate Biocides, Adv. Appl. Microbiol. 33 (1988) 223 – 277. 4. Masanobu, K., T. Matsuda, T. Yagi, Genotoxicity of formaldehyde: molecular basis of DNA damage and mutation, Frontiers in Environmental Science, 2 (2014), 1 – 8. 5. Wilson, K. A., J. L. Garden, N. T. Wetmore, L. R. Felske, St. D. Wetmore, DFT and MD Studies of Formaldehyde-Derived DNA Adducts: Molecular-Level Insights into the Differential Mispairing Potentials of the Adenine, Cytosine, and Guanine Lesions, 6. J. Phys. Chem. A, 123 (2019), 6229 – 6240.

Individual profile/alert	
Name	Four- and Five- membered Lactones
Type of profile	Structural alert
Description/applicability domain	 (Y can be -H and/or -CH_3, C_2H_5) 
Mechanism	Ring opening S_N2 reaction (alkylation) and A_N2 Michael-type addition on α,β-unsaturated carbonyl compounds SN2 Acylation
The following mechanistic Scheme 1 for the DNA adducts formation at the N1 site of adenosine nucleotide elicited by four-membered lactones of high reactivity can be outlined based on literature:	

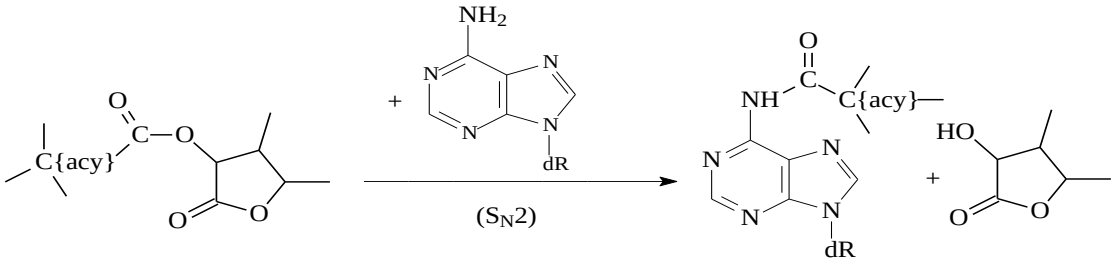


Scheme 1

The conjugated system in the molecular structure of some alpha-methylidene-γ-butyrolactone derivatives might actually cause bacterial mutagenicity by expertly assumed (hypothetic) mechanistic Scheme 2, similar to that for some α,β-unsaturated systems:

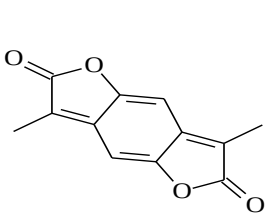
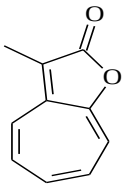


For some acylated five-membered hydroxylactones, another mechanistic scheme of interaction with DNA fragment, which is based on an expertly suggested activation of the acyloxy group attached to the lactone ring could be proposed:

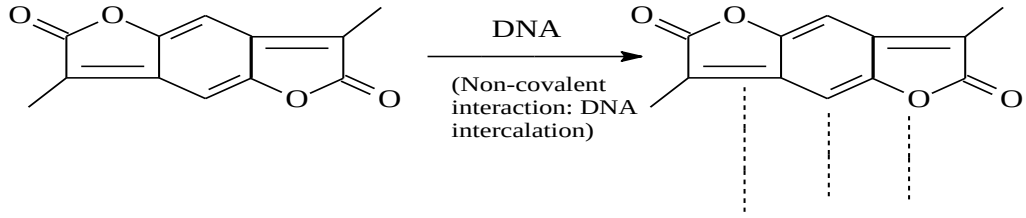


Set of chemicals used for profile development	Four- and Five-Membered Lactones
Data/Knowledge used for	An extensive review of the literature was performed enabling the

profile development	chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Hemminki, Chem. Biol. Interact. **34** (3), 1981, 323 - 331. 2. Beta-Butyrolactone (CAS 3068-88-0), The Carcinogenic Potency Project; http://potency.berkeley.edu/chempages/beta-BUTYROLACTONE.html Last visited: June, 2021. 3. Sawatari, Industrial Health **39**, 343 (2001), 341 – 345. 4. Chen, Carcinog. **2**(2) (1981), 73 – 80. 5. Kupchan, J. Med. Chem. **14**(12) (1971), 1147 – 1152. 6. Picman, Biochem. System. Ecol. **14**(3) (1986), 255 – 281.

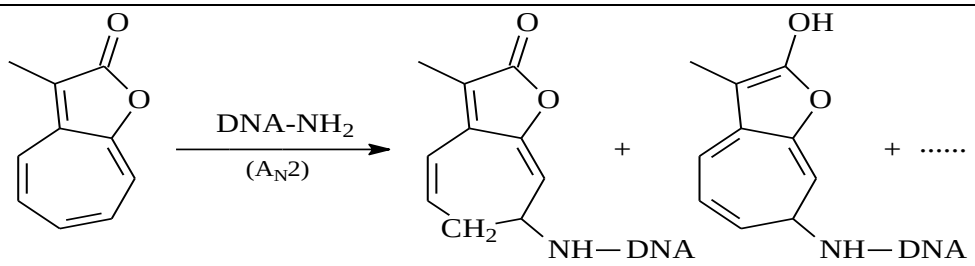
Individual profile/alert	
Name	Fused-Ring Conjugated Lactones
Type of profile	Structural alert
Description/applicability domain	 (Conjugated Benzodifurandione)  (Fused-Ring Conjugated Bicyclic Lactone)
Mechanism	Mechanistic Domain: AN2 Mechanistic Alert: Michael-type nucleophilic addition to conjugated unsaturated fused-ring lactones Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation

Generally, two mechanistic schemes for interaction with prokaryotic DNA in Salmonella typhimurium bacteria can be expertly proposed to explain the positive mutagenicity results. According to the first mechanism, the long-chain conjugated system with polar functionality interacts with the prokaryiotic DNA via AN2 Michael-type addition with formation of covalent adduct(s). This is more likely to occur with the second chemical in Table 1 (2-Oxo-2H-cyclohepta[b]furan-3-carboxylic acid methyl ester), which is sterically less hindered at the conjugated double bonds, particularly, after metabolic activation. According to the second mechanism, the strongly polarized polycyclic conjugated system undergoes non-covalent interaction, causing DNA intercalation (Scheme 1):



DNA
(DNA intercalative adduct with fused-ring conjugated lactone)

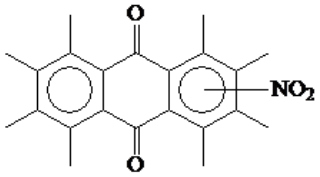
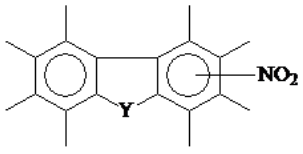
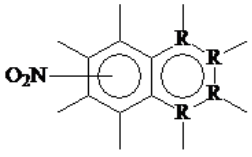
For more compact and less sterically hindered conjugated fused ring lactone systems, AN2 Michael-type additions with formation of covalent adduct(s) are also plausible (Scheme 2):

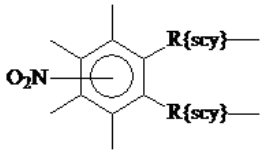
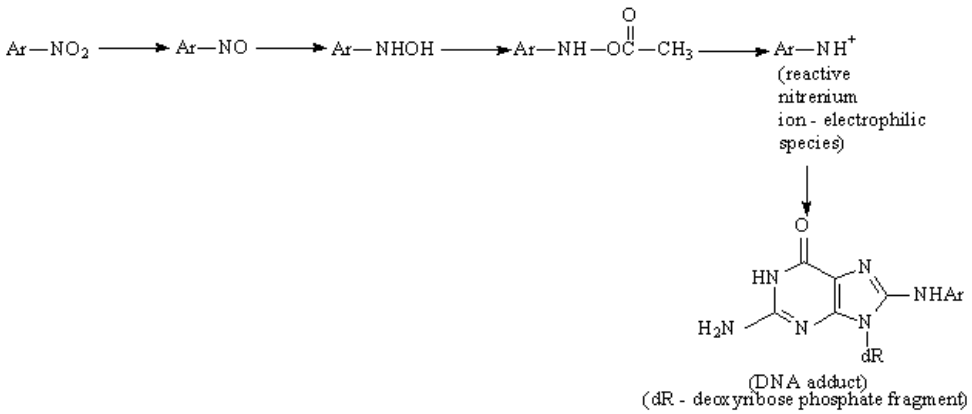


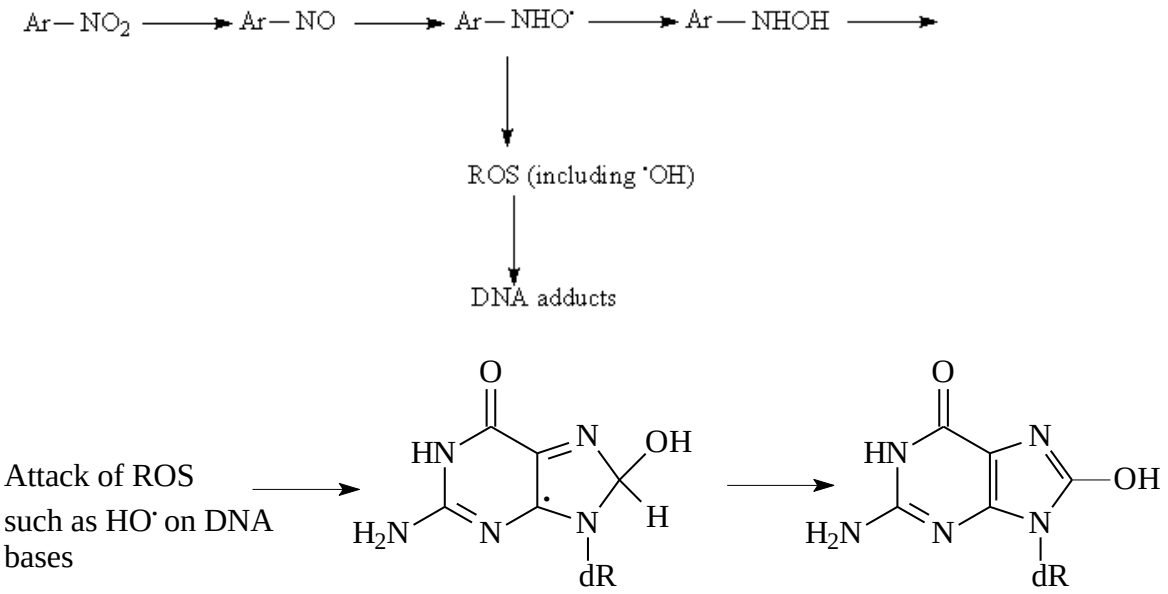
(Some possible DNA adducts)

(DNA-NH_2 refers to purine/pyrimidine base with -NH_2 group as functionality of nucleophilic attack along the conjugated system)

Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Methyl 2-oxo-2H-cyclohepta[b]furan-3-carboxylate, National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/compound/10081616). Last visited: June, 2021. 2. 3-Phenyl-7-[4-(tetrahydrofurfuryloxy)phenyl]-1,5-dioxa-s-indacen-2,6-dione; ECHA Registration Dossier; https://echa.europa.eu/bg/registration-dossier/-/registered-dossier/16617/7/7/2. Last visited: June, 2021.

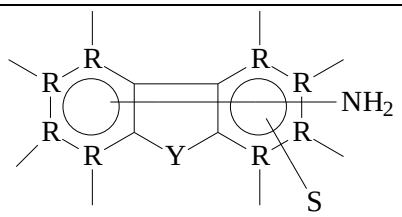
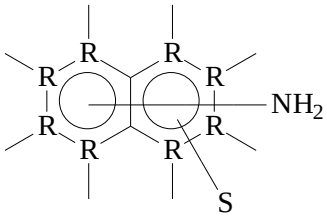
Individual profile/alert	
Name	Fused-Ring Nitroaromatics
Type of profile	Structural alert
Description/applicability domain	Nitroantraquinones  Nitrofluorenes and their heterocyclic analogues  **Y= C or S(V2) , N(V3) (sp^3)** Other fused-ring nitroaromatics  R= C or N(number of N is 1 or 2) ; Can't have SO_3H group attached

	to the ring, bearing NO_2  R(scy)= C or N(V3) or S(V2) or a combination as part of a fused cyclic fragment
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases. (**Nucleophilic attack after reduction and nitrenium ion formation)** **Radical (Homolytic) Mechanism.** This is one of the mechanisms (but not the most important) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks) **(Radical mechanism *via* ROS formation (indirect)**
Heterolytic  **Homolytic**	

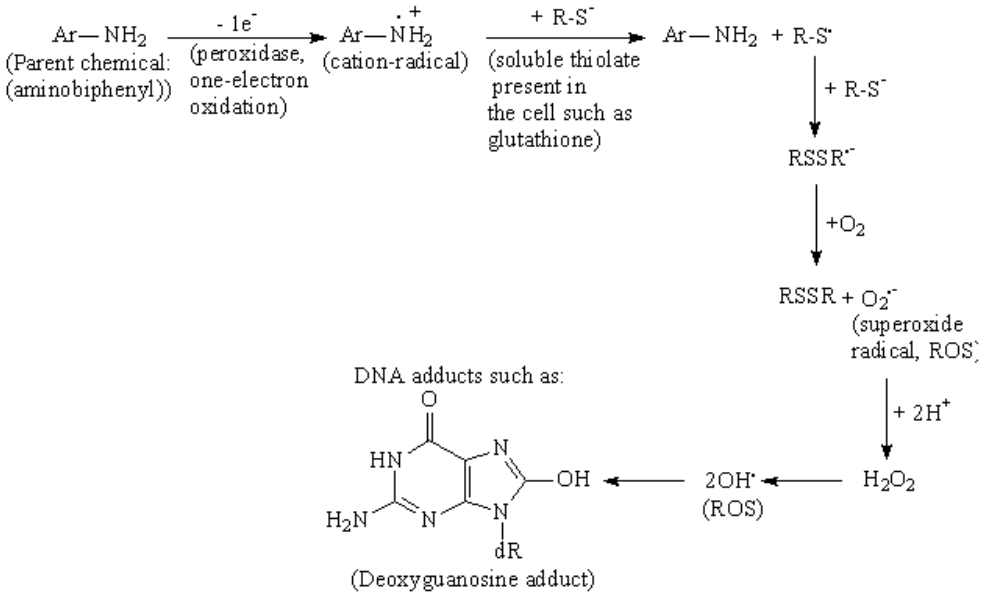


Set of chemicals used for profile development	Fused-Ring Nitroaromatics
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2.Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3.Aiub, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4.Einisto, Mutat. Res. **259** (1991), 95 – 102. 5.Kovacic, Current Med. Chem. **8**, (2001), 773 – 796. 6.Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7.Wiseman, Biochem. J. **313** (1996), 17 – 29. 8.Purohit, Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9. Rosenkranz, Mutat. Res. **114** (1983), 217 – 267. 10. Brown, J. P., Mutat. Res. **66** (1979), 9 – 24. 11.Vance, W. A., Environ. Mutag. **6** (1984), 797 – 811.

Individual profile/alert	
Name	Fused-Ring Primary Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	

	  (S can be -C{sp3}, no more than three C{sp3}; -O-C{sp3}in alkyl chain, no more than three C{sp3}; -NH- or -H or -OH or $-NO_2$ (-OH only if N{ar} is present); S can be attached anywhere to an aromatic ring; Y is CH_2 or -NH; R can be C{ar} only or combinations of C{ar} and N{ar}; no more than two N{ar}in a molecular structure). No electron-withdrawing substituents attached such as $-SO_3H$, CN, C=O, $-CF_3$, $-SO_2$, N{V3}{sp2}, halogen (F, Cl, Br). No more than four fused rings)
Mechanism	S_N1 Nucleophilic attack after metabolic nitrenium ion formation, Radical ROS generation (indirect) & Non-covalent interactions DNA intercalation

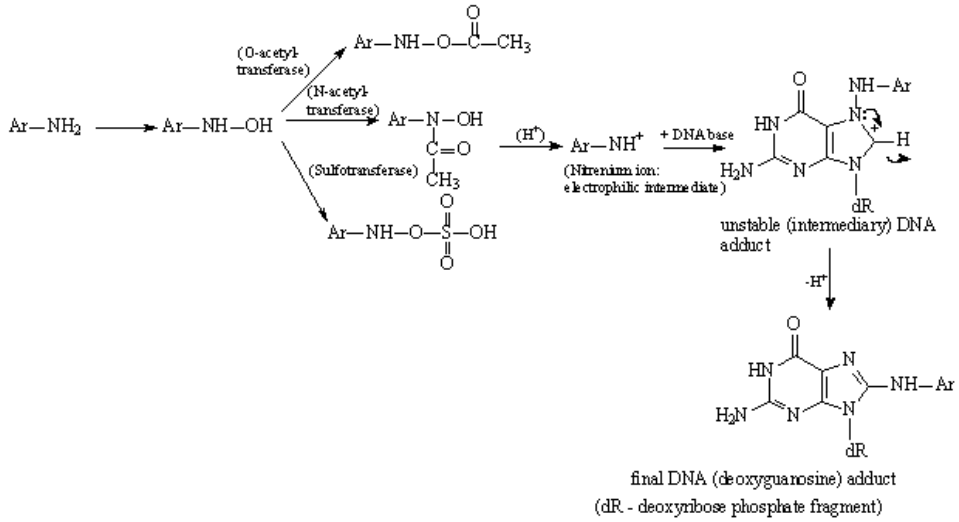
It is expertly assumed that the presence of electron-donating substituents with either +I or +M-effects, together with the planar structure and conjugation effects may determine the positive mutagenicity of some polycyclic aromatic amines as parent chemicals. In addition, endogenous generation of reactive oxygen species can be assumed, due to the presence of peroxidase enzymes in bacterial cells, and this process can be mediated by thiols shown below in Scheme 1 [5, 6]:



Scheme 1

For all sub-classes of primary aromatic amines, including the polycyclic ones, there is strong evidence that, in many cases, metabolic activation with the external microsomal S9 system is required for eliciting mutagenicity and carcinogenicity. According to an excellent review on the bioactivation pathways of organic functional groups, the obligatory step in the bioactivation of all aniline derivatives involves enzymatic N-hydroxylation on the primary amine nitrogen, leading to the formation of *N-hydroxylamine* intermediate. These reactive N-hydroxylamine derivatives (metabolites) can undergo phase II conjugation, to generate the more reactive N-O sulfate and/or N-O acetyl conjugates. The excellent leaving group capability of sulfonyloxy- and acetoxy-functionalities in these conjugates is believed to lead to a highly reactive *nitrenium ion*. The nitrenium ion

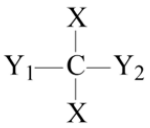
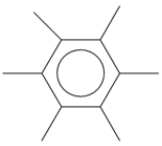
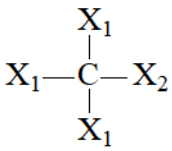
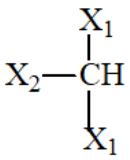
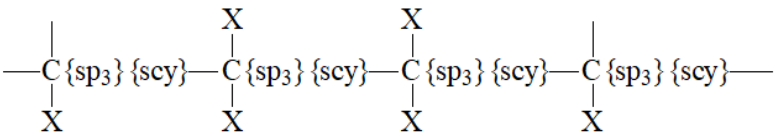
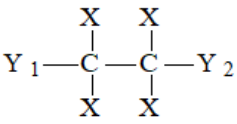
electrophilic species may readily bind covalently to cellular DNA and RNA [9]. The principal *in vitro* metabolic pathway causing mutagenicity of aromatic amines is therefore associated with metabolic activation induced by interactions with the CYP450 isoenzyme CYP1A2, and can be outlined as follows shown below in Scheme 2 [10]:

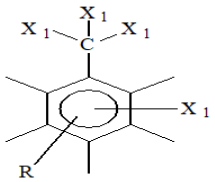
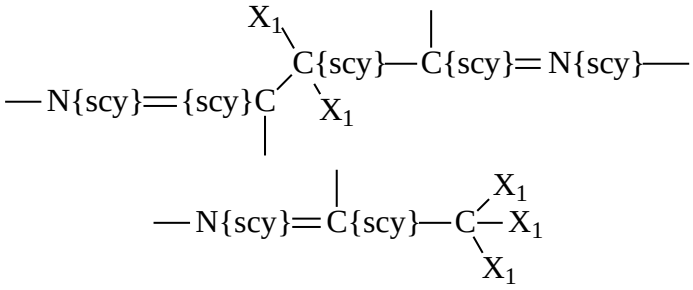
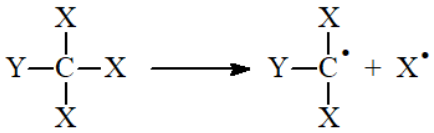


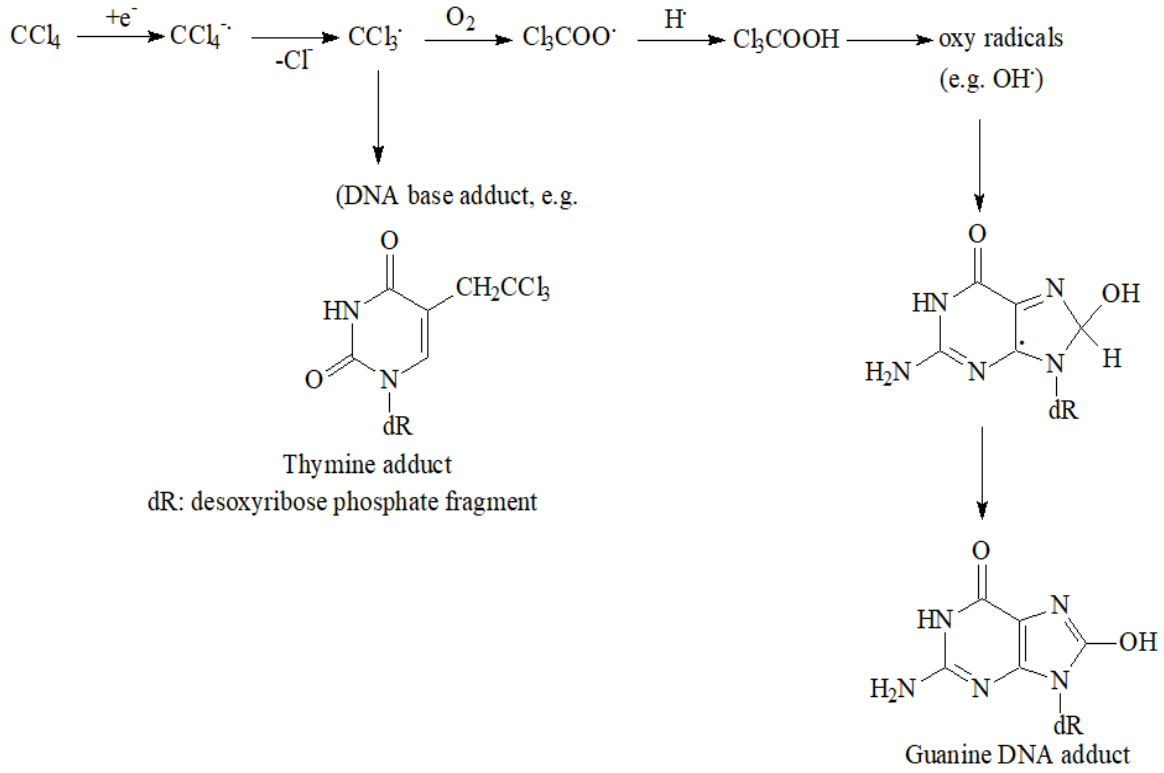
Scheme 2

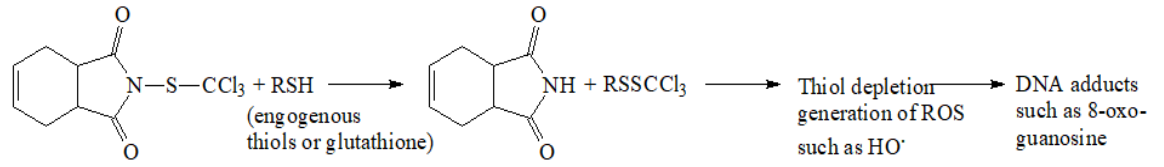
Set of chemicals used for profile development	Fused-Ring Primary Aromatic Amines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Double, J. Pharm. Pharmac. **28** (1976), 166 – 169. 2. Shapiro, Chem. Res. Toxicol. **11** (1998), 335 – 341. 3. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/ 4. Hoffman, Chem. Res. Toxicol. **10**(4) (1997), 347 – 359). 5. Subrahmany, V. V., Chem.-Biol. Interactions **56** (1985), 185 – 199. 6. Makena, Environ. Molec. Mutagenesis **48** (2007), 404 – 413. 7. Guerin, Environ. Res. **23** (1980), 42 – 53). 8. Chung, K. T., App. Environ. Microbiol. **42**(4) (1981), 641 – 648. 9. Kalgutkar, Curr. Drug Metabol. **6**(3), 2005, 161 – 225. 10. Shamovsky, JACS **133** (2011), 16168 – 16185 11. Glatt, H., FASEB J. **11**(5) (1997), 314 – 321. 12. Chung, Mutat. Res. 387 (1) 1997, 1 – 16. 13. Franke, R. , Carcinogenesis **22**(9) (2001), 1561. 14. Fu, Mutat. Res. **94** (1982), 13 – 21.

Individual profile/alert	
Name	Geminal Polyhaloalkane Derivatives
Type of profile	Structural alert

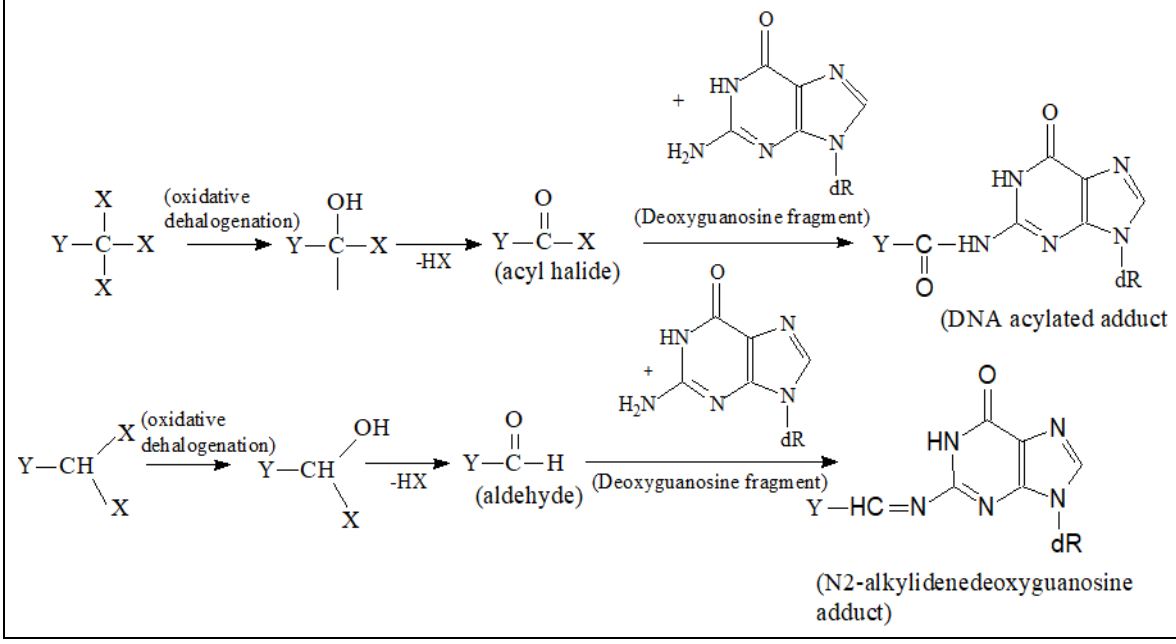
Description/applicability domain	 X can be Cl, Br, I or F; Y_1 can be X or H; Y_2 can be -H, -CH-O-, S{V2}, -CN, -CHO, -CH-X_2, -C(O)X, NO_2, -CH_3,  -C(O)-O- (carbonyl group attached *via* C-atom), -CO, -CF_2-O-; (no electron-withdrawing halogens or –CF_3 attached; no more than two substituents in the phenyl ring)  (X_1 = F or Cl; X_2 = Br or I)  X_1 is F or Cl; X_2 is Cl or Br  C{scy}: cyclic carbon atom X= Cl, Br  (Y_1 is C or H or combinations or S{V2}; Y_2 is C or H; X is Cl or Br

	 (X is Cl or Br; R is –CH_3 or –C_2H_5; No more than two X_1; No more than totally four substituents on phenyl ring)  (X_1 is Cl or Br)
Mechanism	S_N2 Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation, Radical ROS generation, S_N2 Acylation involving a leaving group after metabolic activation & A_N2 Shiff base formation by aldehyde formed after metabolic activation Radical ROS generation after radical CYP-induced dehalogenation
Radical mechanisms: Free-radical pathways for biactivation of some polyhaloalkanes have been suggested: 	

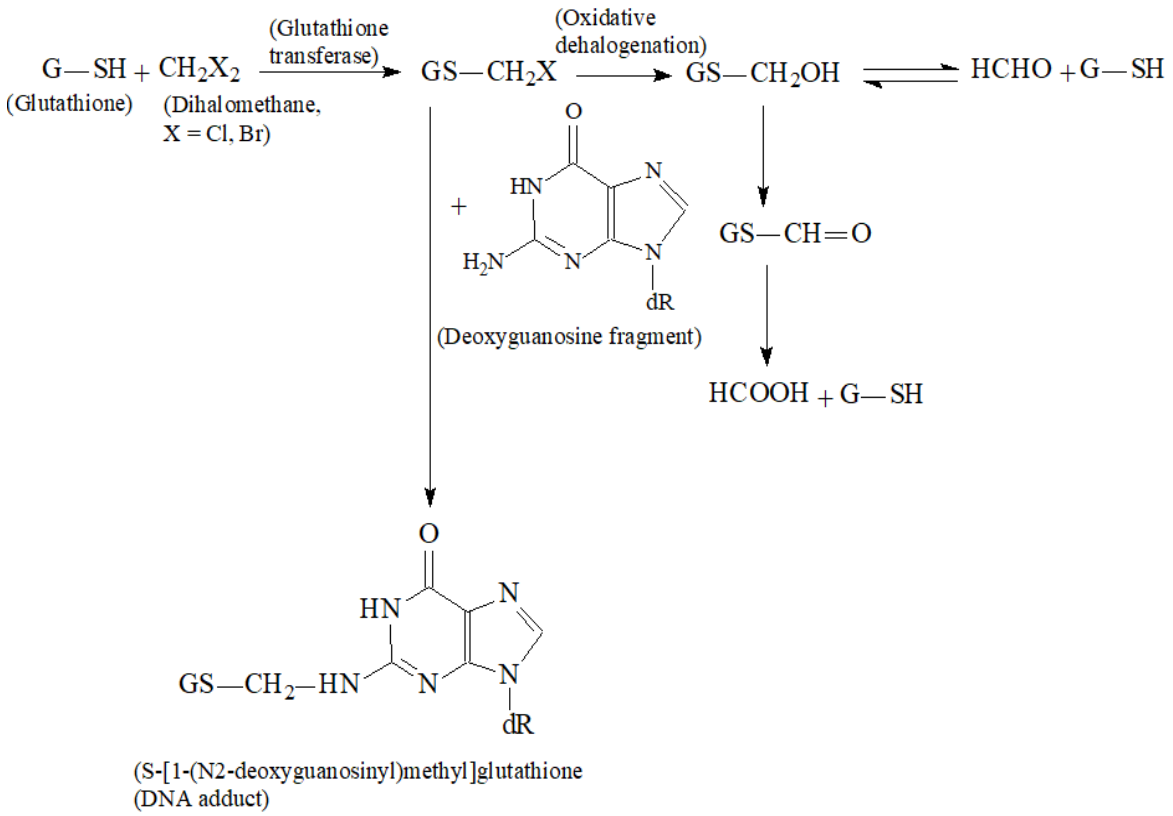




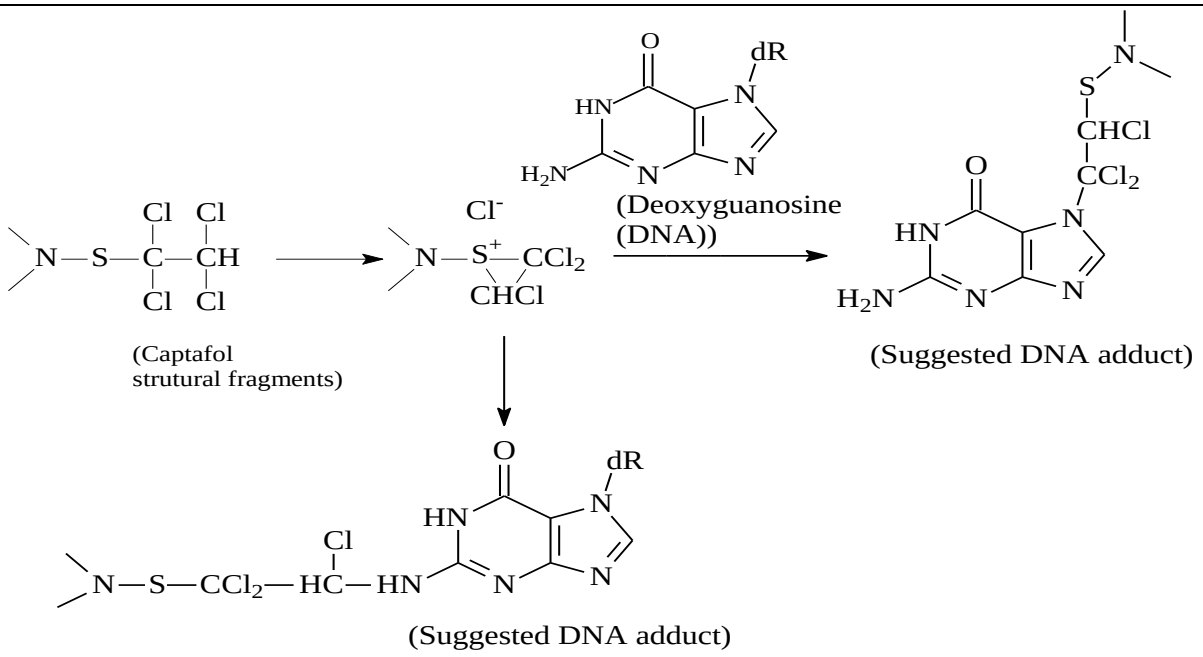
Non-Radical Mechanisms: Phase I metabolic activation –Oxidative dehalogenation



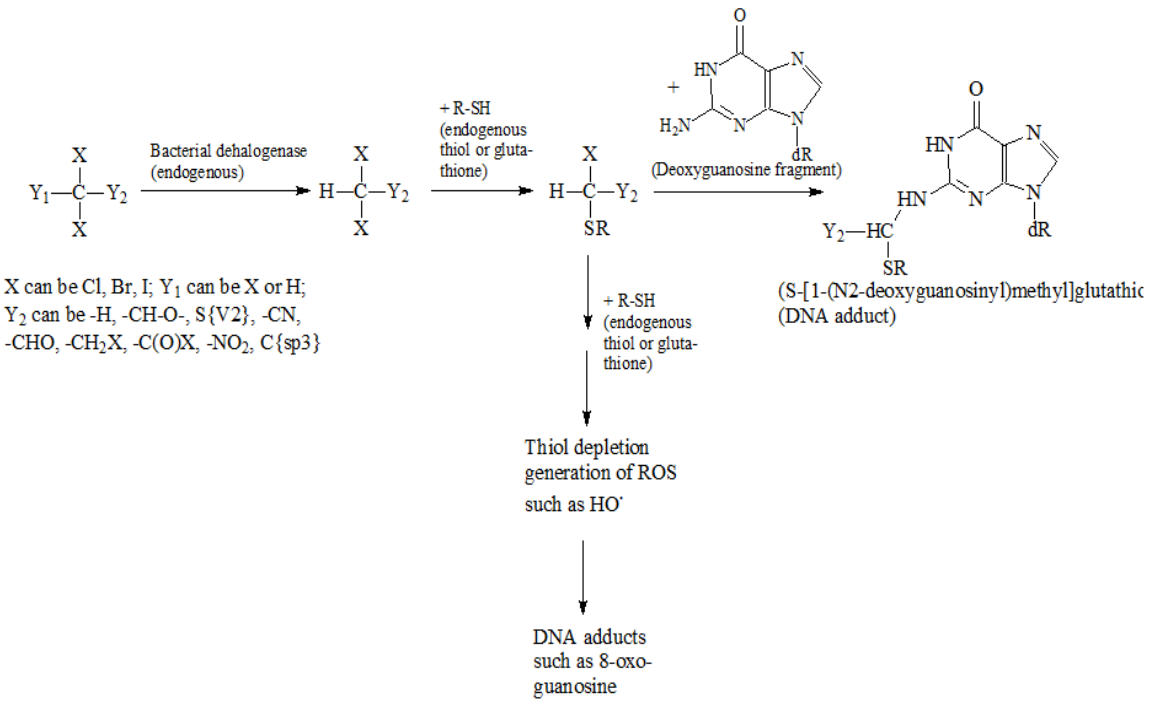
Non-Radical Mechanism - Thiol (glutathione)-dependent bioactivation:



Episulfonium ion, which is known carcinogenic electrophile and derives from the glutathione-dependent bioactivation of some geminal polyhaloalkane derivatives such as captafol has been suggested as an active electrophilic species, which could explain the toxicity and mutagenicity of this chemical [30]:



Non-Radical or Radical Mechanisms: Reductive dehalogenation and thiol/glutathione-dependent bioactivation:



Set of chemicals used for profile development	Geminal Polyhaloalkane Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Strubel, K., Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128. 2. *Chemical Carcinogenesis Research Information System (CCRIS)*;

https://chem.nlm.nih.gov/chemidplus/. Last visited: June, 2021.
3. Longstaff, E., *Toxicol. Lett.*, **1978**, **2**(1), 1 – 4.
4. Anders, M. W., Environ. Health Persp. **96** (1991), 185 – 191.
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6. A.D. Mitchell, Genetic Toxicity Evaluation of Iodotrifluoromethane (CF_3I), Vol. 1. Results of Salmonella typhimurium Histidine Reversion Assay. Govt. Reports Announcements & Index (GRA & I) Issue 06, 1996).
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11. Hosey, K. M. Quinn, J. Environ. Protection **3** (21012), 902 – 914.
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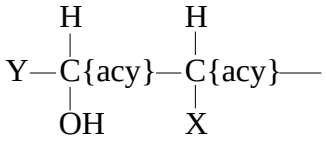
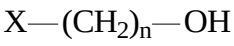
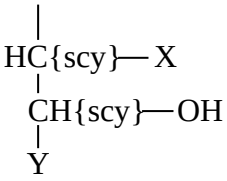
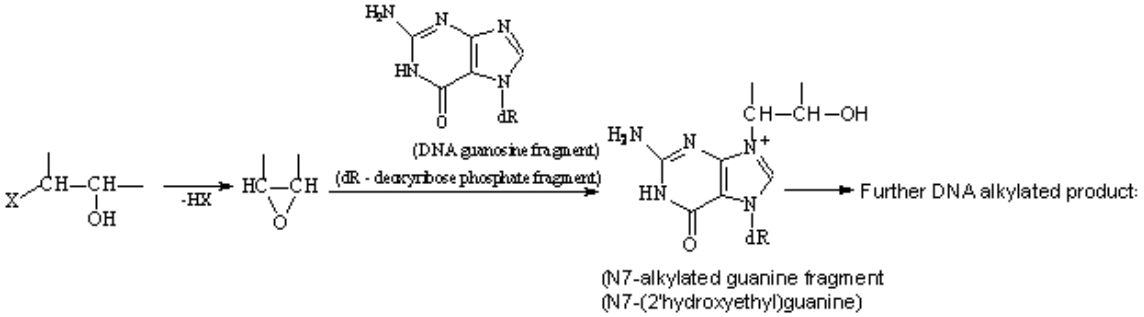
18. Bagchi, D., Toxicol. **104** (1995), 129 – 140.
19. Kovacic, P., Current Medic. Chem. **8**, 2001, 773 – 796.
20. Wiseman, H., Biochem. J. **313** (1996), 17 – 29.
21. Gerardo, D. C., Chem.-Biol. Interact. (1994), 13 – 22.
22. *Public Health Goal for Carbon Tetrachloride in Drinking Water*, Office of Environmental Health Hazard Assessment, California EPA, pesticide and Environmental Toxicology Section, September 2000;
23. Di Ilio, C., Biochem. Pharmacol. **52** (1996), 43 – 48.
24. Yasuo, K., Mutat Res. **58**(2-3) (1978), 143 - 150; http://www.sciencedirect.com/science/article/pii/0165121878900034. Last visited: July, 2021.
25. *Some Industrial Chemicals and Dyestuffs (Benzotrichloride; Benzal Chloride), Summary of Data Reported and Evaluation*, IARC Monographs on Evaluation of Carcinogenic Risk to Humans, Vol. 29, April 13, 1999; https://monographs.iarc.fr/wp-content/uploads/2018/06/mono29.pdf. last visited July.2021
26. *Trihalomethanes in Drinking Water*, Background Document for Development of WHO Guidelines for Drinking-Water Quality, WHO/SDE/WSH/05.08/64, World Health Organization 2005; http://www.who.int/water_sanitation_health/dwq/chemicals/THM200605.pdf
27. Chiu, C. W., L. H. Lee, C. Y. Wang, G. T. Bryan, *Mutagenicity of*

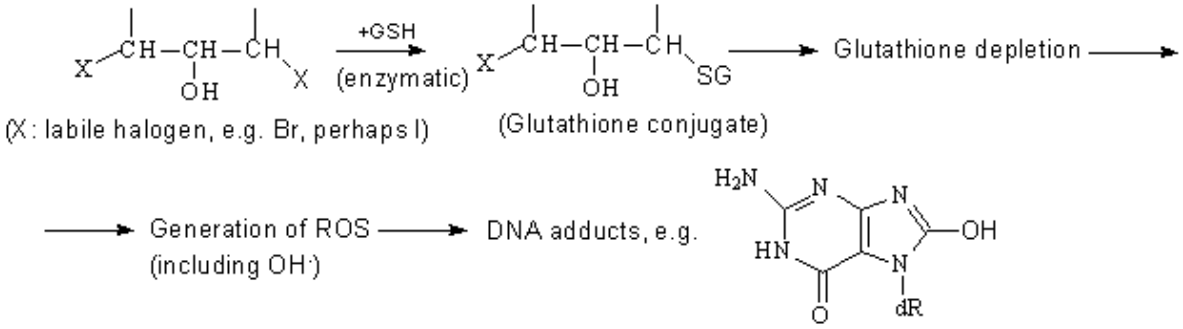
	Some Commercially Available Nitro Compounds for Salmonella Typhimurium, Mutat Res. **58(**1) (1978), 11 – 22; DOI: 10.1016/0165-1218(78)90090-3, Last visited: July, 2021. 28. Wang, M., Chem. Res. Toxicol. **13**(11) (2000), 1149 – 1157; http://pubs.acs.org/doi/abs/10.1021/tx000118t. Last visited: July, 2021. 29. Anders, M. W., Drug Metabol. Rev. **36** (3 – 4) (2004), 583 – 594. 30. Bernard, Br. K., Inter. J. Toxicol. **19** (2000), 43 – 61. 31. Mejer, J., Chem.-Biol. Interact. **31** (1980), 247 – 254. 32. Morita, T., Mutat. Res. **802** (2016), 1 – 29. 33. Sato, T., The Science of Total Environment **46** (1985), 229 – 241.

Individual profile/alert	
Name	Haloalcohols
Type of profile	Structural alert
Description/applicability domain	 (Y can be C{sp3} or -H) (X = Cl, Br, J)  (X is Cl, Br, n = 3 - 10)  X is Cl, Br or I; Y can be one of the following: C{sp3} or -H
Mechanism	S_N2 Alkylation, direct-acting epoxide formed after E2 reaction and Radical ROS formation after GSH depletion (indirect)
The metabolism of 1,3-dichloropropan-2-ol is likely to produce a reactive epoxide intermediate that could damage DNA, and this compound was found to be mutagenic to *Salmonella typhimurium* strains TA1535 and/or TA 100. 2,3 Dichloropropan-1-ol, on the other hand, was also mutagenic *in vitro* in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation [1]. The formation of epoxide intermediate (mutagenicity alert group) can be influenced by *haloalcohol dehalogenases* which are bacterial enzymes that catalyze the cofactor-independent dehalogenation of vicinal haloalcohols. Typical example in this respect is again the genotoxic environmental pollutant 1,3-dichloro-2-propanol, which produces epoxide, chloride ion and proton [2]. Then the epoxide is likely to exert its DNA alkylation capability shown in Scheme 1 [3]:	



Scheme 1

Some authors have assumed genotoxicity mechanism, associated with glutathione depletion as glutathione S-transferase was used as the enzyme source, especially with bromohydrins such as 1,3-dibromopropanol [4]. It is likely that the protection afforded by glutathione against the toxicity of this chemical is mediated through the activity of cytosolic glutathione S-transferase. While 1,3-dichloro-2-propanol is relatively poor substrate for glutathione S-transferase, the dibromo-analogue causes extensive glutathione depletion [4]. According to another study, dichloropropanols such as 1,3-dichloropropan-2-ol, 2,3-dichloropropan-1-ol, 1,3-dibromopropan-2-ol, 1,4-dibromopropan-2-ol, 1-bromopropan-2-ol, other haloalcohols and their metabolites such as epichlorohydrin have been proved to deplete glutathione when incubated with liver fractions obtained from rats. However, difluoropropanols did not deplete glutathione [5]. It is therefore expertly assumed that glutathione depletion would further give rise to formation of ROS and DNA adducts in Scheme 2:



Such mechanistic scheme could also apply to haloalcohols of the type:

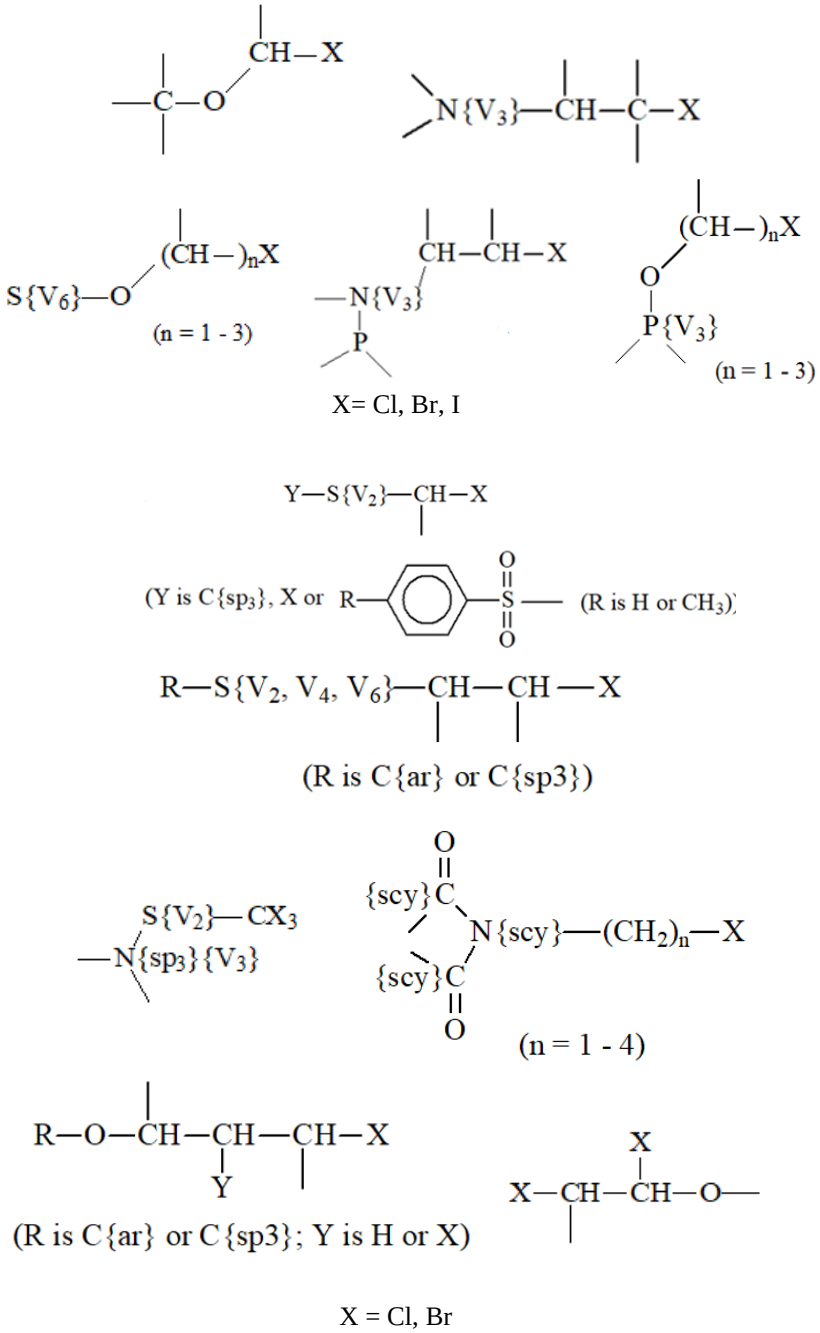
$$X—(CH_2)_n—OH$$

(X is Cl, Br, n = 3 - 10)

Scheme 2

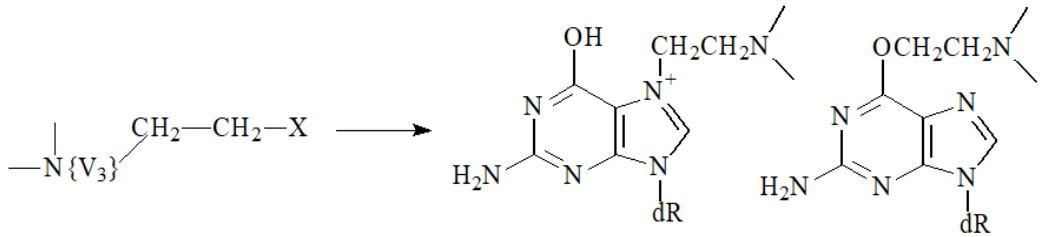
Set of chemicals used for profile development	Haloalcohols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.*Carcinogenicity of 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3-Dichloropropan-1-ol (2,3-DCP)*, Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, COC/04/S2

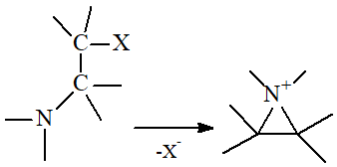
	– June 2004; http://www.iacoc.org.uk/statements/statement123dichloropropanjune2004.htm. Last visited: June, 2021. 2. De Jong, The EMBO Journal **22**(19) (2003), 4933 – 4944. 3. Saha, J. Chromatogr. A **712** (1995), 345 – 354. 4. Hammond, Toxicol. Appl. Pharmacol. **155**(3), 1999, 287-291. 5. Garle, Xenobiotica **29**(5) (1999), 533 – 545.

Individual profile/alert	
Name	Haloalkane Derivatives Containing Chain Heteroatom
Type of profile	Structural alert
Description/applicability domain	
Mechanism	S_N2 Alkylation, nucleophilic substitution at sp3 carbon atom & Radical

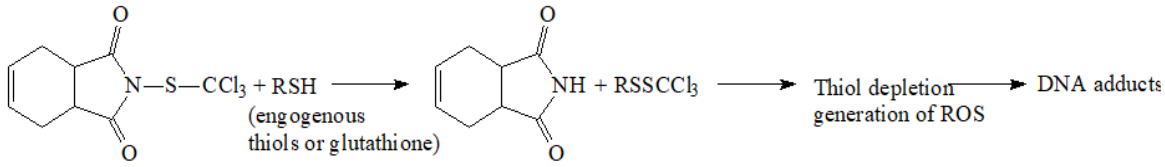
	Generation of ROS by glutathione depletion

1. Compounds with halogen in β-position with respect to a heteroatom



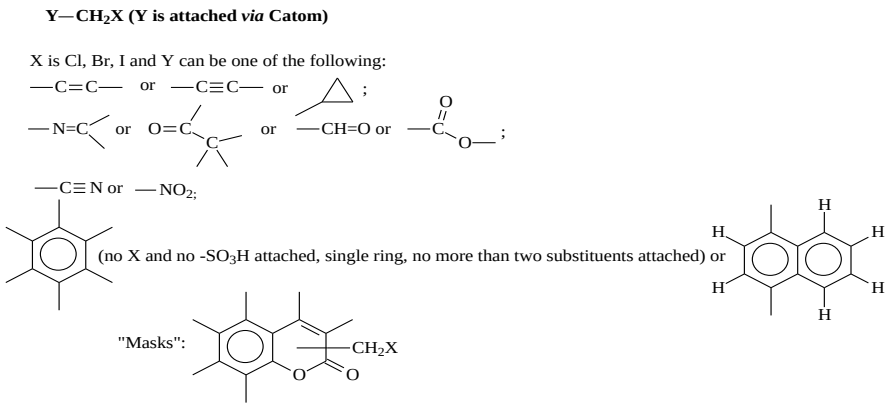
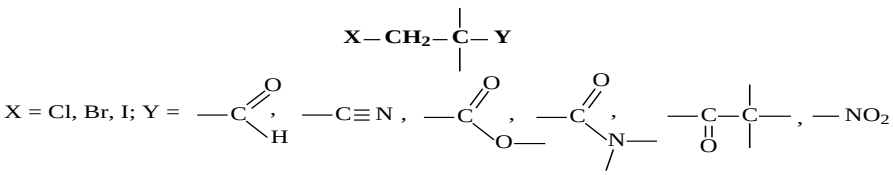


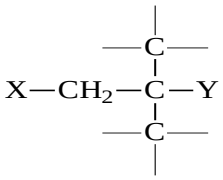
2. Compounds with halogen in α-position with respect to a heteroatom

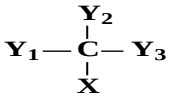
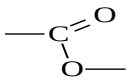
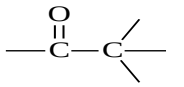
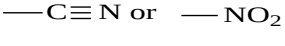
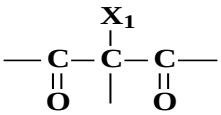
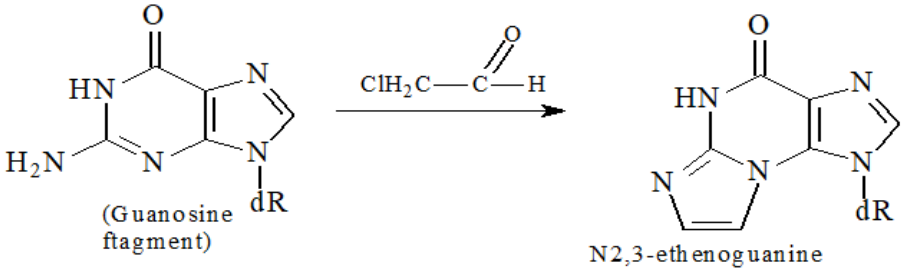


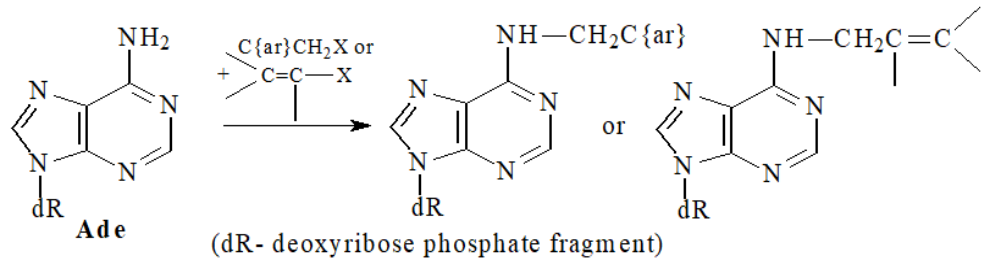
Set of chemicals used for profile development	Haloalkane Derivatives Containing Chain Heteroatom
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kovacic, P., Medical Hypoth.**64** (2005), 104 - 111. 2. *Evidence on the Carcinogenicity of Technical Grade Bis(2-Chloro-1-Methylethyl) Ether*, Final November 1999 (Reproductive and Cancer Hazard Assessment Section Office of Environmental Health Hazard Assessment, California EPA; https://oehha.ca.gov/media/downloads/crnr/bcmeef_1.pdf, last visited 06. 2021. 3. Dacre, J. C., R. Beers, M. Goldman (Geo-Centers Inc. Newton Centre, MA), *Toxicology and Pharmacology of the Chemical Warfare Agent Sulfur Mustard – A Review* (1995). 4. Theiss, J. C., Canc. Res. **39** (1979), 391-395. 5. B. Ringdahl, Pharmacol. Exper. Ther. **240 (**2) (1987), 370-375. 6. *Selected Chloroalkyl Ethers*, World Health Organization, International Programme on Chemical Safety, Environmental Health Criteria 201, (1998); http://www.inchem.org/documents/ehc/ehc/ehc201.htm. last visited 06. 7. Van Duuren, Ann. New York Acad. Sci **163** Biological Effects of Alkylating Agents No. 2 (1969), 633 – 650; DOI: 10.1111/j.1749-6632.1969.tb24883.x. 8. Ruiz, M. J., Mutat. Res. **390** (1997), 245 – 255.

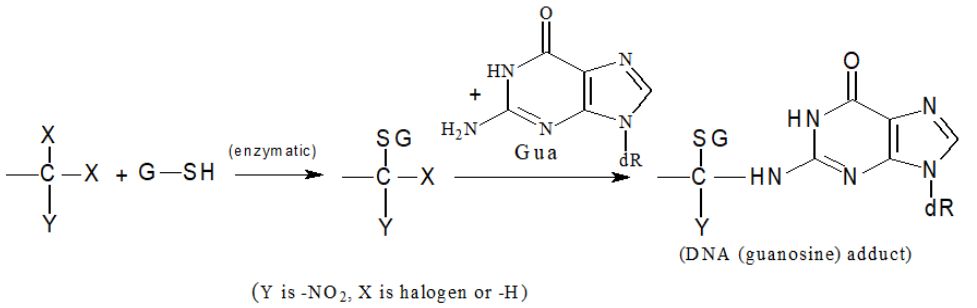
	9. DeBaun, J. R., Xenobiotica **4**(2) (1974), 101-119. 10. D. Morte, R., Boll. Soc. Ital. Biol. Sper. **70**(8-9) (1994), 185 – 192 (Abtsract); http://www.ncbi.nlm.nih.gov/pubmed/7893475. last visited 06. 11. CCRIS: Mephalan, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=148-82-3. Last visited: June, 2021. 12. CCRIS: Chlomaphazine, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=494-03-1. Last visited: June, 2021. 13. CCRIS: Uracil Mustard, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=66-75-1. Last visited. June, 2021. 14. CCRIS: Acrylic Acid, 2-Bromoethyl Ester, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=4823-47-6. Last visited: June, 2021.

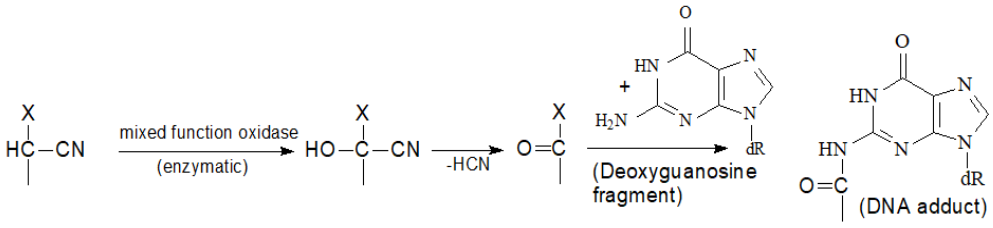
Individual profile/alert	
Name	Haloalkane Derivatives with Labile Halogen
Type of profile	Structural alert
Description/applicability domain	A. Primary haloalkane derivatives with labile halogen and alpha-activating group:  B. Primary haloalkane derivatives with labile halogen and beta-activating group  X = Cl, Br, I; Y = —C(=O)H, —C≡N, —C(=O)—O—, —C(=O)—N—, —C(=O)—C—, —NO2

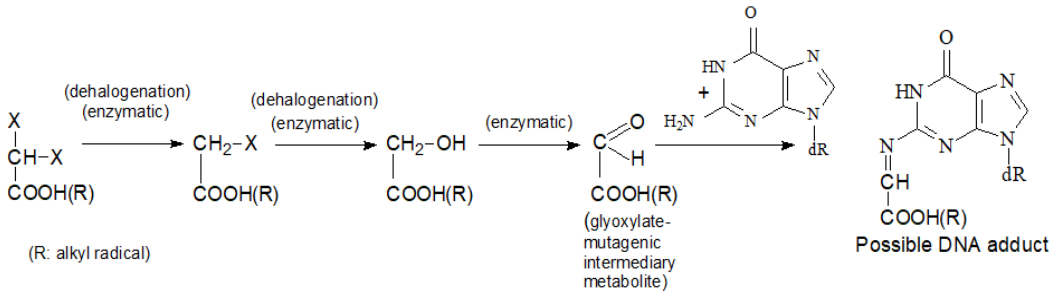
<table>
<tr><td></td><td>"Mask":
 
(Note: If additional one or two more –CH₂X-functionalities are attached to the central C-atom, the "mask" is not valid).

C. Secondary haloalkane derivatives with labile halogen and alpha-activating group:
 
X is Cl, Br, I
Y₁ is C; Y₂ is H or X or CH₃;
Y₃ is -CH=O or 
or  or 
 
(X₁ is Cl, Br, F)</td></tr>
<tr><td>Mechanism</td><td>S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom, A_N2 Shiff base formation for aldehydes & S_N2 Acylation involving a leaving group</td></tr>
<tr><td colspan="2">A. Primary haloalkane derivatives with labile halogen and alpha-activating group:
 
(Guanosine ftagment)
N2,3-ethenoguanine
(dR - deoxyribose phosphate fragment)</td></tr>
</table>

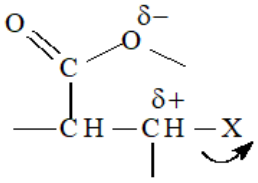


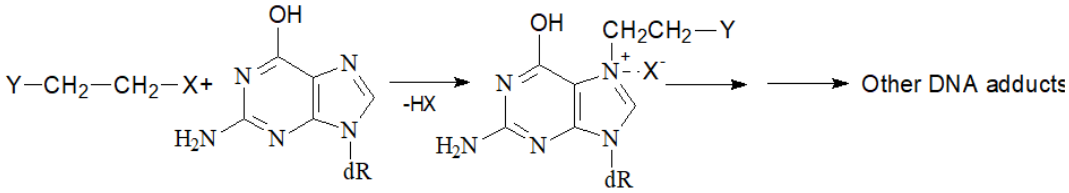






B. Primary haloalkane derivatives with labile halogen and beta-activating group





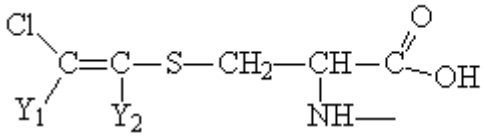
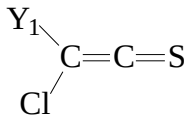
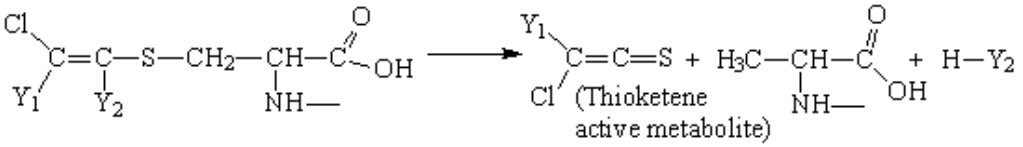
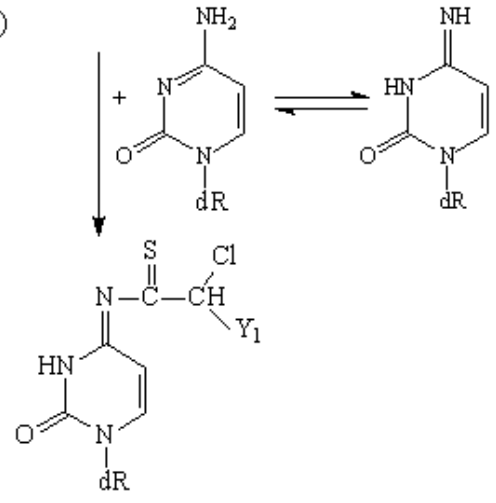
(Deoxyguanosine fragment) (N7-Alkylated DNA adduct)

C. Secondary haloalkane derivatives with labile halogen and alpha-activating group

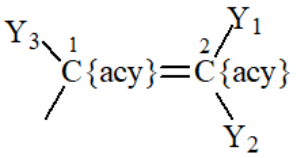
Despite the lack of relevant mechanistic data, it could be expertly assumed that the mechanisms of interaction with prokaryotic DNA may predominantly involve S_N2-type alkylation *via* the C{sp3} atom, bearing halogen and connected to strong EWGs. In the case of aldehyde group attached, A_N2 mechanism of Schiff base formation may be also involved.

Set of chemicals used for profile development	Haloalkane Derivatives with Labile Halogen
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Woo, Y. T., Environ. Health Persp. **110** (2002), 75 – 87. 2. Kargalioglu, Y., Teratog. Carcinog. Mutag. **22**(2) (2002), 113-128; DOI: 10.1002/tcm.10010. 3. Plewa, M. J., Environ. Sci Technol. **38**(18), 2004, pp. 4713-4722. 4. Giller, S., Mutagenesis **12**(5) (1997), 321 - 328. 5. Oesch, Fr., Carcinogenesis **3** (6) (1982), 663 – 665; DOI: 10.1093/carcin/3.6.663. Last visited: June, 2021. 6. Cheng, K. C., Proc. Natl. Acad. Sci USA **88** (1991), 9974 - 9978. 7. Fall, M., Mutat. Res. 633(1) (2007), 13 – 20; DOI: 10.1016/j.mrgentox.2007.04.017. Last visited: June, 2021. 8. Eder, E., Xenobiotica **12**(12), 1982, 831-848; DOI: 10.3109/00498258209038955. Last visited: June, 2021. 9. Lin, E. L. C., Environ. Health Persp. **69** (1986), 67 – 71. 10. Kundu, B., Mutat. Res. **562**(1-2) (2004), 39 - 65. 11. Schneider, M., Mutat. Res. **439**(2) (1999), 233 - 238. 12. Brominated Acetic Acids in Drinking Water (Background Document for Development of WHO Guidelines for Drinking Water Quality, WHO/SDE/WSH/03.04/79 (2004); 13. *Toxicological Review of Dichloroacetic Acid (CAS No. 79-43-6)*, In Support of Summary Information on the Integrated Risk Information Systme (IRIS), US EPA, Washington DC, August 2003; 14. *Monochloroacetic Acid in Drinking Water* (Background Document for Development of WHO Guidelines for Drinking Water Quality), WHO/SDE/WSH/03.04/85, WHO, 2004; 15. Theiss, J. C., Canc. Res. **39**, 1979, 391 - 395. 16. Colburn, N. H., Canc. Res. **28** (1968), 653 – 660. 17. Fall, M., Mutat. Res. 633(1) (2007), 13 – 20; DOI: 10.1016/j.mrgentox.2007.04.017. 18. *Allyl Bromide CAS No. 106-95-6, CSWG Evaluation* (12/16/94); http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/AllylBromide.pdf Last visited: June, 2021.

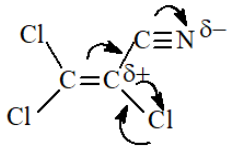
	19. Eder, E., Xenobiotica 12(12), 1982, 831-848; http://pubget.com/paper/6763406. Last visited: June, 2021. 20. McCoy, E. C., Mutat. Res./Fund. Molec. Mechan. Mutag. 57(1) (1978), 11 – 15; http://www.sciencedirect.com/science/article/pii/0027510778902294. Last visited: June, 2021.

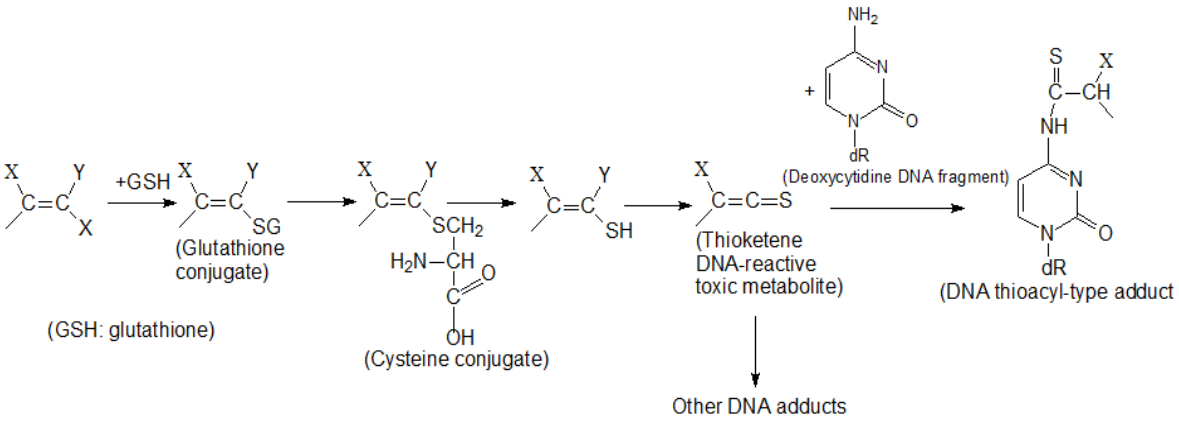
Individual profile/alert	
Name	Haloalkene Cysteine S-Conjugates
Type of profile	Structural alert
Description/applicability domain	 (Y_1 can be -Cl, $-CCl_3$, -H, -C=C- or $-CF_3$; Y_2 is -Cl or -F) 
Mechanism	A_N2 Nucleophilic addition to metabolically formed thioketenes
 (Y_1 can be -Cl, $-CCl_3$, -H, -C=C- or $-CF_3$; Y_2 is -Cl or -F)  (Cytidine DNA adduct)	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Evidence on the Carcinogenicity of 1,3-Hexachlorobutadiene* (Final), December 2000,

	Reproductive and Cancer Hazard Assessment Section Office of Environmental Health Hazard Assessment California Environmental Protection Agency; https://oehha.ca.gov/media/downloads/proposition-65/chemicals/hcbd-final.pdf, last visited 06.2021 2. Dreessen, Mutat. Res. **539** (2003), 157 – 166. 3. Vamvakas, Chem.-Biol. Interact. **65** (1988), 59 – 71. 4. Muller, Chem. Res. Toxicol. **11** (1998), 464 – 470.

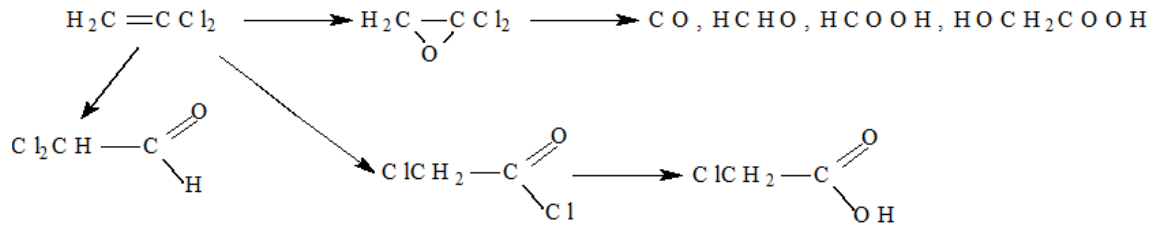
Individual profile/alert	
Name	Haloalkene Derivatives with Electron-Withdrawing Groups
Type of profile	Structural alert
Description/applicability domain	 Y_2 can be $–NO_2$ or –CN or **-C=C-** or Cl or Br or -C(O)O- (attached *via* the carbon of carbonyl group C(O)), or -C(O)C (attached *via* the carbon of carbonyl group C(O)); Y_3 is Cl or Br or H C(O) corresponds to carbonyl group C=O No $–SO_3H$ or -COOH groups attached to the C_1-atom;
Mechanism	S_N2 Direct alkylation or alkylation by metabolically formed epoxides & A_N2 Thioacylation *via* nucleophilic addition after thioketene formation

1. Haloalkenes containing halogen(s) and other electron-withdrawing group(s) (EWG).

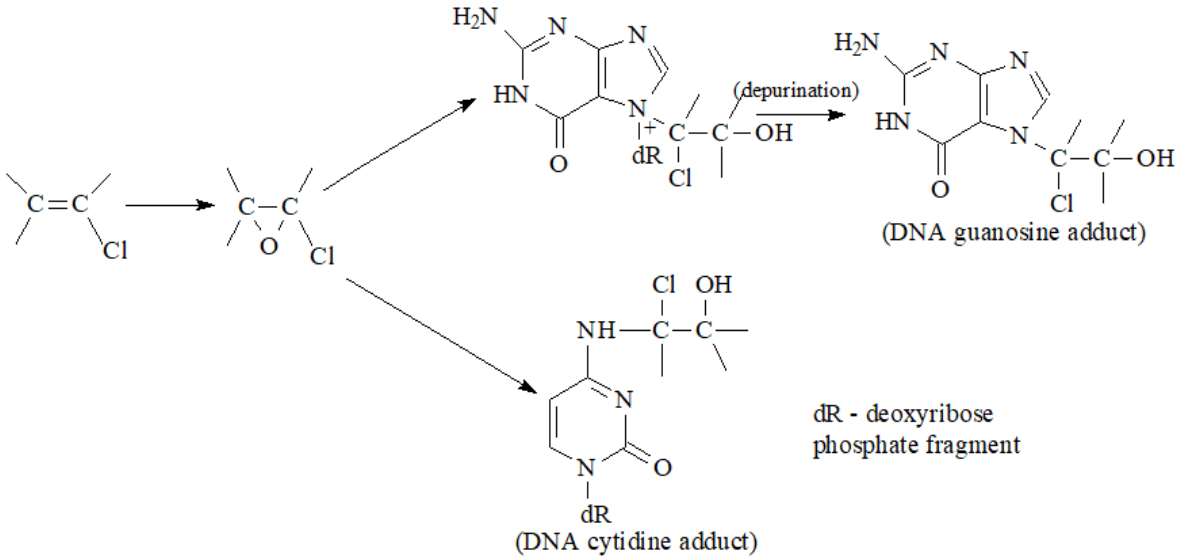




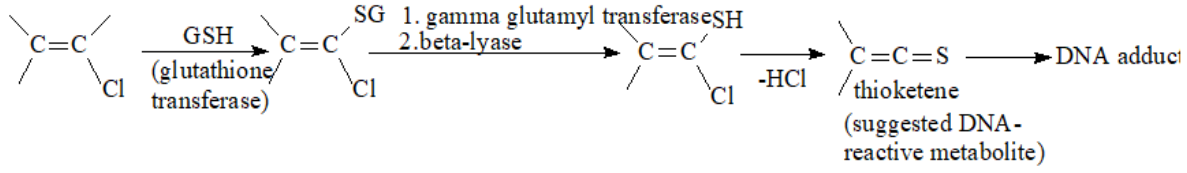
2. Vinyl-type haloalkenes, not containing other EWGs

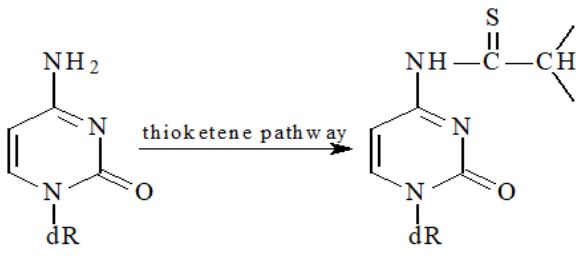


3. Formation of epoxide intermediate that binds covalently to DNA *via* electrophilic mechanism of alkylation towards the biological macromolecule:



4. Glutathione or thiol activation pathway. In this case, the formation of reactive product that binds to DNA *via* electrophilic mechanism [11] takes place:

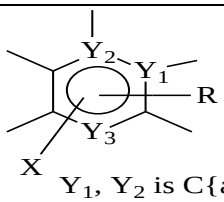
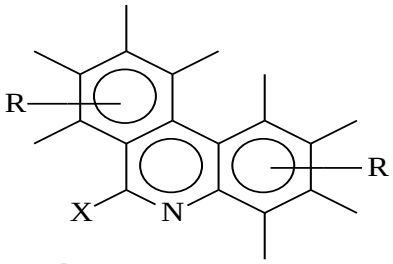




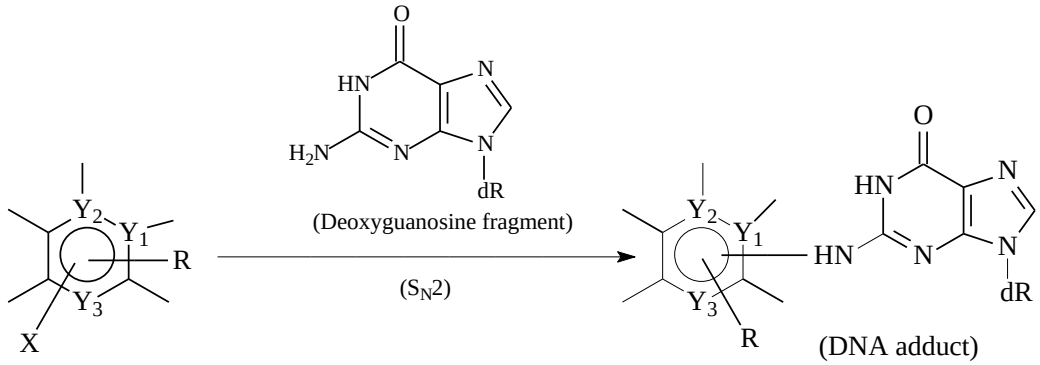
Set of chemicals used for profile development	Haloalkene Derivatives with Electron-Withdrawing Groups
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.

References	1. Woo, Y. T., Environ. Health Persp. **110** (Suppl. 1) (2002), 75 - 87. 2. Kim, D., Drug Metab. Dispos. **34**, 2006, 2020 – 2027. 3. Decant, W., Environ. Health Persp. **88** (1990), 107 – 110. 4. Muller, M., Chem. Res. Toxicol. **11**(5) (1998), 464 – 470; DOI: 10.1021/tx9701440. 5. *Vinyl Chloride, An Annotated Bibliography with Emphasis on Genotoxicity and Carcinogenicity* (Prepared by Dr. Michael F. Salamone and Dr. Gary Westlake), Ontario Ministry of Environment, September 1998; 6. Lijinsky, W., Teratog. Carcinog. Mutag. **1** (1980), 259 – 267. 7. *Trichloroethylene*, International Programme on Chemical Safety, Environmental Health Criteria 50; http://www.inchem.org/documents/ehc/ehc/ehc50.htm#SectionNumber:5.3 8. Fahrig, R., Mutat. Res. 340 (1995), 1 – 36. 9. *Vinylidene Chloride* International Programme for Chemical Safety, Environmental Health Criteria 100; http://www.inchem.org/documents/ehc/ehc/ehc100.htm#SubSectionNumber:6.1.4 Last visited: June, 2021. 10. *Toxicological Profile for Hexachlorobutadiene*, US Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (May 1994); http://www.atsdr.cdc.gov/toxprofiles/tp42.pdf Last visited: June, 2021. 11. Muller, M., Chem. Res. Toxicol. **11**(5) (1998), 464 – 470; http://pubs.acs.org/doi/abs/10.1021/tx9701440. Last visited: June, 2021. 12. Strubel, K., Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128. 13. Rannug, U., Chem.-Biol. Interact. **12** (1976), 251 – 263. 14. Mucochloric Acid, PubChem Open Chemistry Database, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/compound/Mucochloric_acid#section=Top Last visited: June, 2021. 15. Dichlorvos, ChemPlus, A Tooxnet Database, U.S. National Library of Medicine; https://chem.nlm.nih.gov/chemidplus/rn/62-73-7 Last visited: June, 2021. 16. Bucher, J. R., *NTP Technical Report on Toxicity Studies of β-Bromo-β-Nitrostyrene (CAS No. 7166-19-0) Administered by Gavage to F344/N Rats and B6C3F Mice*, NIH Publication, August 1994; https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox040.pdf Last visited: June, 2021.

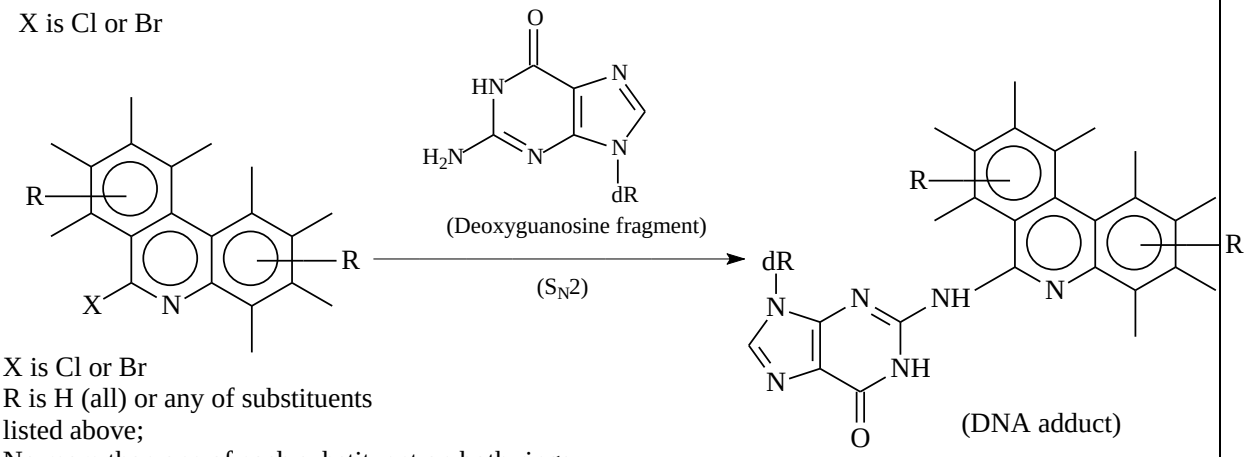
Individual profile/alert	
Name	Haloazaarene and Fused-Ring Haloquinoline Derivatives
Type of profile	Structural alert

Description/applicability domain	 Y_1, Y_2 is C{ar} (both) or N{ar} (one only, the other is C{ar}); Y_3 is N{ar} or N{ar}=O; R is $-NO_2$ or $-C\equiv N$ or $-CH_3$ or $-OCH_3$ or N{V3}{sp3} or $-CCl_3$ or Cl; X is Cl or Br At least two non-substituted H-atoms attached to the ring  X is Cl or Br R is H (all) or any of substituents listed above; No more than one of each substituent on both rings
Mechanism	S_N2 Arylation, nucleophilic substitution on activated heteroaromatic carbon atom

According to one publication, it is possible that the toxicity of chloropyridines is due to the reactivity of chlorine attached to the pyridine ring. Under the experimental conditions associated with metabolic activation, N-oxidation may contribute to the toxicity effects, more particularly, genotoxicity [1]. These assumptions were more profoundly investigated in another publication [2]. Here the Salmonella/microsome assay with strains TA97, TA98, TA100 and TA102 was used to examine the potential mutagenicity and structure-activity of a number of mono- and di-halogenated pyridines. The chemical reactivity of the halopyridines suggests that nucleophilic displacement of halogens can easily occur with halogens at positions 2, 4 and 6. Especially, 2-Chloropyridine gave a positive result with rat liver metabolic activation, and 2-fluoropyridine produced equivocal results under these conditions. Mutagenic responses were also obtained with 2-chloromethyl pyridine and 3-chloromethyl pyridine, in both the presence and absence of rat-liver $9. These results suggest that the halogenated pyridines, especially with halogens at the 2-position, and on a methyl substituent, have mutagenic activity in the Salmonella assay. Since the N-oxidation is a microsomal metabolic process, the formation of N-oxide is regarded as bioactivation reaction. According to the authors, it is possible that the electron-withdrawing effect of the N-oxide functionality would make the halogen, particularly, in position 2 more labile and susceptible to nucleophilic attack. The positive results of 2- and 3-chloromethyl pyridines without metabolic activation suggest a direct nucleophilic substitution by these compounds [2]. It is also expertly assumed that the presence of other electron-withdrawing groups such as –NO2, -CN, -OCH3, -CCl3, N{V3}{sp3}, etc. attached to the heteroaromatic ring, in combination with the metabolic N-oxidation, and the presence of other electron-withdrawing nitrogen heteroatoms would additionally contribute to the reactivity towards DNA and mutagenicity.
Based on the above discussions, the following simplified mechanistic schemes can be expertly proposed:



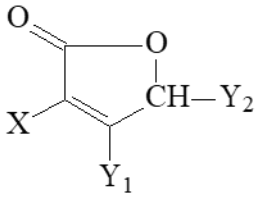
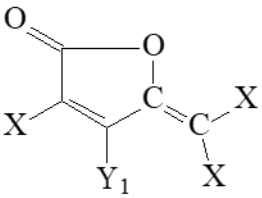
Y_1, Y_2 is C{ar} (both) or N{ar} (one only, the other is C{ar});
Y_3 is N{ar} or N{ar}=O;
R is $-NO_2$ or —C≡N or
$-CH_3$ or $-OCH_3$ or N{V3}{sp3}
or $-CCl_3$ or Cl;
X is Cl or Br

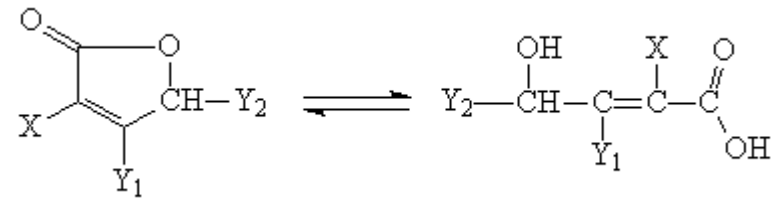


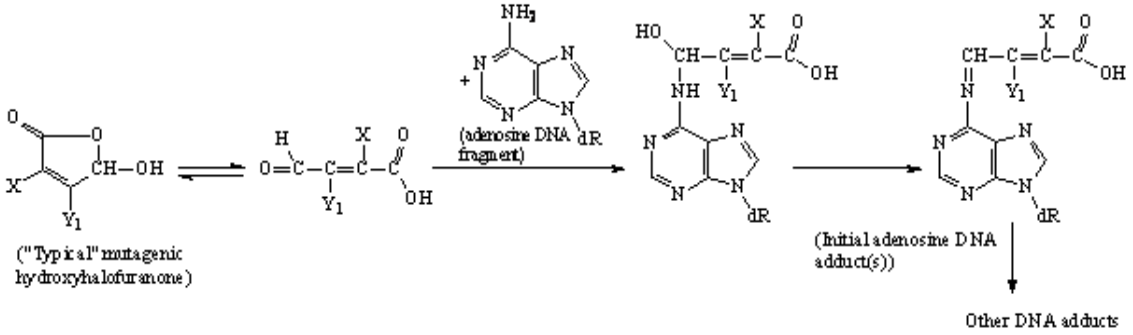
X is Cl or Br
R is H (all) or any of substituents
listed above;
No more than one of each substituent on both rings

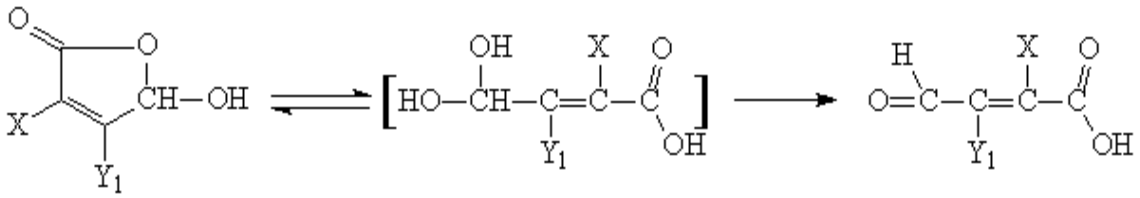
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Anuszewska, E. L., J. H. Koziorowska, Role of Pyridine N-Oxide in the Cytotoxicity and Genotoxicity of Chloropyridines, Toxicol. in Vitro 9(2) (1995), 91 – 94. 2. Glaxton, L. D., K. L. Dearfield, R. J. Spanggord, E. S. Riccio, K. Mortelmants, Comparative mutagenicity of halogenated pyridines in the Salmonella typhimurium/mammalian microsome test, Mutat. Res. 176 (1987), 185 – 198.

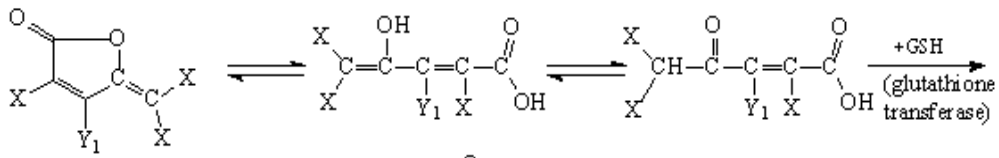
Individual profile/alert	
Name	Halofuranones
Type of profile	Structural alert

Description/applicability domain	 **(I)**  **(II)** (X is Cl or Br; Y_1 is $-CHX_2$, -X, $-CH_3$, -H, $-CH_2X$; Y_2 is -H or -OH or $-OCH_3$)
Mechanism	S_N2 Nucleophilic substitution at sp3 carbon atom & A_N2 Shiff base formation

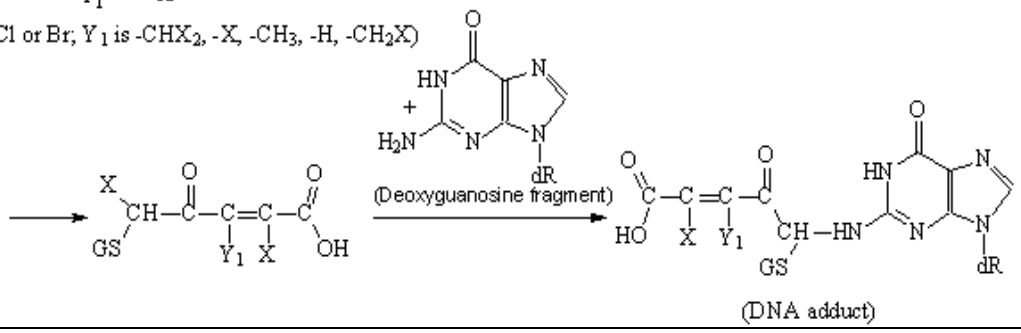


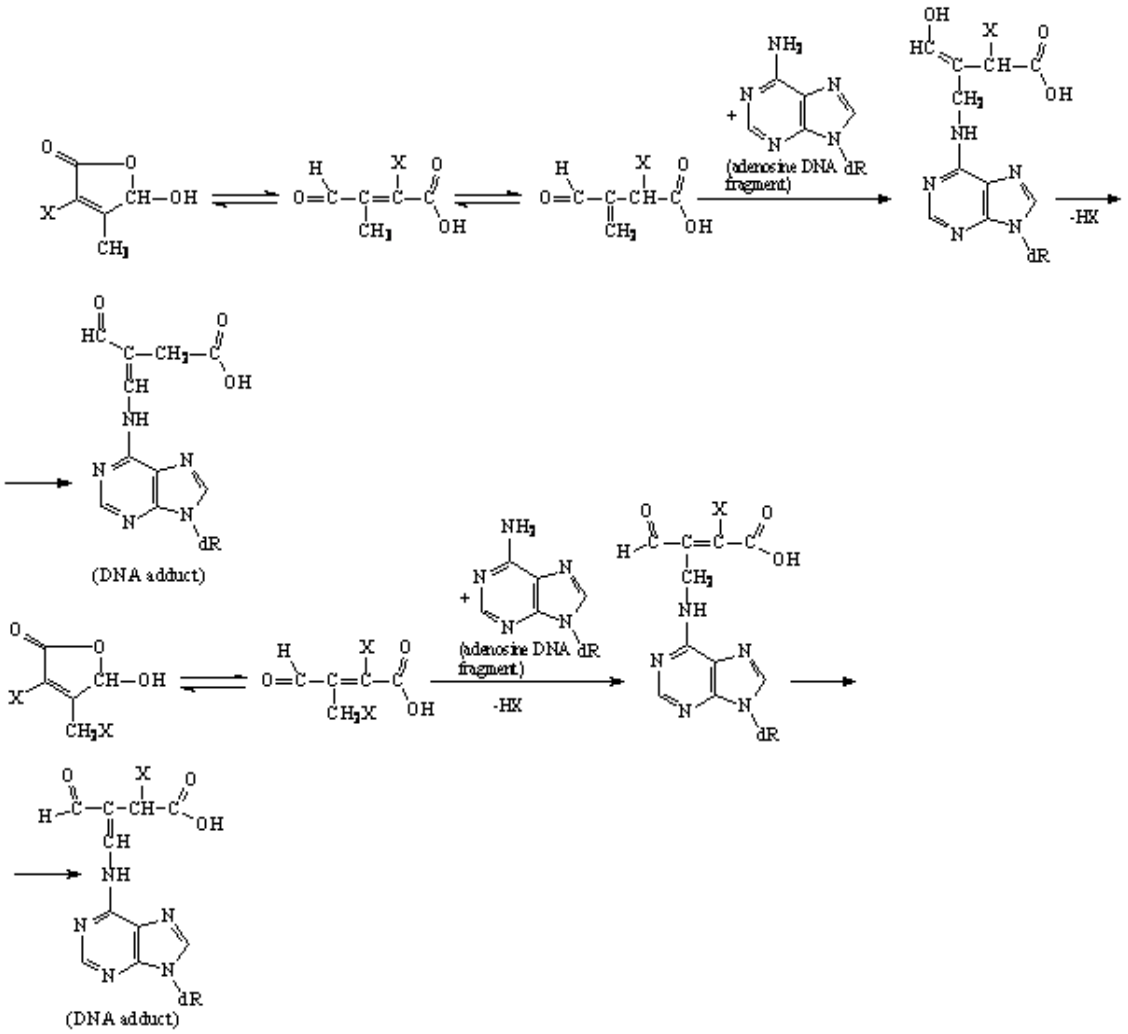






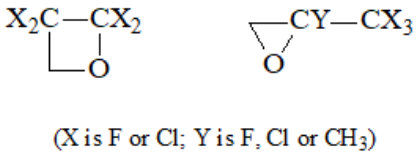
(X is Cl or Br; Y_1 is $-CHX_2$, -X, $-CH_3$, -H, $-CH_2X$)





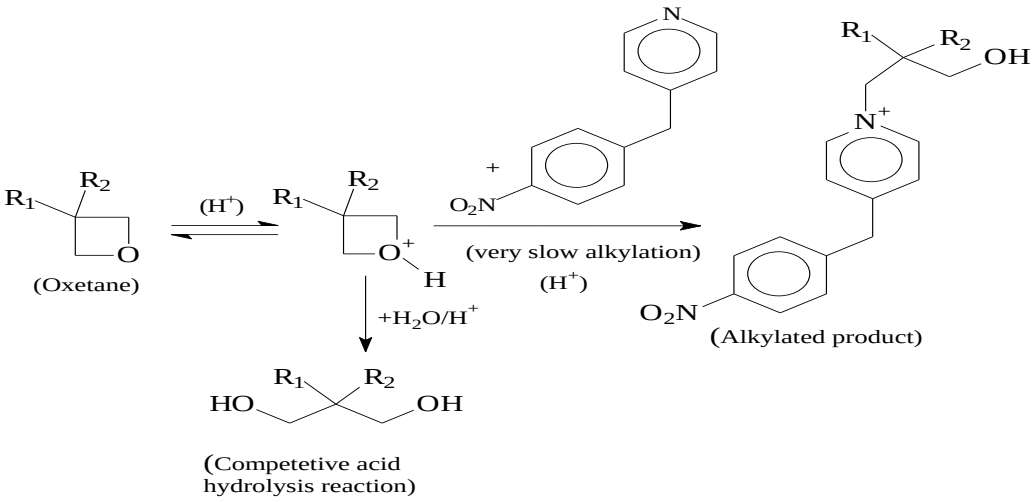
Set of chemicals used for profile development	Halofuranones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Woo, Y. T., Environ. Health Persp. **110** (Suppl. 1) (2002), 75-87. 2. Tuppurainen, K. *A Plausible Mechanism for the Mutagenic Activity (Salmonella typhimurium TA100) of MX Compounds: A Formation of CG-CG$^+$-CG Radical Cation by One-Electron Reduction*, SAR and QSAR in Environ. Res. **7**(1-4) (1997), 281 – 286. 3. Bombarelli, R. G., Env. Sci. Technol. **45** (2011), 9009 – 9016. 4. Bombarelli, R. G., Environ. Sci Technol. **46** (2012), 13463 – 13470. 5. Anders, M. W., Drug Metabol. Rev. **36** (3 – 4) (2004), 583 – 594. 6. Bombarelli, R. G., *Chemical Processes That Can Damage Cellular DNA: Reactivity and Alkylating Potential of Some O-Heterocycles*, PhD Thesis, Departamento de Quimica Fisica Facultad de Ciencias Quimicas, Salamanca, December 2011.

Individual profile/alert

Name	Halogenated Oxetanes and Haloepoxides: DNA Reactivity
Type of profile	Structural alert
Description/applicability domain	 (X is F or Cl; Y is F, Cl or CH_3)
Mechanism	S_N2: Alkylation, direct acting epoxides and related

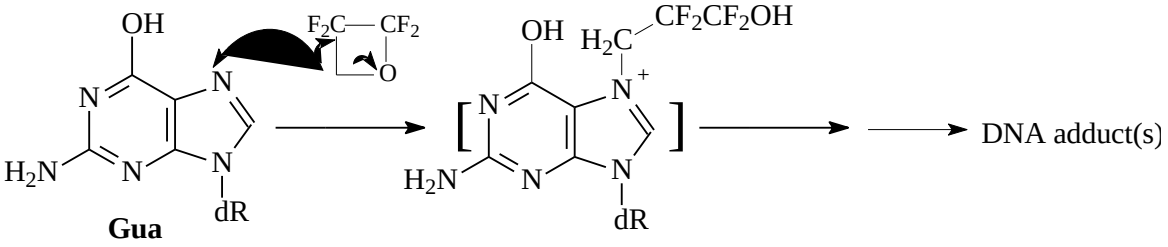
I. Halogenated Oxetanes

Alkylation of the model compound 4-(p-nitrobenzyl)pyridine (NDP) with hydrocarbon-type (non-fluorine-containing) oxetanes occurs very slowly under acidic conditions as illustrated by the following scheme:



(Scheme 1)

Introduction of electron-withdrawing fluorine (or, possibly, chlorine) atoms bound to the cyclic carbons would enhance the elctrophilicity, and the ring-opening DNA alkylating capacity of the partially fluorinated oxetanes by heterolytic cleavage of the CH_2-O bond (Scheme 2):



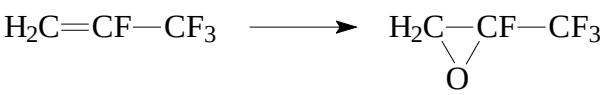
(dR - deoxyribose phosphate fragment;
Gua: Guanine nucleosides)

(Scheme 2)

II. Haloepoxides

The chemical 2,3,3,3-tetrafluoropropene (HFO-1234yf) was reported positive in the bacterial mutagenicity test with Salmonella typhimurium strain TA100 and E. coli (WP2 uvrA) with metabolic activation only [4]. On the other hand, the biotransformation studies showed the epoxide (Scheme 3)

as the primary active metabolite of HFO-1234yf [5] (Scheme 3):

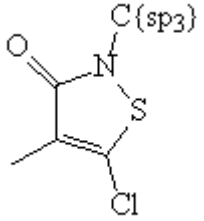


2,3,3,3-Tetrafluoropropene (HFO-1234yf)

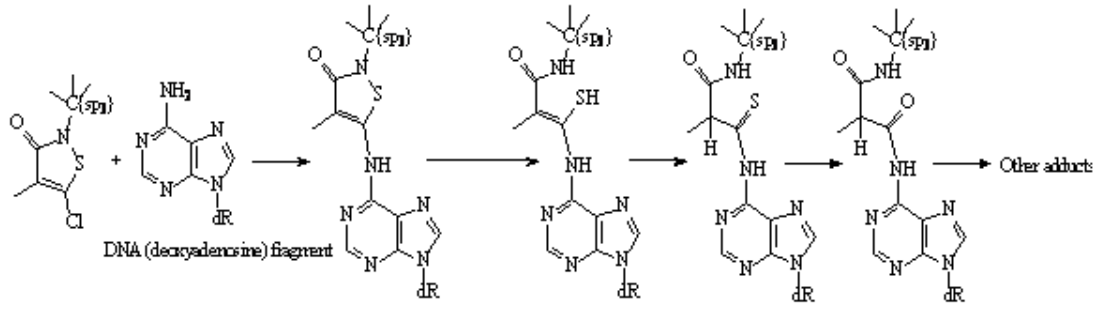
2,3,3,3-Tetrafluoropropene epoxide (active metabolite)

(Scheme 3)

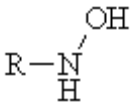
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Bombarelli, R. G., B. Br. Palma, C. Martins, M. Kranendonk, A. C. Rodriges, E. Calle, J. Rueff, J. Casado, Alkylating Potential of Oxetanes, Chem. Res. Toxicol. 23 (2010), 1275 – 1281) 2. 2,2,3,3-Tetrafluorooxetane, CAS No 765-63-9. ECHA Legal Notice, Registration Dossier. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/6126/7/7/2); Last visited: June, 2021. 3. List of Mutagenic Substances, Japan National Center for Occupational Safety and Health; https://www.jniosh.johas.go.jp/icpro/jicosh-old/english/topics/mutagenicchemicals/mutagenicchemicals.html. Last visited: June, 2021. 4. Tveit, A., G. M. Rusch, H. Muijser, M. M. Tegelenbosch-Shouten, The Acute, Developmental, Genetic and Inhalation Toxicology of 2,3,3,3-tetrafluoropropene (HFO-1234yf), Drug Chem. Toxicol. 36(4) (2013), 412 – 420. 5. T. Schmidt, Biotransformation of trans-1-Chloro-3,3,3-Trifluoropropene and 2,3,3,3-Tetrafluoropropene, Dissertation zur Erlangung des Naturwissenschaftlichen Doktorgrades der Julius-Maximilians-Universitat Wurzburg, Bad Kissingen, Wurzburg, 2013. 6. Wade, D.R., Airy, S.C., Sinsheimer, J.E., Mutagenicity of aliphatic epoxides. Mutat. Res. 58(2-3) (1978), 217 - 223.

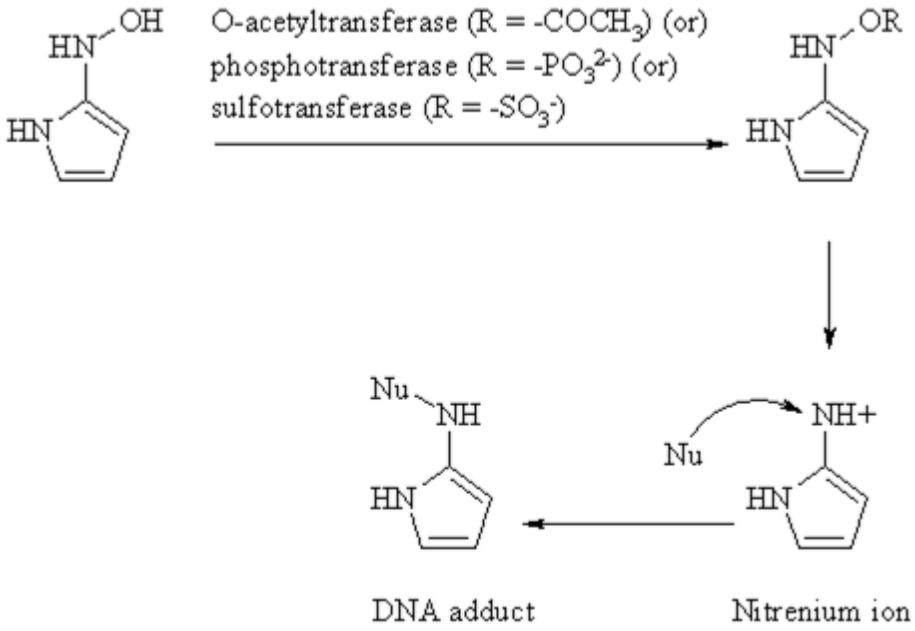
Individual profile/alert	
Name	Haloisothiazolinones
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**Ring opening S_N2 reaction**
Despite the fact that no mechanistic schemes for DNA adduct formation with this class of chemicals	

have been found in the literature so far, it may be suggested that some potential DNA reactivity and adduct formation are possible. For example, the adenine base in DNA would perhaps react as nucleophile *via* its primary amino group with the haloizothiazolone chemical. This interaction is probably promoted by the thiol groups of CYP450 enzymes in the S9/microsomal fraction. It may happen, according to the following expertly assumed scheme, similar to that, proposed for the reaction with lysine primary amino group fragments in proteins [4]:



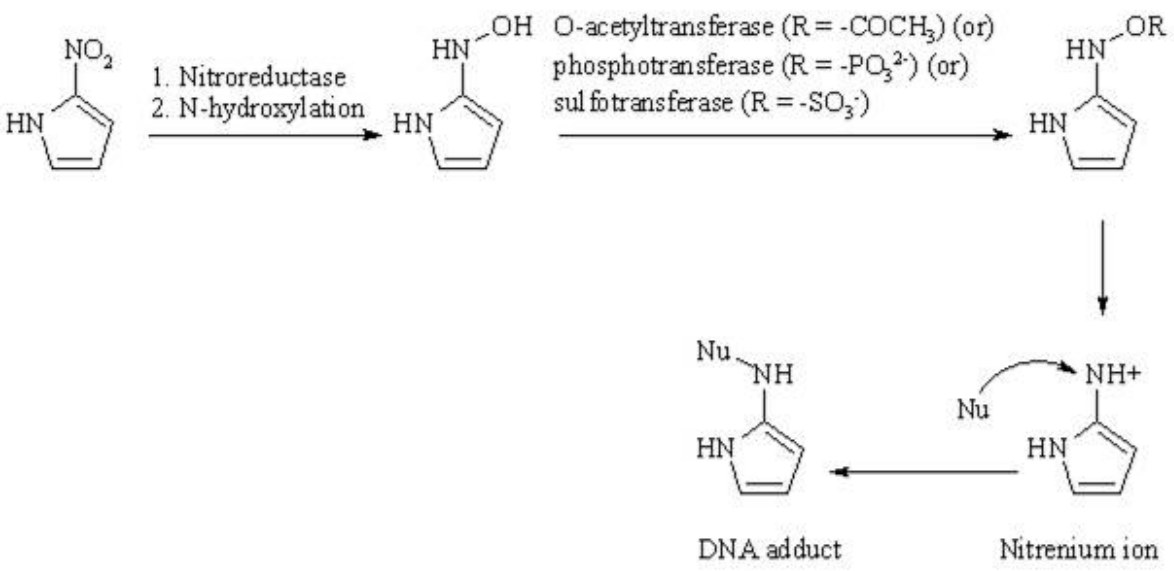
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Scribner, Mutat. Res./Gen. Toxicol. 118(3) (1983), 129 – 152. 2. Connor, Environ. Molec. Mutag. 28 (1996), 127 – 132. 3. Williams, PowerPlant Chemistry 9(1) (2007), 14 – 22. 4. Sanchez, Chem. Res. Toxicol. 17(9) (2004), 1280 – 1288.

Individual profile/alert	
Name	Heterocyclic N-Hydroxylamines
Type of profile	Structural alert
Description/applicability domain	 R = aromatic carbon atom
Mechanism	SN1 reaction Nitrenium ion formation
Heterocyclic N-hydroxylated groups have the potential to be metabolised by either acetyl-, phospo- or sulfotransferase. These species then produce the electrophilic nitrenium ion which is capable of reacting with DNA via an SN1 mechanism (Kalgutkar 2005).	



Set of chemicals used for profile development	Heterocyclic N-Hydroxylamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225

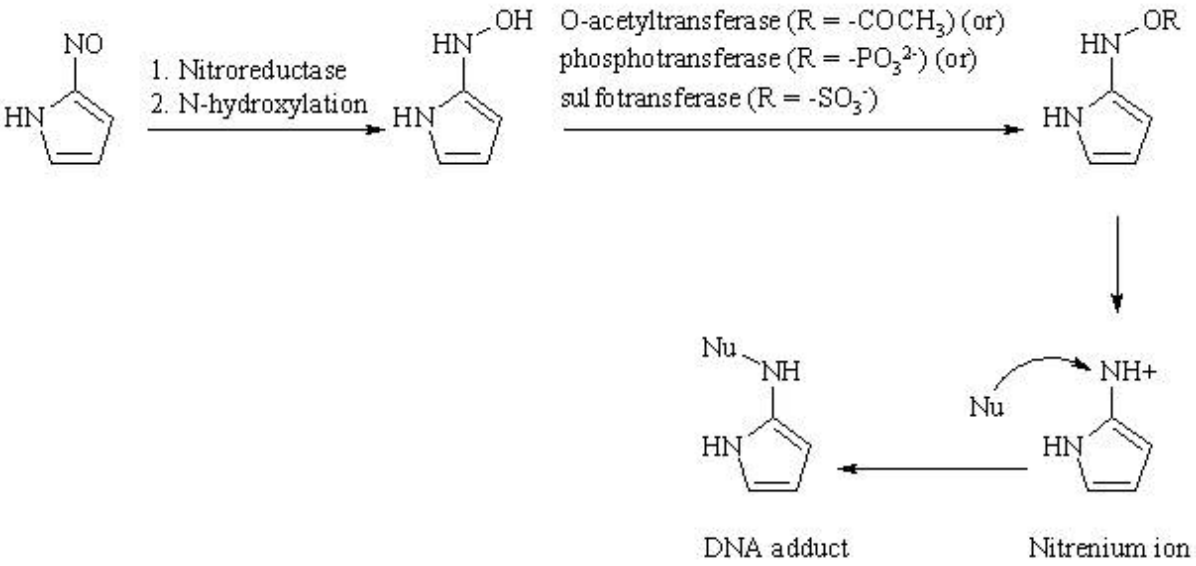
Individual profile/alert	
Name	Heterocyclic nitro compounds
Type of profile	Structural alert
Description/applicability domain	$R—NO_2$ R = aromatic carbon atom
Mechanism	SN1 reaction Nitrenium ion formation
Heterocyclic nitro groups can be metabolised into an N-hydroxylated intermediate which subsequently undergoes either acetyl-, phospo- or sulfotransferase. This is an analogous reaction to that which occurs for aromatic nitro chemicals. This species then produces the electrophilic nitrenium ion which is capable of reacting with DNA via an SN1 mechanism (Kalgutkar 2005).	



Set of chemicals used for profile development	Heterocyclic nitro compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225.

Individual profile/alert	
Name	Heterocyclic Nitroso compounds
Type of profile	Structural alert
Description/applicability domain	R – NO R = any five membered heterocyclic ring system (the heterocyclic ring can contain any combination of carbon, nitrogen, oxygen or sulphur in which R is connected via a carbon atom)
Mechanism	SN1 reaction Nitrenium ion formation

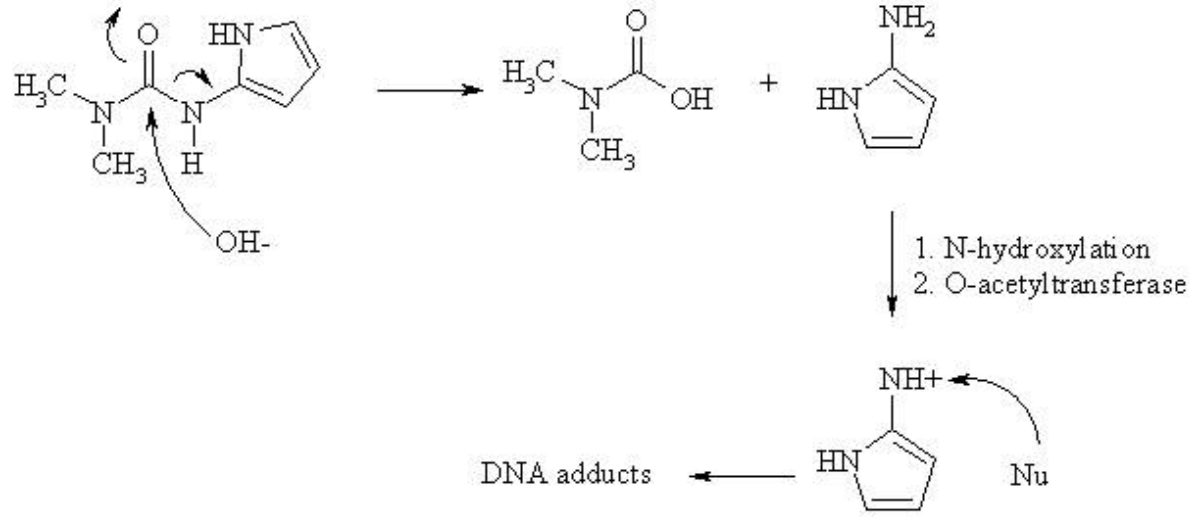
Heterocyclic nitroso compounds have the potential to be reduced, and then hydroxylated to an N-hydroxylamine intermediate. This species is then further metabolised by one of three potential transferases, which themselves produce the reactive nitrenium ion which can bind DNA via an S_N1 mechanism (Kalgutkaer 2005).



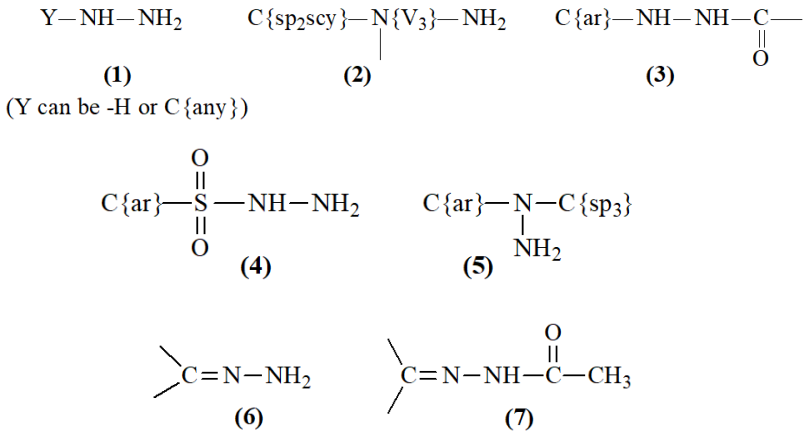
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225

Individual profile/alert	
Name	Heterocyclic urea derevatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	SN1 reaction Nitrenium ion formation

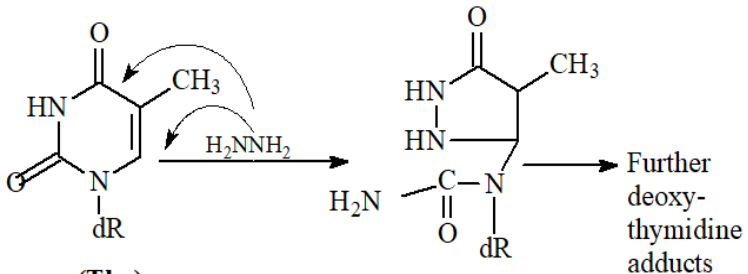
Hydrolysis of the amide bond to produce an aromatic amine moiety has been suggested to be responsible for the toxicity of chemicals containing this alert. The formation of the nitrenium ion results in DNA binding via an SN1 mechanism (Guengerich et al 1997).



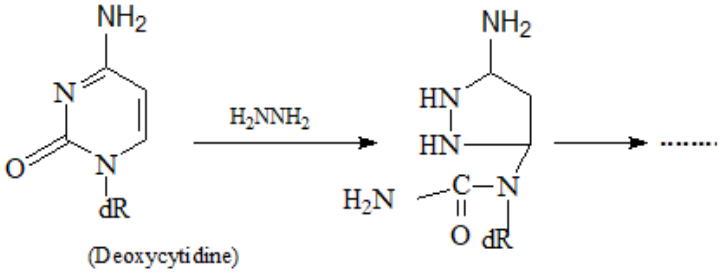
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Guengerich FP et al (1997) Drug Metabolism and Disposition, 25, p1234-1241

Individual profile/alert	
Name	Hydrazine Derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS generation (indirect), A_N2 Nucleophilic addition reaction with cycloisomerization & S_N2 Direct nucleophilic attack on diazonium cation

The mechanism of the direct formation of the initial DNA adduct with hydrazine is complex, accompanied by an array of DNA adducts [3]:

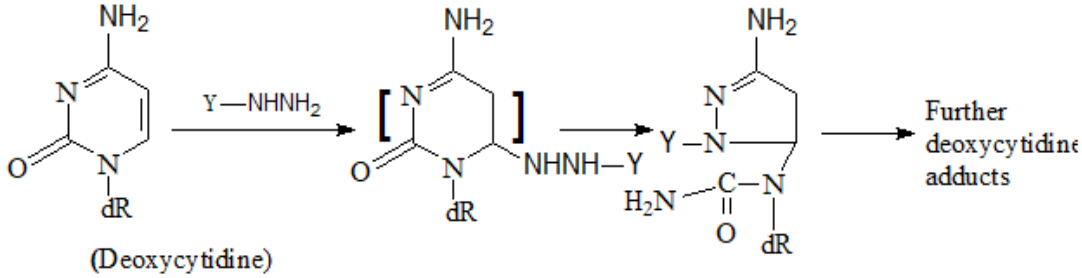


Similar mechanism has been proposed elsewhere, as illustrated by the formation of adduct(s) with the cytidine fragment of DNA [4]. According to the authors, the initial attack of hydrazine is likely to be predominantly at C6 of the pyrimidine ring, followed by ring closure at C4 (cycloisomerization). The resulting intermediates are substituted dihydropyrazoles, which undergo further chemical transformations with formation of other types of adducts:



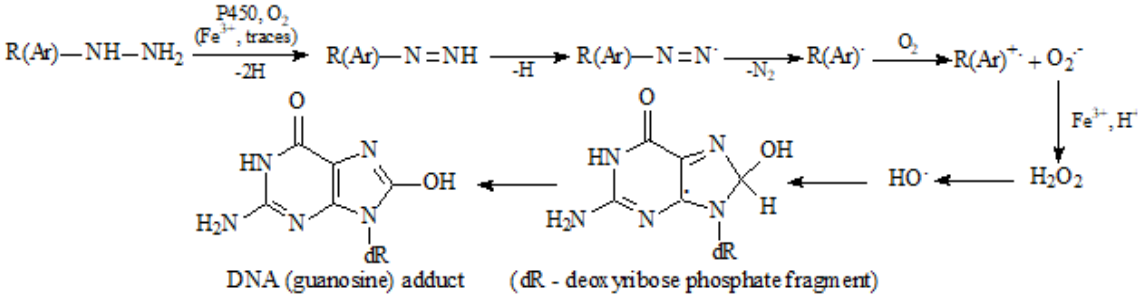
(Deoxycytidine)
(dR - deoxyribose phosphate fragment)

On the basis of the above data, a more general mechanism for the formation of initial adducts with pyrimidine bases of DNA can be expertly suggested:



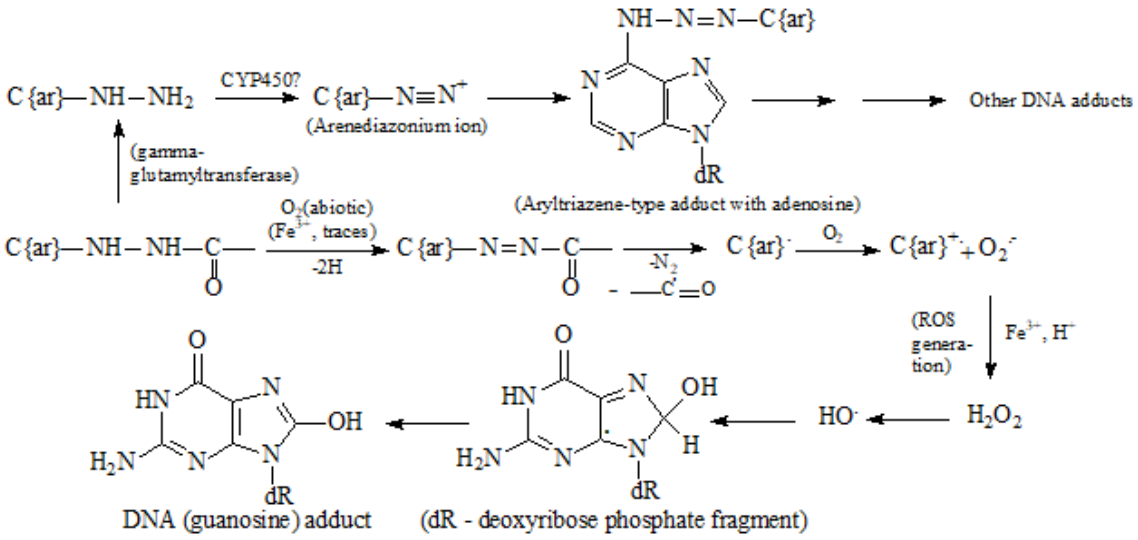
(Deoxycytidine)
(dR - deoxyribose phosphate fragment)
(Y can be alkyl, aryl or -H)

On the basis of the available literature data, the following generalized scheme is likely to operate *via* radical mechanism by ROS formation [5, 6, 7 - 9]:



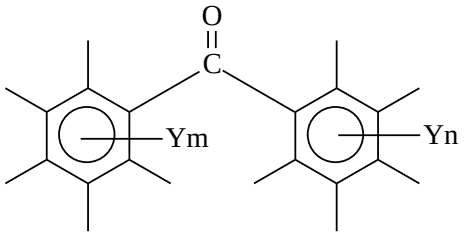
DNA (guanosine) adduct (dR - deoxyribose phosphate fragment)

Based on the established abiotic oxidative consumption of agaritine and structurally similar chemicals, the following mechanistic scheme for the explanation of its mutagenicity can be expertly suggested:

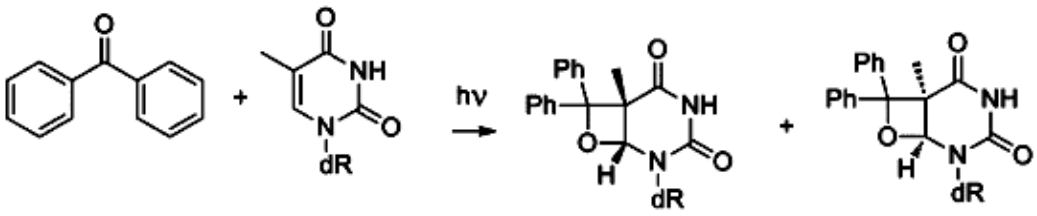


Set of chemicals used for profile development	Hydrazine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Phenylhydrazine, ICPS Inchem, Concise International Chemical Assessment Document 19; http://www.inchem.org/documents/cicads/cicads/cicad_19.htm#PartNumber:7 Last visited: June, 2021. 2. Parodi, S., Canc. Res. **41** (1981), 1469 – 1482. 3. Gilbert, W., *DNA Sequencing and Gene Structure*, Nobel Lecture, 8 December 1980; DOI: 10.1007/bf01116186. 4. Cashmore, A. R., Nucleic Acids Research **5**(7) (1978), 2485 – 2491. 5. Kalgutkar, A. S., Current Drug Metabol. **6** (2005), 161 – 225. 6. Kovacic, P., Current Med. Chem. **8** (2001), 773 – 796. 7. Rumyantseva, G., J. Biol. Chem. **266**(32) (1991), 21422 – 21427. 8. Quintero, B., Ars Pharmaceutica **41**(1) (2000), 27 – 46. 9. Gannet, P. M., Chem. Biol. Interact. **80**(1) (1991), 57 – 72. 10. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=86-54-4. Last visited: June, 2021. 11. Friedrich, U., Z. Lebensm. Unters Forsch **183** (1986), 85 – 89. 12. Walton, K., Carcinog. **18**(8) (1997), 1603 – 1608. 13. Hajslova, H., Food Additives and Contaminants, 19(11) (2002), 1028 – 1033. 14. Sinha, B. K., J. Drug Metabol. & Toxicol. **5**(2) (2014), 1 – 6.

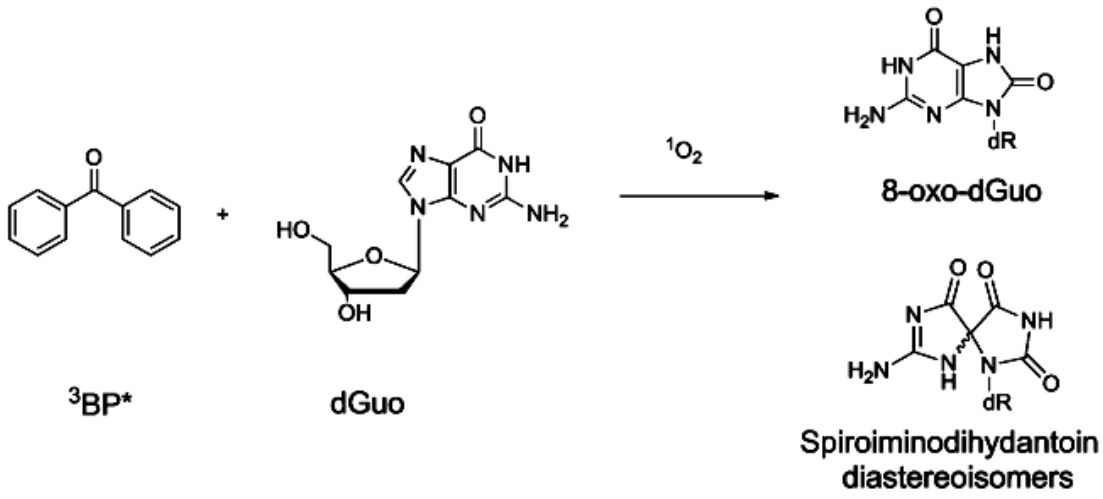
Individual profile/alert	
Name	Hydroxybenzophenone Derivatives
Type of profile	Structural alert

Description/applicability domain	 (Y is -H, -OH, -OR, R (R is CnH2n+1 (n = 1 or more), -Cl, -F, -NO2, -COOH, -OC(O)-, -S(O2)O, etc.)
Mechanism	DNA intercalation; : [2+2] photoinduced AN2-type cycloaddition; ROS generation

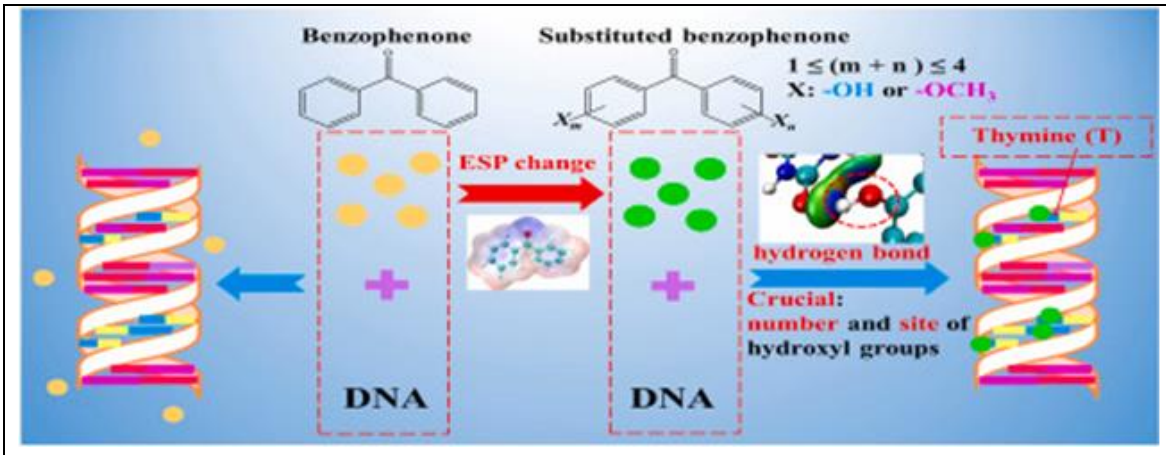
• Carbonyl compounds may react with olefins through a [2+2] AN2-type photocycloaddition giving rise to oxetane derivatives. Thus upon irradiation of Benzophenone (BP) in the presence of thymidine DNA nucleobase (Thd), two stereoisomeric oxetane derivatives have been isolated:



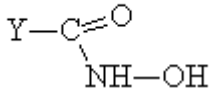
A photosensitizer in its triplet excited state may interact with molecular oxygen, generating singlet oxygen 1O2, which is a very potent oxidizing agent. This is the case for BP; it produces 1O2 which in turn reacts with guanine yielding spiroiminodihydantoin diastereoisomers or 8-oxo-deoxyguanosine (Guo), as double stranded DNA and DNA lesions:



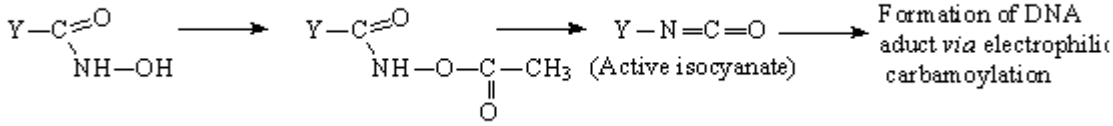
The following scheme of interaction of BPs with DNA has been finally proposed:



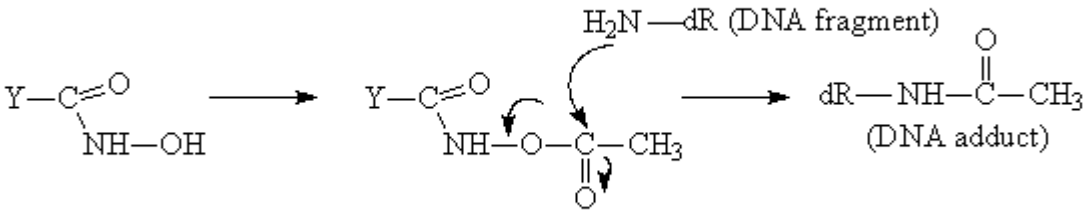
Set of chemicals used for profile development	Hydroxybenzophenone Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Wang, W.-Q., H.-X. Duan, Zh.-T. Pei, R.-R.- Xu, Ze-T. Qin, G.-C. Zhu, Li-W. Sun, Evaluation by the Ames Assay of the Mutagenicity of UV Filters Using Benzophenone and Benzophenone-1, Int. J. Environ. Res. Public Health 15 (2018), 1907; doi:10.3390/ijerph15091907. 2. Addendum to the Final Report on the Safety Assessment of Benzophenones-1, -3, -4, -5, -9, and -11 to Include Benzophenones-2, -6, and -8, JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOCY Volume 2, Number 5, 1983 Mary Ann Liebert, Inc., Publishers; https://journals.sagepub.com/doi/pdf/10.3109/10915818309140715. 3. Robinson, St. H., M. R. Odio, E. D. Thompson, M. Aardema, A. Kraus, Assessment of the In Vivo Genotoxicity of 2-Hydroxy 4-Methoxybenzophenone, Environ. Mol. Mutagenesis 23 (1994), 312 – 317. 4. Zhang, J., Zh. T. Pei, Ya-Ni Zhao, M. Zhang, L.-L. Zhang, W.-Q. Wang, J.-Ya Wu, R. Yu, L.-W. Sun, Mutagenicity evaluation to UV filters of benzophenone-6, benzophenone-8, and 4- methylbenzylidene camphor by Ames test, PLoS ONE 16(9) (2021); e0255504. https://doi.org/10.1371/journal.pone.0255504). 5. Cuquerella, M. C., V. L.-Vallet, J. Cadet, M. A. Miranda, Benzophenone Photosensitized DNA Damage, Acc. Chem. Res. 45(9) (2012), 1558 – 1570; doi: 10.1021/ar300054e. Epub 2012 Jun 14. 6. Ma, J., Ch. Qin, M. G. Waigi, Y. Gao, X. Hu, A. Mosa, W. Ling, Functional group substitutions influence the binding of benzophenone-type UV filters with DNA, Chemosphere 299 (2022), 134490; https://doi.org/10.1016/j.chemosphere.2022.134490. 7. Wang, H., Y. Xiao, Z. Xie, H. Sun, J. Wang, R. Huang, 2-Hydroxybenzophenone Derivatives: ESIPT Fluorophores Based on Switchable Intramolecular Hydrogen Bonds and Excitation Energy–Dependent Emission, Frontiers in Chemistry, 2021 Oct 19; 9:766179 doi: 10.3389/fchem.2021.766179. eCollection 2021.

Individual profile/alert	
Name	Hydroxamic Acids
Type of profile	Structural alert
Description/applicability domain	 (Y can be C, N or C-O-)
Mechanism	A_N2 Carbamoylation after isocyanate formation and S_N2 Acylation

A number of pyridine and quinoline carbohydroxamic acids have been tested for mutagenicity on *Salmonella typhimurium* strains TA100 and TA98. According to the authors, the mechanism for the mutagenicity of hydroxamic acids is associated with the so-called *Lossen rearrangement* of the acid conjugates produced by enzymatic acylation of the hydroxamic acids, followed by carbamoylation of the target (DNA) molecule by the resulting isocyanate [4].

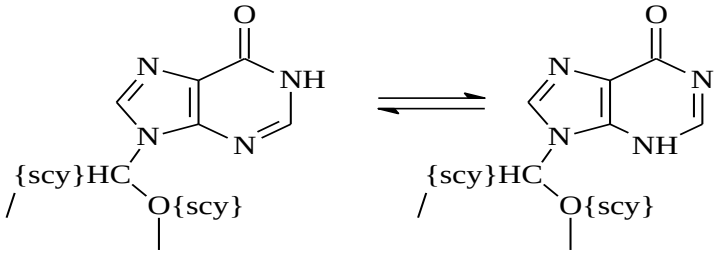


Another possible mechanism may involve enzymatic activation (O-acylation) and subsequent acylation reaction with DNA for acetohydroxamic acid derivatives (Y is alkyl, O-alkyl or N-alkyl) [5]:

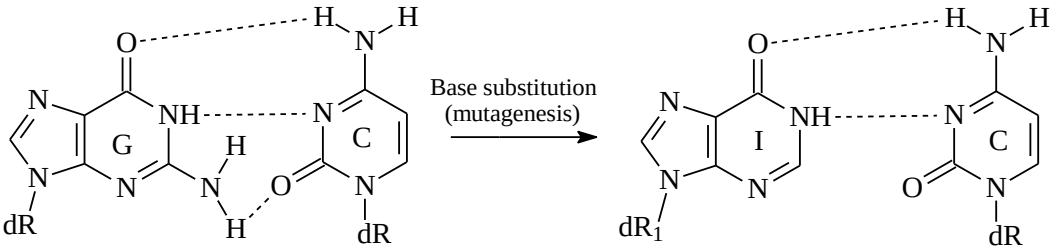


Set of chemicals used for profile development	Hydroxamic Acids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Wang, Mutat. Res. **56** (1977) 7 – 12. 2. Wang, Antimicr. Agents Chemother. **11**(4) (1977), 753 – 755. 3. Skipper, Canc. Res. **40** (1980), 4704 – 4708. 4. Kochany, Mutat. Res. **135** (1984), 139 – 148. 5. Enoch, Mutat. Res. **743** (2012) 10 – 19.

Individual profile/alert

Name	Hypoxanthine Derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Non-Covalent Interactions with DNA Base Substitution

Inosine is a nucleoside that is formed when hypoxanthine is attached to a ribose ring (also known as a ribofuranose) via a β-N9-glycosidic bond. In some publications, inosine-induced mutations have been reported. Inosine can be formed by oxidative deamination of adenosine, which represents spontaneous mutation process. It was reported that, in the first round of DNA replication, DNA polymerase enzyme recognized inosine as the structurally similar guanosine, which initiated the mutation process [1]. The substitution of guanine with inosine base results in 17-fold increase of transient base pairs, which is accompanied by loss of the hydrogen bonds in the DNA double strand and mutations [2]. This can be illustrated by the following simplified mechanistic scheme (Scheme 1 below):

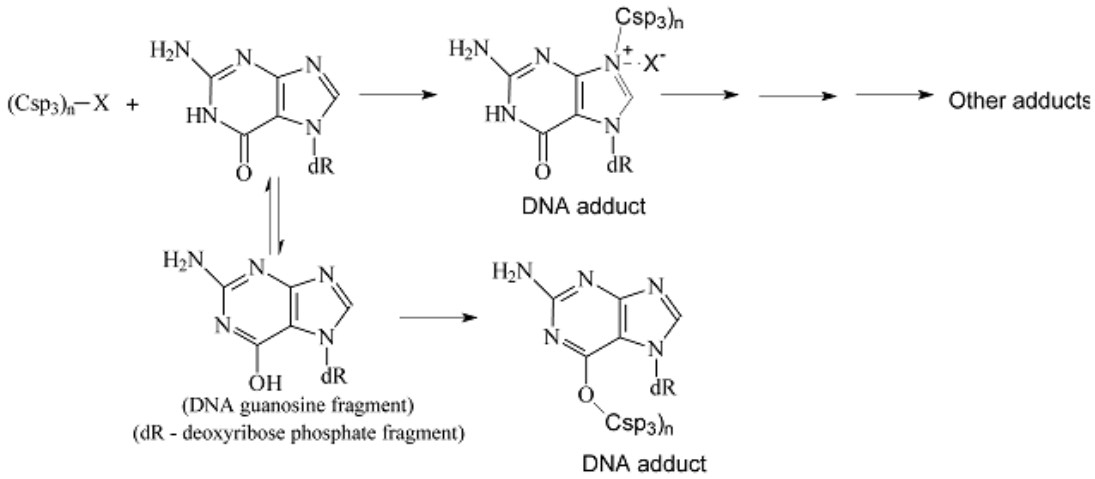


(G: guanine; C: cytosine; I: inosine; dR: deoxyribose fragment; dR_1: ribose fragment)

Set of chemicals used for profile development	Hypoxanthine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Nordmann, P., J. C. Makris, W. S. Reznikoff, Inosine Induced Mutations, Mol. Gen. Genet. 214 (1988), 62 – 67. 2. Nikolova, E. N., Fr. Stull, H. M. Al-Hashimi, Guanine to Inosine Substitution Leads to Large Increases in the Population of a Transient G·C Hoogsteen Base Pair, Biochem. 53 (2014), 7145−7147.

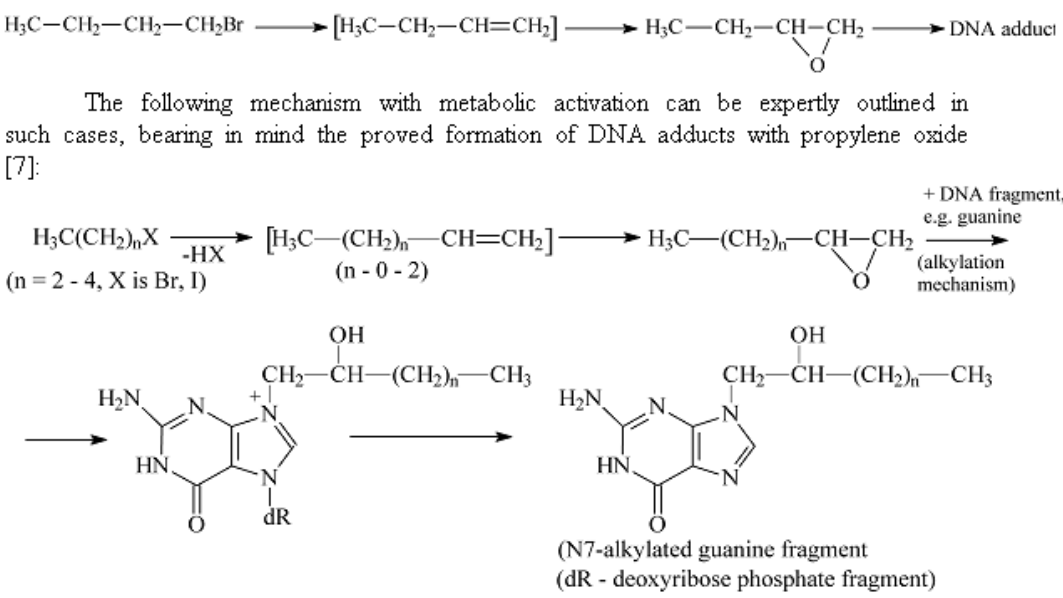
Individual profile/alert	
Name	Monohaloalkanes
Type of profile	Structural alert
Description/applicability domain	$(C(sp^3)(acy))_n$-X(n=1-4; X=-Cl, -Br, -I)
Mechanism	S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom, S_N1 Nucleophilic substitution after carbenium ion formation and S_N2 Alkylation by epoxide metabolically formed after E2 reaction

Direct-Acting Mutagens – DNA alkylation in Scheme 1:



Scheme 1

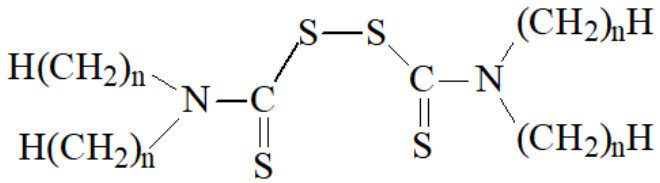
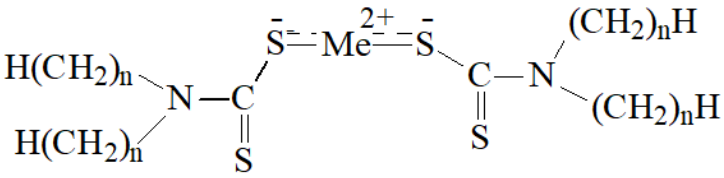
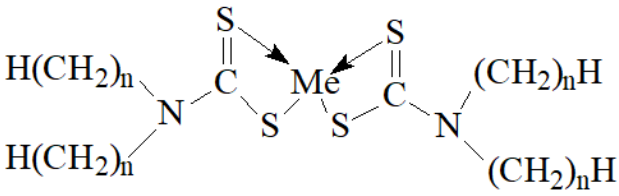
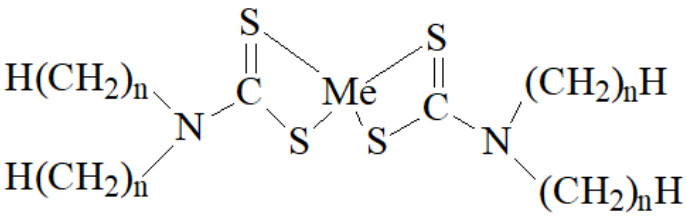
Metabolic Activation (Bioactivation) (Exogenous S9 System Added) in Scheme 2:

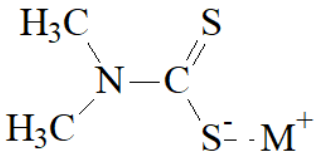
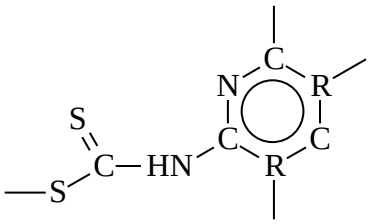
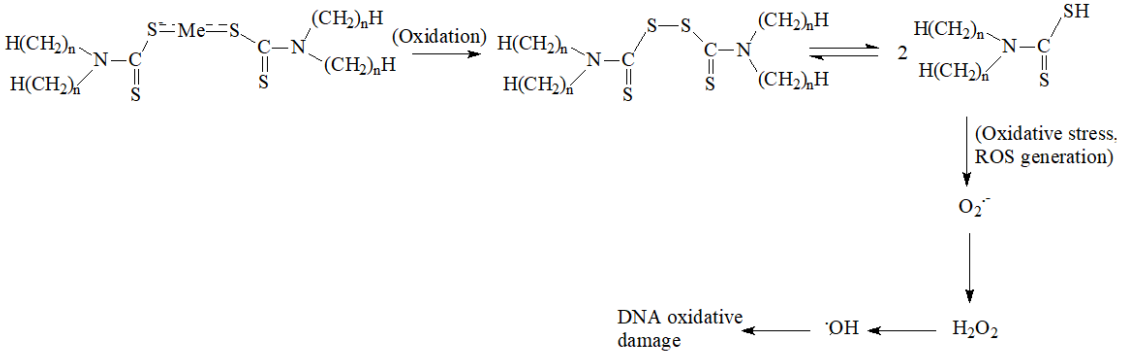


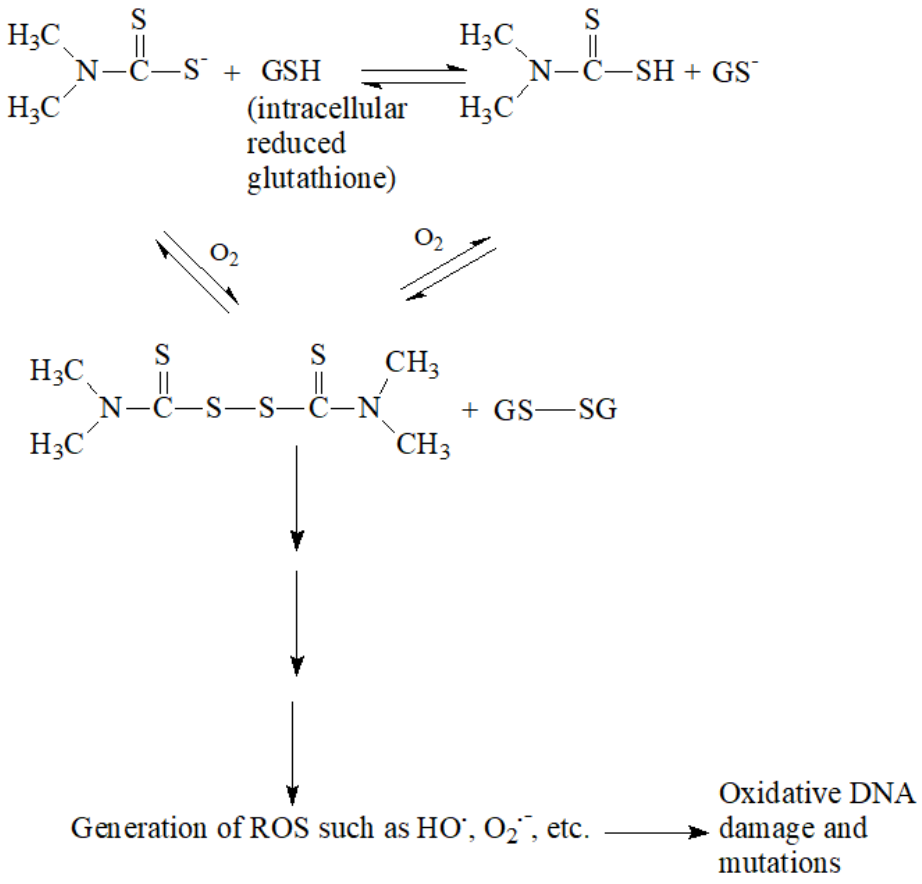
Scheme 2

Set of chemicals used for profile development	Monohaloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Woo, Environ. Health Persp. **110** (2002), 75 – 87. 2. Ballering, Mutagenesis **9**(4) (1994), 387 – 389; DOI: 10.1093/mutage/9.4.387. 3. *Toxicology and Carcinogenesis Studies of Bromoethane (Ethyl Bromide) (CAS No. 74-96-4) in F344/N Rats and B6C3F₁ Mice (Inhalation Studies)*, NTP Technical Report Series No. 363, US Department of Health and Human Services, Public Health Service, National Institute of Health, October 1989.

	4. Guengerich, Jap. J. Toxicol. Environ. Health **43**(2) (1997), 69-82; http://sc.chat-shuffle.net/paper/uid:110003642293. Last visited: June, 2021. 5. Warwick, Canc. Res. **23** (1963), 1315 -1333. 6. Sobol, Z., M. E. Emgel, E. Rubitski, W. W. Ku, J. Aubrecht, R. H. Schiestl, Genotoxicity Profiles of Commion Alkyl Halides and Esters with Alkylating Capability, Mutat. Res. 633 (2007), 80 – 94. 7. Solomon, Environ. Health Persp. **81** (1989), 19 – 22. 8. Strubel, Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128.

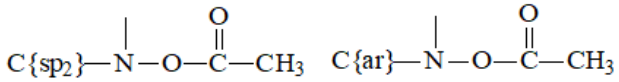
Individual profile/alert	
Name	N,N-Dialkyldithiocarbamate Derivatives
Type of profile	Structural alert
Description/applicability domain	  or  or  (n = 1, 2; Me^{2+} can be Zn^{2+}, Cd^{2+}, Cu^{2+} or Pb^{2+} or Me can be Zn, Cd(II), Cu(II) or Pb(II)

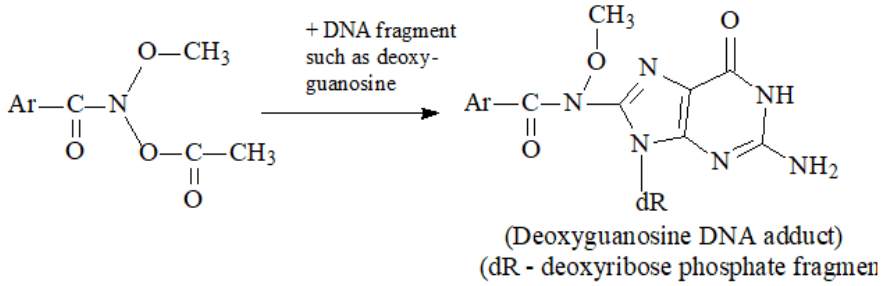
	(depending on the structural representation of metal complexes))  (M^+ can be Na^+, K^+, Li^+)  (R is Het_1: can be N or C-atom)
Mechanism	Radical ROS generation
 Mutagenicity of tetramethylthiuram disulfide (thiram), which can be obtained by mild oxidation of dimethyldithiocarbamate has been experimentally proved for both frameshift and base-substitution sensitive strains of Salmonella typhimurium. The following reversible equilibrium and redox cycling effects seem to be established for the interaction of sodium dimethyldithiocarbamate with endogenous (intracellular) thiols such as glutathione under biological conditions:	

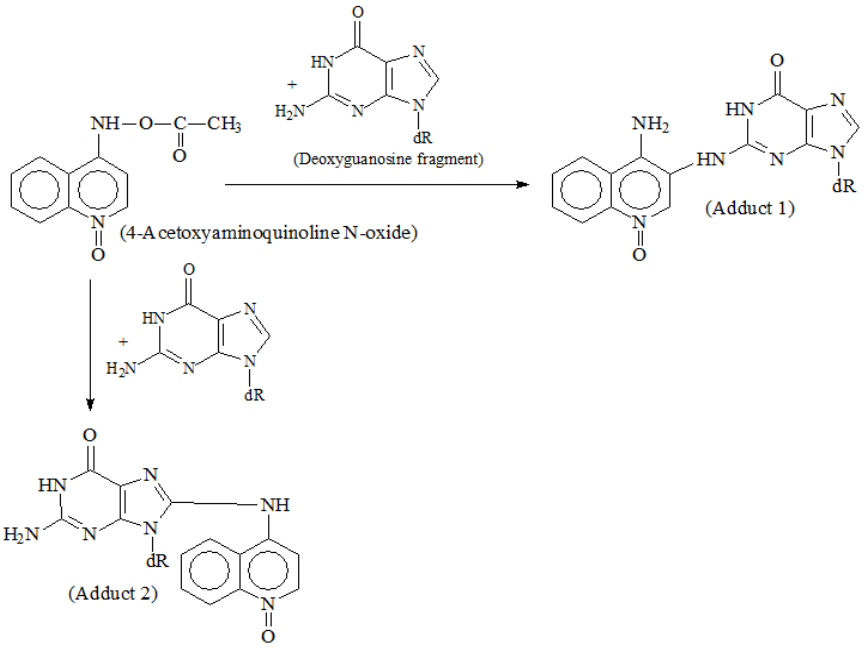


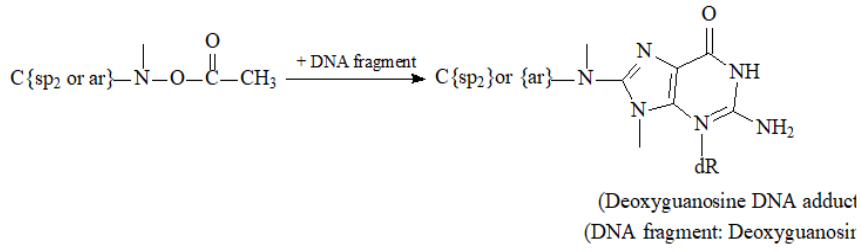
Set of chemicals used for profile development	N,N-Dialkyldithiocarbamate Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Rannug, Chem. Biol. Interact. **49**(3), (1984), 329 - 340. 2. Franekic, Mutat. Res. **325**(2 - 3), (1994), 65 - 74. 3. Hedenstedt, Mutat. Res. **68**(4), (1979), 313 - 325. 4. Wild, Biochem. J. **338** (1999), 659 - 665. 5. Johnson, Toxicol. Sci **76**, (2003), 65 - 74. 6. Grebelli, Mutag. **12** (1992), 97 – 112. 7. Moriya, Mutat. Res./Environmental Mutagenesis and Related Subjects **54**(2) (1978), 221. 8. Staron, Arch. Toxicol. **86** (2112), 1841 – 1850. 9. CCRIS: *Sodium Dimethyldithiocarbamate RN 128-04-1*, Toxicology Data Network, U.S. National Library of Medicine**.** 10. *Test Plan Sodium Dimethyldithiocarbamate CAS Registry Number 128-04-1* Rubber and Plastic Additives Panel American Chemistry Council December 2003;

Individual profile/alert	
Name	N-Acetoxyamines
Type of profile	Structural alert

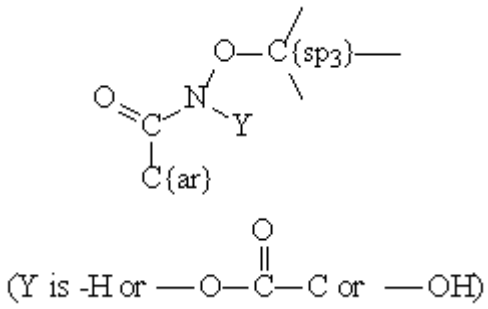
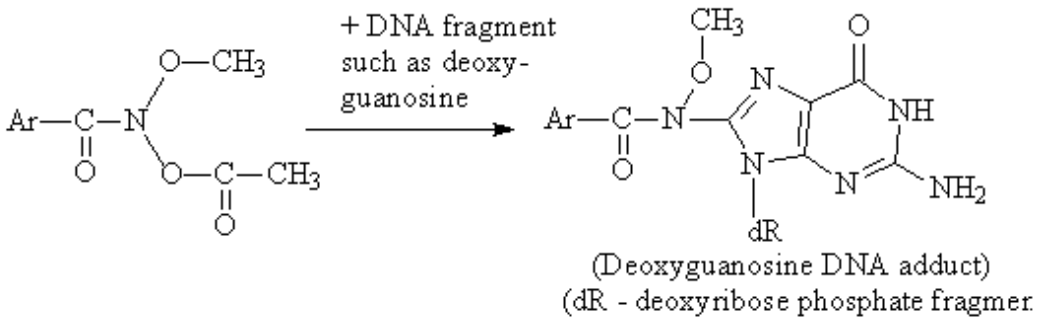
Description/applicability domain	
Mechanism	S_N2 reaction on a nitrogen-atom bound to a good leaving group

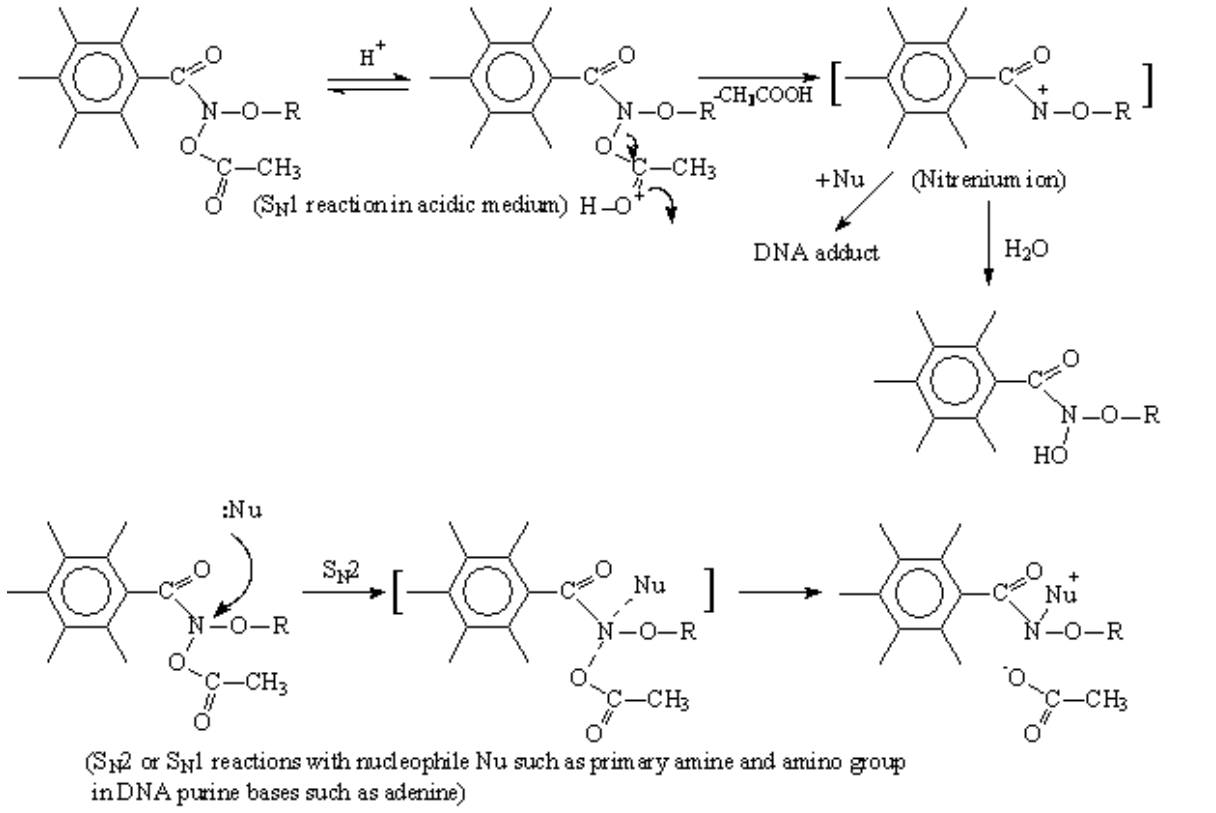




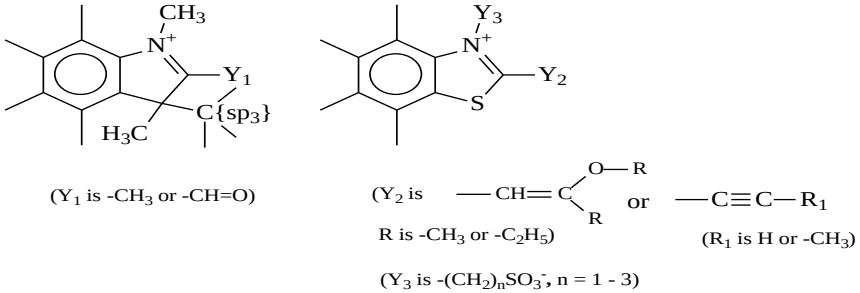


Set of chemicals used for profile development	N-Acetoxyamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Glatt, H., Carcinogenesis **25**(5) (2004), 779 – 786. 2. Banks, T. M., Org. Biomolec. Chem. **1**(13) (2003), 2238 – 2246. 3. Zoultina, S. G., Canc. Res. **45** (1985), 520 – 525.

Individual profile/alert	
Name	N-Acyloxy(Alkoxy) Arenamides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	S_N2 or S_N1 reaction at nitrogen-atom bound to a good leaving group or on nitrenium ion
	



Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Glatt, Carcinogenesis **25**(5) (2004), 779 – 786. 2. Banks, Org. Biomolec. Chem. **1**(13) (2003), 2238 – 2246. 3. Bonin, Mutat. Res. **494** (2001), 115 – 134.

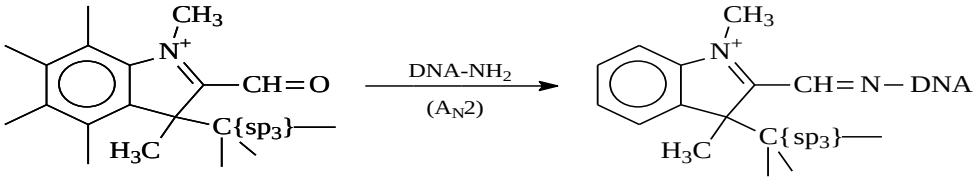
Individual profile/alert	
Name	N-Alkylindolinium and N-Alkylbenzothiazolium Salts
Type of profile	Structural alert
Description/applicability domain	
Mechanism	AN2 Schiff base formation AN2 Nucleophilic addition to activated double bond Non-covalent interactions DNA intercalation
There are very few published data on the toxicity of chemicals, belonging to this sub-class. 2-Alkylindolinium salts were regarded as activated carbon species used for the synthesis of cyanine dyes [1]. N-Alkylindolinium structural fragments have been synthetically introduced into the	

chemical structure of organic fluorophores used for molecular imaging for cancer diagnostics [2]. N-Propargyl-2-alkynylbenzothiazolium derivatives were proved to interact with DNA via AN2-type mechanism causing DNA strand breaks and cleavage [3].

Both target chemicals possess positively charged tetraalkylammonium-type nitrogen atom, which acts as electron-withdrawing moiety along the conjugated double bonds. This is believed to enhance the electrophilic reactivity of the side acyclic double bonds. In the first case (Chemical 1, Table 1), the formation of mutagenic DNA adduct would occur via AN2-type Schiff base formation, which is facilitated by the positively charged nitrogen. For the second chemical (Chemical 2, Table 1), the side C=C bond could be also activated towards AN2 attack for the same reason. The same type of AN2-mechanism applies to the side acyclic C-C-triple bond conjugated with the positively charged cyclic C=N+-bond for the same case. On the other hand, with the polycyclic zwitterion structure of the highly polar target Chemical 2 (Table 1), the process of DNA non-covalent intercalation may occur easily, which could also result in mutagenicity effects.

Therefore, despite the obvious lack of relevant information, the following simplified mechanistic schemes can be expertly proposed:

Mechanistic scheme A:

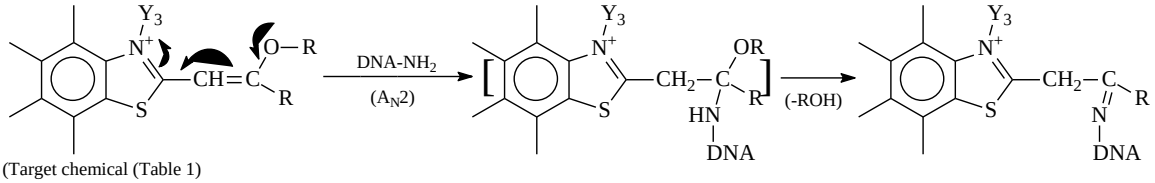


(Assumed active metabolite of the target chemical (Table 1)

DNA-NH_2: purine/pyrimidine nucleobase with exocyclic -NH_2 groups)

(Expertly assumed DNA adduct, possibly eliciting mutagenicity)

Mechanistic scheme B:



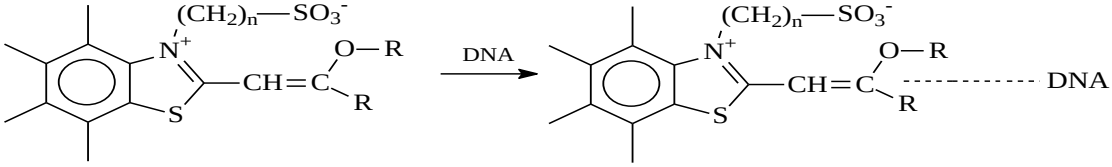
(Target chemical (Table 1)

R is -CH_3 or -C_2H_5)

(Y_3 is -$(CH_2)_nSO_3^-$, n = 1 - 3)

(Expertly assumed DNA adduct, possibly eliciting mutagenicity)

Mechanistic scheme C:

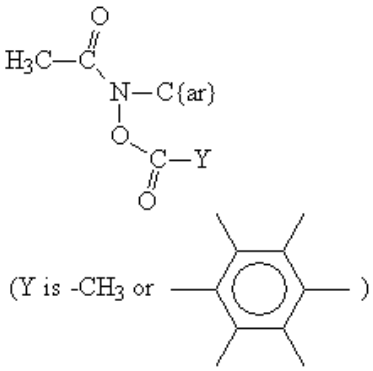


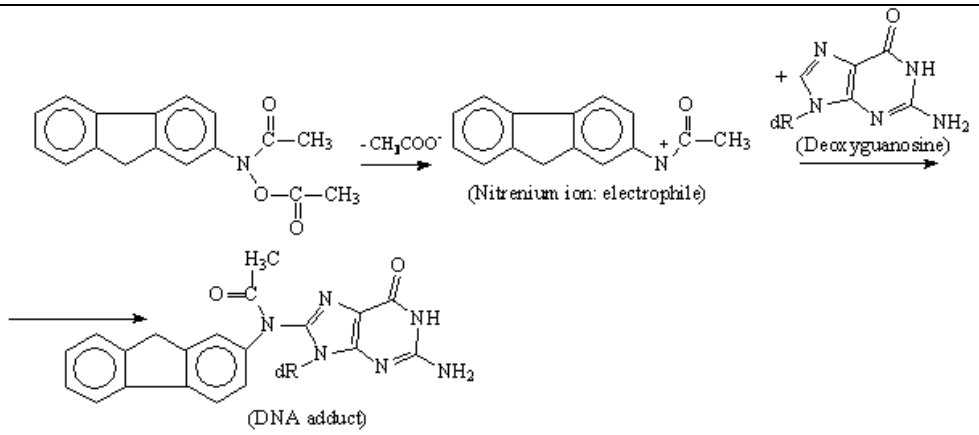
R is -CH_3 or -C_2H_5)
(Target chemical (Table 1)

(Expertly assumed DNA intercalative adduct: non-covalent)

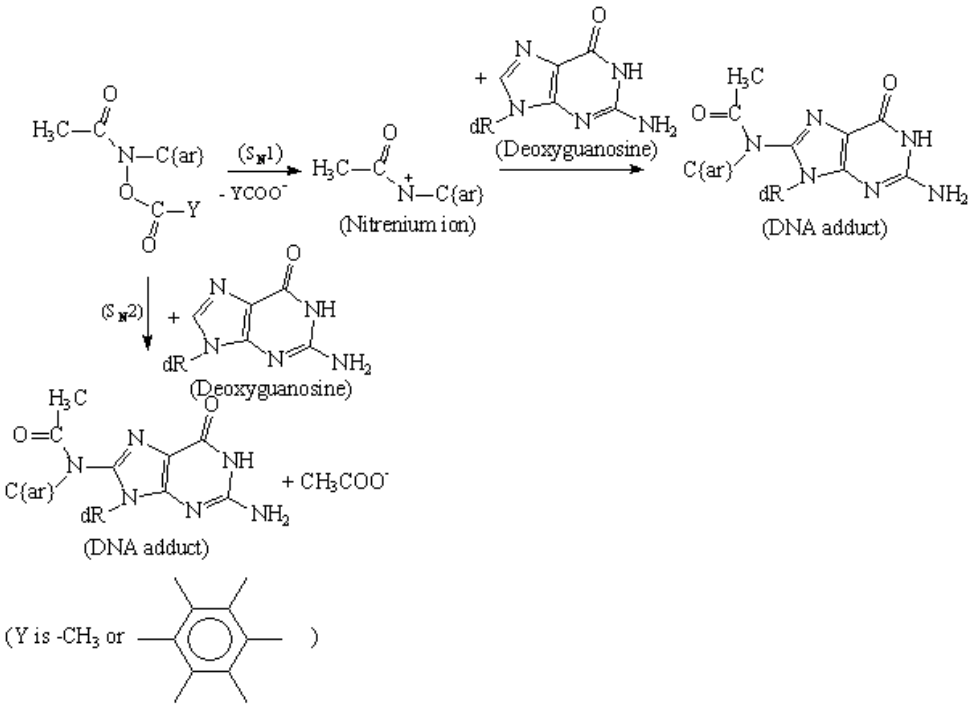
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Lavis, L. D., R. T. Rines, Bright Building Blocks for Chemical Biology, ACS Chem. Biol. 9 (2014), 855 – 866; dx.doi.org/10.1021/cb500078u. 2. Alford, R., H. M. Simpson, J. Duberman, G. Graig Hill, M. Ogawa, C. Regino, H. Kobayashi, P. L. Choyke, Toxicity of Organic Fluorophores Used in Molecular Imaging: Literature Review,

	Molecular Imaging, 8(6) (2009), 341 – 354. 3. Kumar, D., W. M. David, S. M. Kedrwin, N-Propargyl-2-alkynylbenzothiazolium Aza-enediynes: Role of the 2-Alkynylbenzothiazolium Functionality in DNA Cleavage, Bioorg. Med. Chem. Lett. 11 (2001), 2971–2974.

Individual profile/alert	
Name	N-Aryl-N-Acetoxy(Benzoyloxy) Acetamides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	S_N2 or S_N1 reaction at nitrogen atom bound to a good leaving group or on nitrenium ion
The lipid-soluble N-acetoxy and N-benzoyloxy-derivatives of the compound N-2-fluorenylacetamide as well as the N-benzoyloxy derivative of N-methyl-4-aminoazobenzene, and the N-acetoxy derivatives of N-4-stilbenylacetamide , N-4-biphenylylacetamide, and N-2-phenanthrylacetamide are each more carcinogenic at the sites of subcutaneous injection than the correspondinng parent compounds. These acetoxyesters are also much more reactive with nucleophiles such as nitrogen atoms in DNA bases than the corresponding N-hydroxylamine precursors. The nature of the aryl group, however, has a pronounced effect on both the reactivity and carcinogenicity of the hydroxamic acids and their esters. In the presence of nucleophiles that are less basic than acetate ion, the 2-fluorenyl and 4-stilbenyl-N-acetoxyacetamides reacted *via* unimolecular ionization (S_N1 mechanism), and the initial attack on the DNA bases occurs at their nitrogen atoms, followed by rearrangement. The unimolecular mechanistic scheme is shown below in Scheme 1 [1]:	

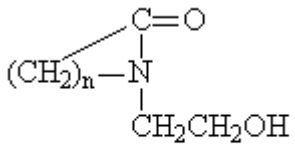


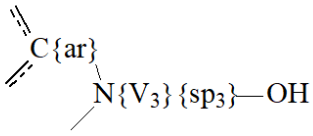
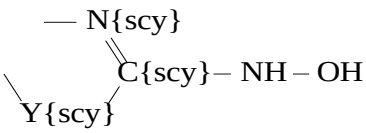
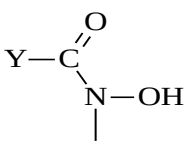
The general scheme for such interactions with DNA fragments could be outlined as follows:

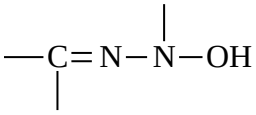


Scheme 1

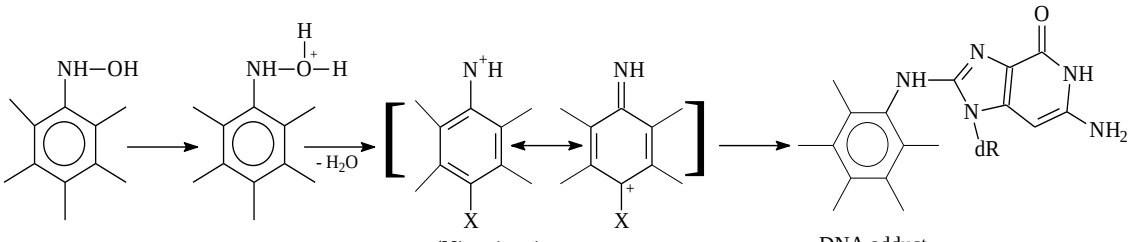
Set of chemicals used for profile development	N-Aryl-N-Acetoxy(Benzoyloxy) Acetamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Scribner, Canc. Res. **30** (1970), 1570 – 1579. 2. Swaminathan, Canc. Res. **52** (1992), 3286 – 3294.
Individual profile/alert	
Name	N-Hydroxyethyl Lactams
Type of profile	Structural alert

Description/applicability domain	 (n = 2 – 4)
Mechanism	Non-covalent interactions DNA intercalation
Positive *in vitro* bacterial mutagenicity test results with *Salmonella typhimurium* strains TA100 and TA1535 were reported for 1-(2-Hydroxyethyl)-2-pyrrolidinone as parent chemical. The chemical is probably frameshift mutagen [1]. According to one publication, the oxopyrrolidine derivatives may interact with DNA as one of their possible mechanisms of action. For example, hydrogen bonds might be formed among the base pairs of DNA (adenine, guanine, cytosine and thymine), the free carbonyl group, and the nitrogen atom of oxopyrrolidine ring [2].	
Set of chemicals used for profile development	**Not appliciable** – all chemicals are private and can`t be disclosed.
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *2-Pyrrololidinone, 1- (2Hydroxyethyl)-, Full Public Report*, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), 14 February 2005; 2. Ali, Chem. Papers **68**(4) (2014), 540 – 552. 3. Duff, J. Phys. Chem. **B 110** (2006), 20693 – 20701. 4. US Pat. 5124444 (*Lactam-Containing Compositions and Methods Useful for the Extraction of Nucleic Acids* (June 23, 1992).

Individual profile/alert	
Name	N-Hydroxylamines
Type of profile	Structural alert
Description/applicability domain	  (Y is N{V3}{sp3} or S{V2})  (Y is C{ar} or C{sp2} or N{V3}{sp3})

	
Mechanism	S_N1 Nucleophilic attack after nitrenium ion formation, Radical ROS formation after GSH depletion (indirect), S_N2 Acylation & A_N2 Carbamoylation after isocyanate formation

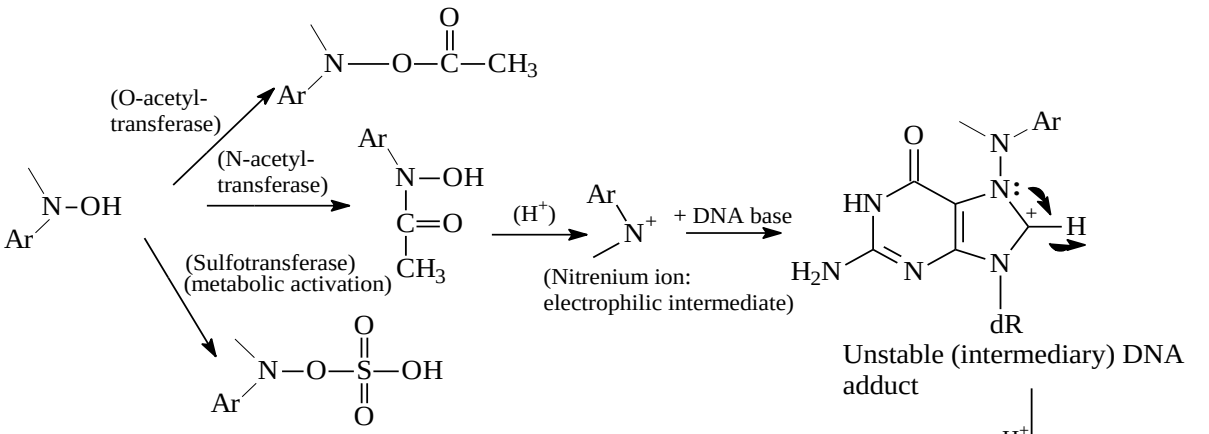
1. Aromatic and Heterocyclic N-Hydroxylamines

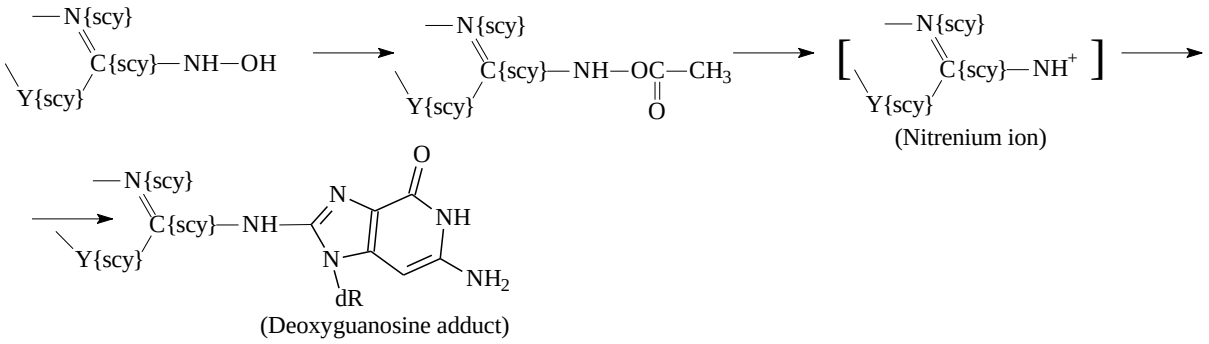


(Nitrenium ion: resonance stabilization)

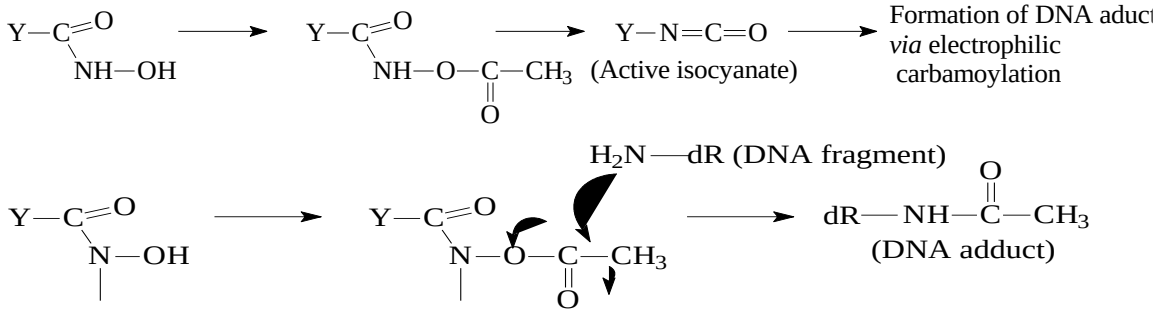
DNA adduct

(dR - deoxyribose phosphate fragment in deoxyguanosine)

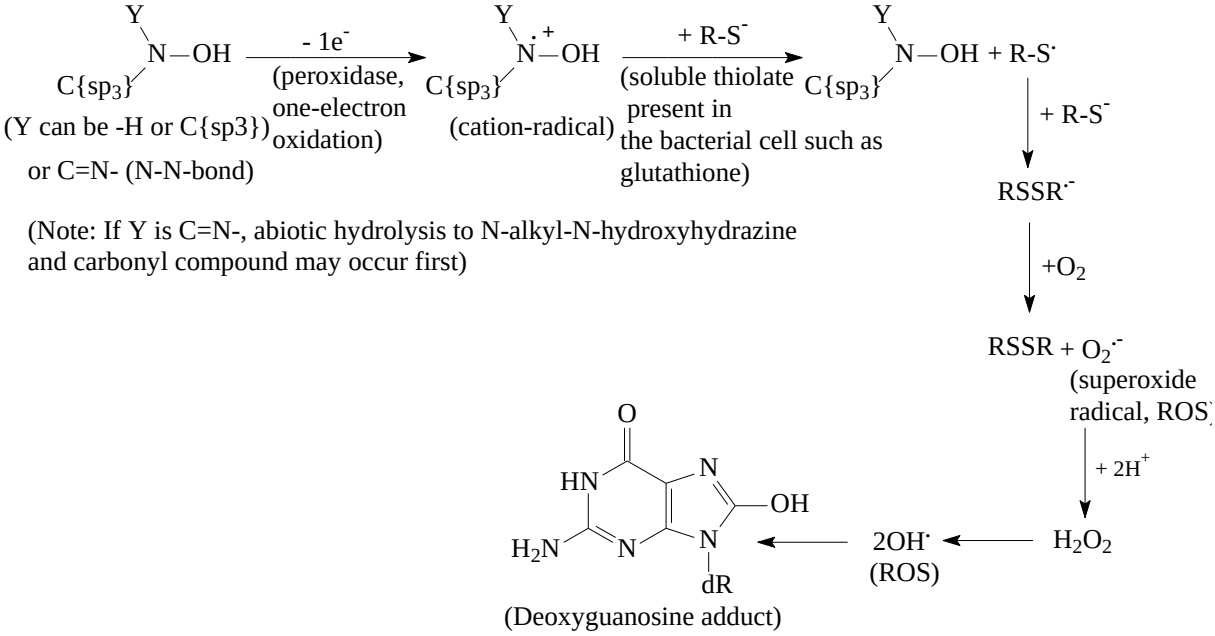




2 Hydroxamic Acids



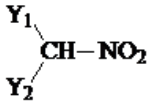
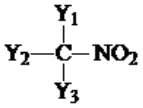
3. Other Non-Aromatic N-Hydroxylamines



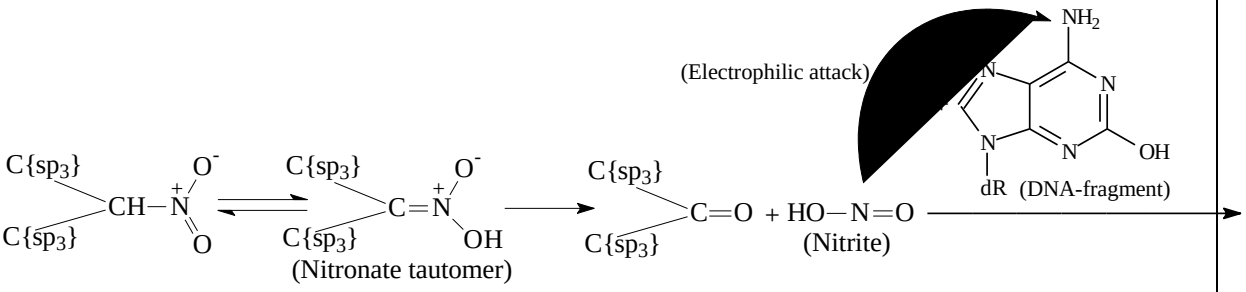
(Note: If Y is C=N-, abiotic hydrolysis to N-alkyl-N-hydroxyhydrazine and carbonyl compound may occur first)

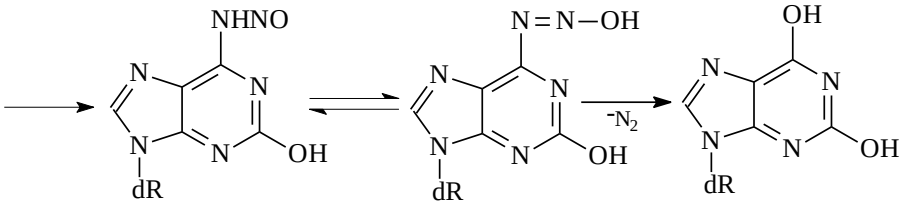
Set of chemicals used for profile development	N-Hydroxylamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Nitrenium Ions*; https://www.wikidoc.org/index.php/Nitrenium_ion. Last visited: June, 2021. 2. Schut, H. A. J., Carcinog. 20, (3) (1999), 353 – 368. 3. Kalgutkar, A. S., Curr. Drug Metabol. **6**(3), 2005, 161 – 225). 4. Saito, K., Arch. Biochem. Biophys. **239**(1) (1985), 286 – 295. 5. Glatt, H., Carcinog. **25**(5) (2004), 779 – 786. 6. Mushtaq, A., J. Biol. Chem. **277**(14) (2002), 12175 – 12181. 7. Chung, K. T., Mutat. Res. **387** (1997), 1 – 16. 8. You, Z., Mutat. Res. **319** (1993), 19 – 30. 9. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/ Last visited: June, 2021. 10. Kato, R., Environ. Health Persp. **49** (1983), 21 – 25. 11. Barnes, W. S., Carcinog. **6**(3) (1985), 441 – 444. 12. Jaen, J. C., Eur. J. Med. Chem. **28** (1993), 547 – 553. 13. Herman, A., Carcinogenesis **20** (3) (1999), 353 – 368. 14. Shamovsky, I., JACS **133** (2011), 16168 – 16185. 15. Glatt, H., *Sulfation and Sulfotransferases 4: Bioactivation of*

	Mutagens via Sulfation FASEB J. **11**(5) (1997), 314 – 321. 16. Franke, R., Carcinogenesis **22**(9) (2001), 1561. 17. Beland, FR., Mutat. Res. **376** (1997) 13 – 19. 18. Wang, Ch. Y., Mutat. Res. **56** (1977) 7 – 12. 19. Wang, Ch. Y., Antimicrob. Agents Chemother. **11**(4) (1977), 753 – 755. 20. Skipper, P. L., Canc. Res. **40** (1980), 4704 – 4708. 21. Enoch, S. J., Mutat. Res. **743** (2012) 10 – 19. 22. Pai, V., Mutat. Res. **151** (1985), 201 – 207. 23. *General Discussion of Common Mechanisms for Aromatic Amines*, IARC Monographs, Vol. 99 (2010); ISBN-13 (PDF): 978-92-832-1599-8. http://monographs.iarc.fr/ENG/Monographs/vol99/mono99-6.pdf. Last visited: June, 2021. 24. Spooren, A. M., Molecules and Diseases **26**(4) (2000), 373 – 386. 25. Kono, Y., Arch. Biochem. Biophys. **186**(1) (1978), 189 – 195. 26. Subrahmany, V. V., Chem.-Biol. Interactions **56** (1985), 185 – 199. 27. Makena, P. SEnviron. Molec. Mutagenesis **48** (2007), 404 – 413. 28. NTP Results Report: Results, Status and Publication Information of All NTP Chemicals Produced from Chemtrack System (08/10/00); https://echa.europa.eu/cs/registration-dossier/-/registered-dossier/16982/7/7/2, last visited 09.2019. 29. *3,4-Dichloroaniline*, The MAK Collection for Occupational Health and Safety, 19 June 2013; http://onlinelibrary.wiley.com/doi/10.1002/3527600418.mb9576e4013/pdf, last visited 06.2021.

Individual profile/alert	
Name	Nitroalkanes
Type of profile	Structural alert
Description/applicability domain	Monoalkanes  Y_1- Me or H Y_2- Me or CH_2OH or CH_2COOH Low Molecular weight germinal Polynitroalkanes  Y_1, Y_2, Y_3 can be NO_2(all) or a combination between $–CH_3$, -H, $-NO_2$. The number of NO_2 groups to be more than one.
Mechanism	Nucleophilic substitution after nitrite formation & Radical mechanism *via* ROS formation (indirect)
The following possible scheme for *in vitro* biotransformation can be therefore proposed for secondary	

nitroalkanes has been tested for mutagenic activity in the *Salmonella/mammalian* microsome assay and showed strong *in vitro* genotoxicity. The mutagenicity was independent of an *in vitro* metabolic activation system; therefore, this chemical is regarded as direct-acting mutagen. Tetranitromethane is a potent protein nitrating agent and has been proposed to have role in the deamination of DNA (deamination of cytosine resulting in base mispair). However, there is insufficient information on the precise mechanism of mutagenicity/carcinogenicity of this compound [6, 7]. According to some publications, tetranitromethane is a new type of carcinogen that induces oxidative DNA damage not by itself but *via* modification (nitrosation) of tyrosine residues in proteins, which in turn generates reactive oxygen species (ROS), capable of forming DNA adducts [8].

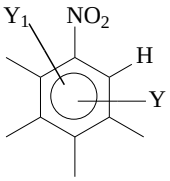




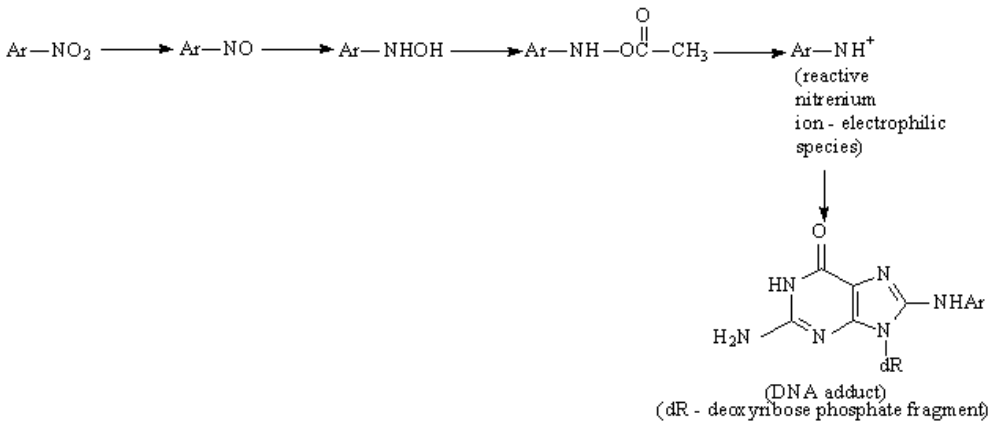
(dR - deoxyribose phosphate fragment)

Set of chemicals used for profile development	Nitroalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Conaway Mutat. Res. **261**(3) (1991), 197 – 207; http://www.ncbi.nlm.nih.gov/pubmed/1719412); DOI: 10.1016/0165-1218(91)90068-w. Last visited: June, 2021. 2. Dayal, R., Fund. Appl. Toxicol. **13**(2) (1989), 341 – 348; http://www.sciencedirect.com/science/article/pii/0272059089902704; DOI: 10.1016/0272-0590(89)90270-4. Last visited: June, 2021. 3. Dalke, C., Toxicol. Lett. **61** (2-3), 1992, pp. 149 – 157. 4. *2-Nitropropane*, International Programme on Chemical Safety, Environmental Health Criteria 138, World Health Organization, Geneva, 1992; www.inchem.org/documents/ehc/ehc/ehc138.htm. Last visited: June, 2021. 5. *Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins. 4. Mechanistic and Other Relevant Data*, IARC Monographs on the Evaluation of Carcinogenic Risk to Humans Vol. 94, 2010, p. 281 (Lyon, France); http://monographs.iarc.fr/ENG/Monographs/vol94/mono94.pdf; ISBN-13 (PDF): 978-92-832-1594-3. Last visited: June, 2021. 6. Wurgler, Mutat. Res. Lett. **244**(1) (1990), 7 – 14. 7. *Toxicology and Carcinogenesis Studies of Tetranitromethane in*

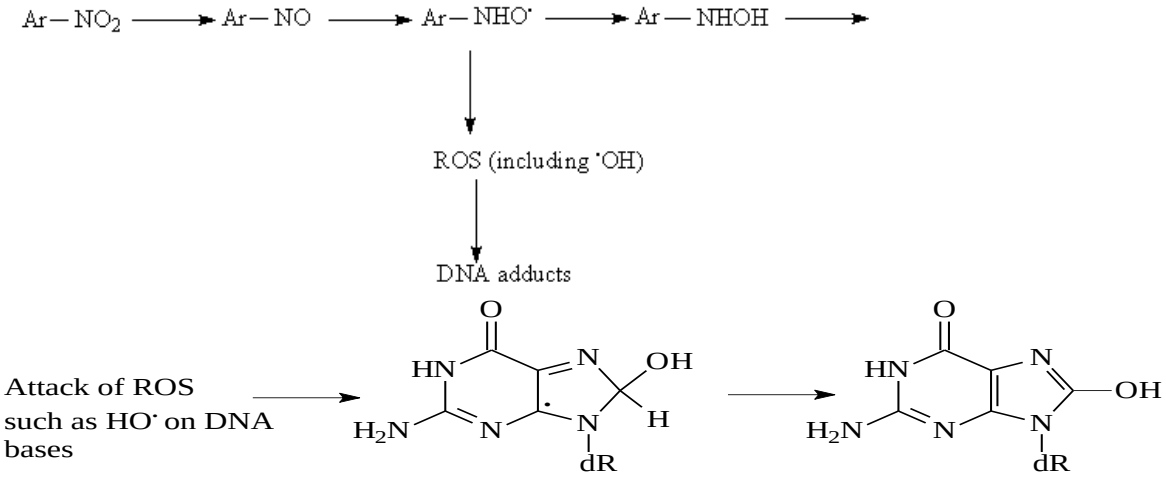
	F344/N Rats and B6C3F1 Mice (Inhalation Studies), NTP Technical Report Series No. 386, March 1990, US Dept. of Health and Human Services, Public Health Service, NIH; http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr386.pdf. Last visited: June, 2021. 8. Murata, M., Chem. Res. Toxicol. **19**(10) (2006), 1379 – 1385. 9. Linhart, I., Chem.-Biol. Interact. **80** (1991), 187 – 210. 10.

Individual profile/alert	
Name	Nitroaniline Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is N{V3}{sp3} (primary, secondary or tertiary amino group) Other substituents (Y_1) that may be present: 1. -NO_2, -NH{sp3}{V3}, -O-C{sp3} (no more than three C{sp3}); -OH, C, -CN, X (Cl, Br) or -H; 2. If hydrocarbon (C-substituent) is present as Y_1 and is C{sp3}, more than one -NO_2 should be available; 3. No more than totally four substituents Y is $N_{(v3)}sp^3$ (Primary, secondary or tertiary amino group) Y_1= NO_2 or $N_{(v3)}Hsp^3$ or $OCsp^3$(3 or less per chain) or OH or C or CN or Cl or Br or H *Note:* In the 2D structures of active fragments, all substituents listed above are attached to phenyl ring *via* their "left" atoms.
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases.(**Nucleophilic attack after reduction and nitrenium ion formation)** **Radical (Homolytic) Mechanism.** This is one of the mechanisms (but not the most important) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks) **(Radical mechanism *via* ROS formation (indirect)**

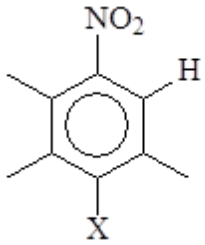
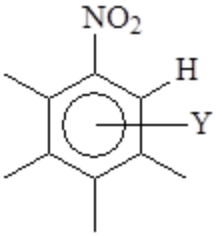
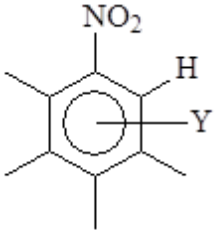
Heterolytic

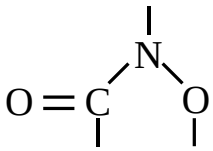
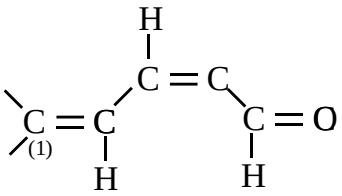


Homolytic

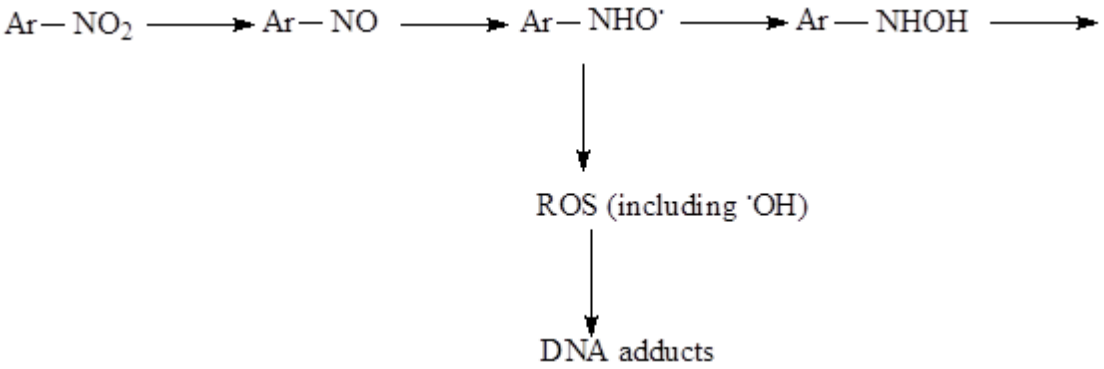


Set of chemicals used for profile development	Nitroaniline Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Sabbioni, G., Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2. Kalgutkar, A. S., Current Drug Metabol. **6** (2005), 161 – 225. 3. Aiub, Cl. A. Fortes, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4. Einisto, P., Mutat. Res. **259** (1991), 95 – 102. 5. Kovacic, P., Current Med. Chem. **8**, (2001), 773 – 796. 6. Witherell, H. L., Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7. Wiseman, H., Biochem. J. 313 (1996), 17 – 29. 8. Purohit, V., Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9. Vance, W. A., Environ. Mutagen, **6** (6) (1984), 797 – 811. 10. Y. Lee, Mol. Cells **19**, No. 1 (2005), 114 – 123 (Abstract); 11. Shimizu, M., Mutat. Res. **170** (1986), 11 – 22. 12. Assmanna, N., Mutat. Res. **395** (1997), 139 – 144. 13. Garner, R. C., Mutat. Res. **44** (1977), 9 – 19. 14. *Opinion on 4-Nitro-o-Phenylenediamine*, Colipa No. 824, Scientific

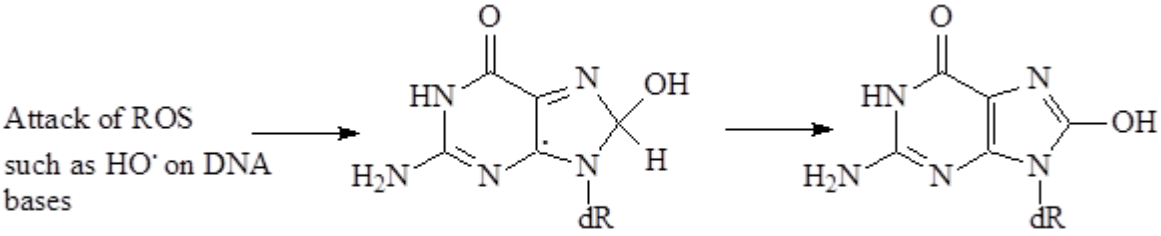
	Committee on Consumer Products, Health&Consumer Protection Directorate-General, EC, December 19, 2006. 15. Chung, K. T., Mutat. Res. **387** (1997), 1 – 16.
Individual profile/alert	
Name	Nitroarenes with Other Active Groups
Type of profile	Structural alert
Description/applicability domain	Halonitroarenes:  (X can be -F, -Cl, -Br, -I; totally no more that four substituents) Nitrobenzyl and Nitrobenzoyl Halides:  (Y can be $-CH_2X$ (X is -Cl, -Br, -F, -I) or —C(=O)X; totally, no more than four substituents) <u>Nitrophenyl Diazonium Salts, Nitrophenyl Triazenes and Other Nitroarenes with Activating Groups</u>:  (Y can be —N=N—N{V3}{sp3}(triazene) or $-N^{+}\equiv N$ (diazonium)); totally no more than four substituents)

	Additional activating substituents Y:  (Note: N, C and O could be atoms in a heterocycle, too)  (attached to the ring via C(1))
Mechanism	A. For the nitro group function: S_N1: Nucleophilic attack after reduction and nitrenium ion formation and Radical: ROS generation (indirect) B. For the alternative active functionalities: S_N2 or S_N1: Nucleophilic attack after diazonium or carbenium ion formation; S_N2 attack on activated carbon Csp3 or Csp2

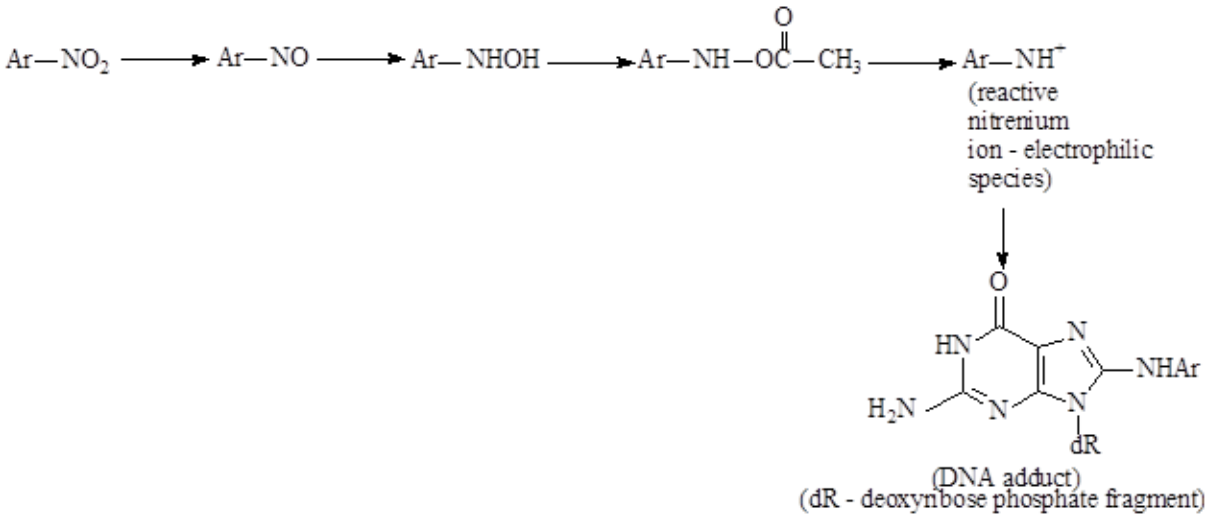
Radical (Homolytic) Mechanism**.** This is one of the mechanisms (*but not the most important*) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds ($ArNO_2$) are implicated in carcinogenesis [5]. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic *Salmonella typhimurium* cell. Several transient *radical intermediates*, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks)**:**



As a result, from the generation of reactive radical species such as $ArNHO^{\cdot}$, an additional formation of ROS such as $O_2^{\cdot-}$ and/or $HO^{\cdot}$ occurs. The hydroxyl radical, for example, is DNA-reactive and adducts, involving pyrimidine and purine nucleoside bases can be formed. The 8-hydroxyguanine adduct is one of the most mutagenic lesions so far discovered, which can induce DNA strands breaks, etc. [6, 7]:



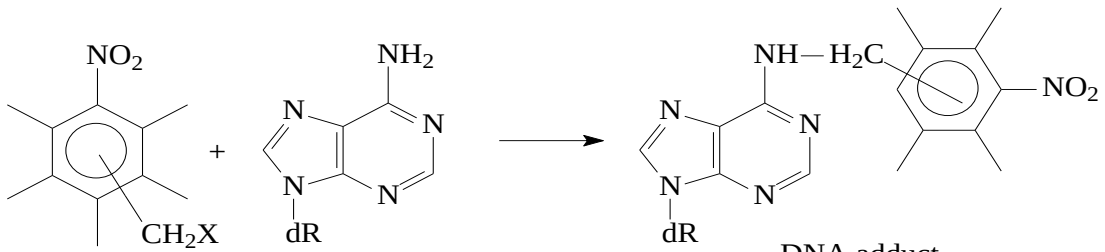
Heterolytic Mechanism. This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases [1, 2, 8]:



Among the isomers of chloronitrobenzenes, only *p*-chloronitrobenzene (4-chloronitrobenzene) showed mutagenicity in *Salmonella typhimurium* when tested in the presence or absence of induced rodent liver S9 [9]. This confirms the importance of *p*-position with respect to the nitro group in eliciting direct mutagenicity through stabilization of electrophilic carbenium ions in the resonance structures, and reduced steric hindrance [10, 11].

Additional chemical mechanistic schemes, other than those associated with nitro group reduction to N-hydroxylamine or generation of ROS (see above) are associated with some nitroarenes, containing other active functionalities and belonging to other classes of *Ames*-positive chemicals involved in the direct mutagenicity effects. Such schemes are outlined below:

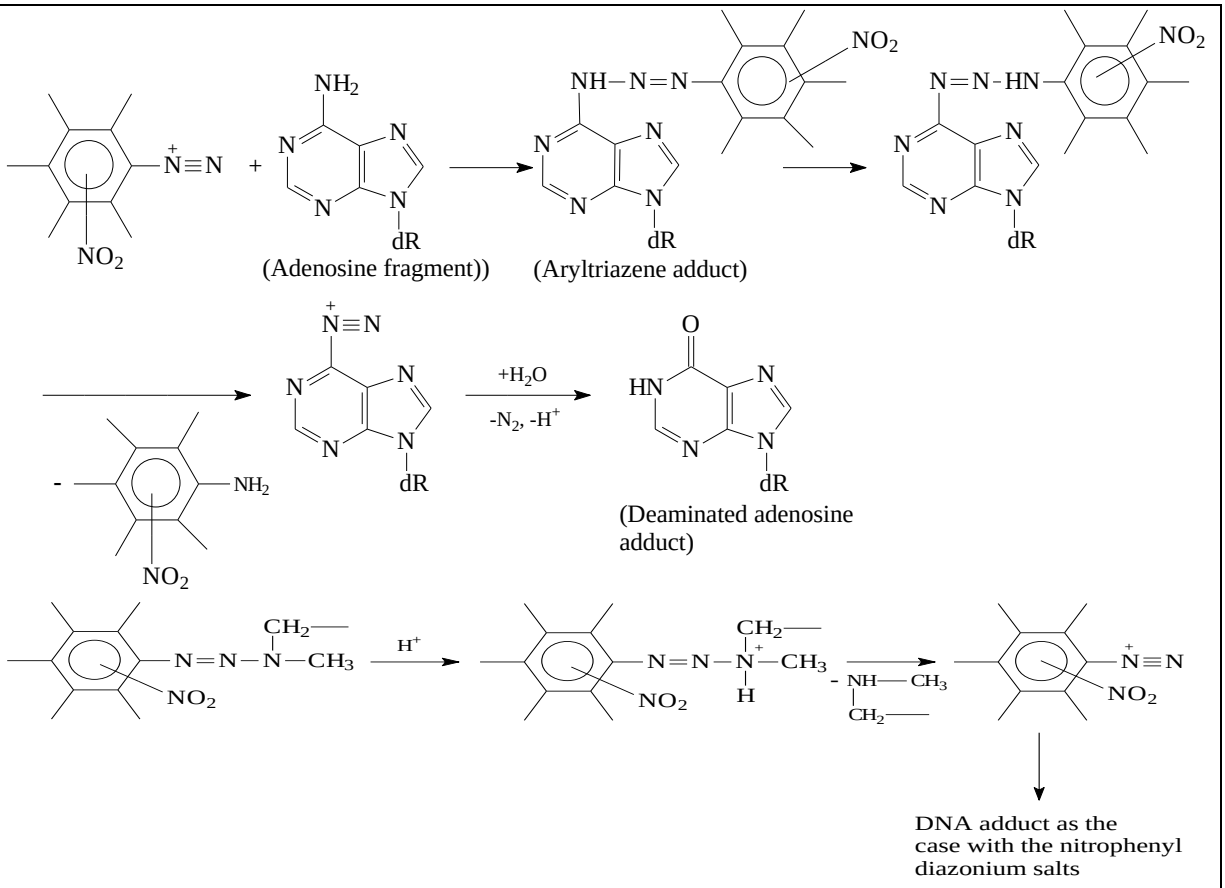
For nitrobenzyl and nitrobenzoyl halides – aralkylation [13]:



(Adenosine fragment; dR - deoxyribose phosphate fragment)

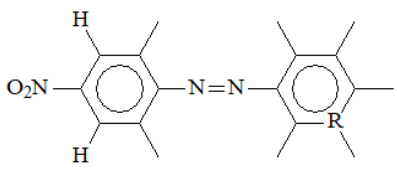
DNA adduct

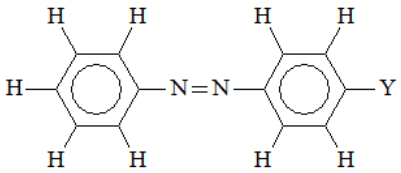
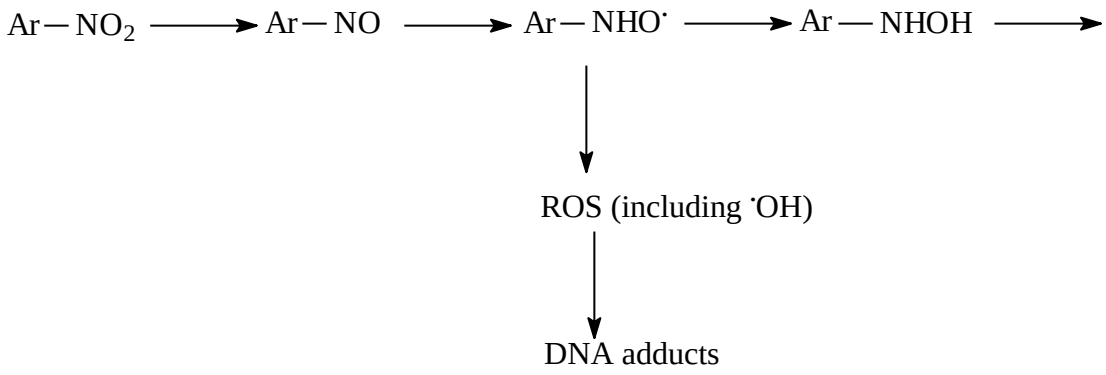
For nitrophenyl diazonium salts and triazenes [14, 15]:

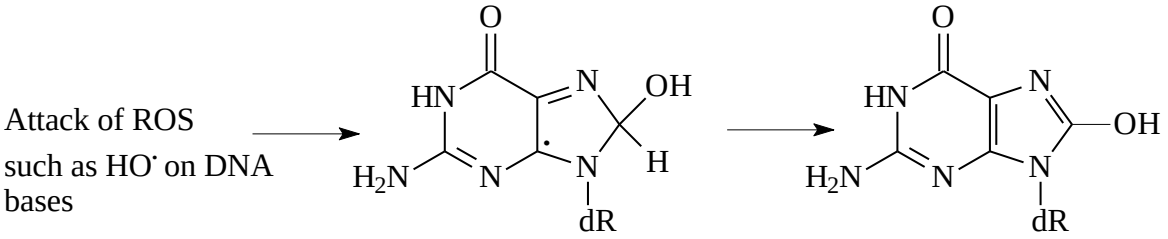


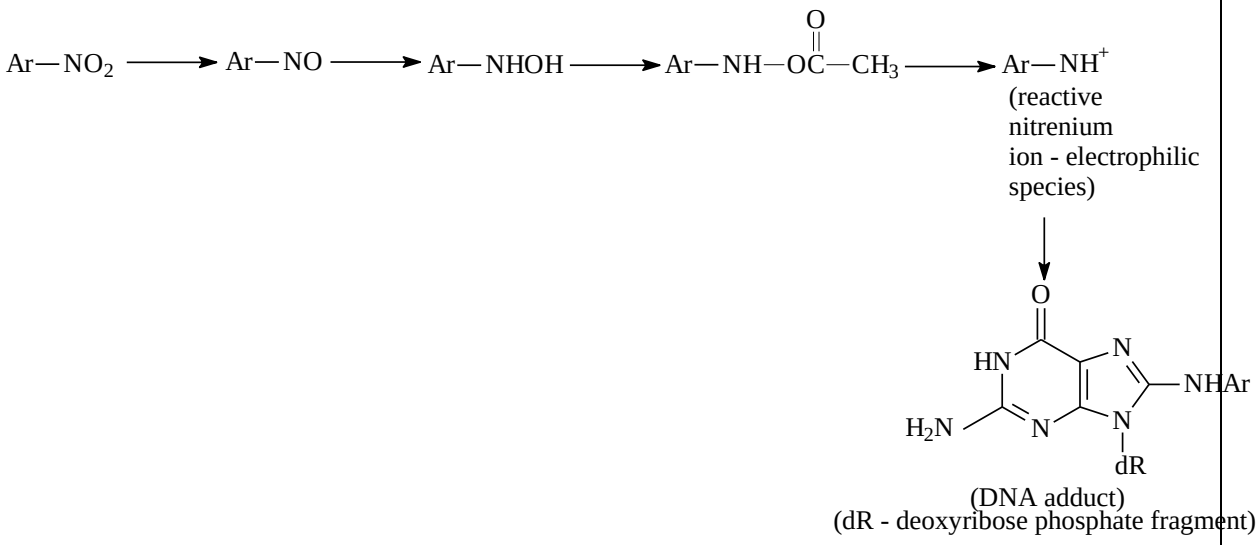
Set of chemicals used for profile development	Nitroarenes with Other Active Groups
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Sabbioni, G., Hemoglobin Binding of Arylamines and Nitroarenes: Molecular Dosimetry and Quantitative Structure-Activity Relationships, Envir. Health Persp. 102, Suppl. 6 (1994), 61 – 67. 2. Kalgutkar, A. S., I. Gardner, R. S. Obach, C. L. Shaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O, Donnell, J. Boer, S. P. Harriman, A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups, Current Drug Metabol. 6 (2005), 161 – 225. 3. Aiub, Cl. A. Fortes, J. L. Mazzei, L. F. R. Pinto, I. Felzenszwalb, Evaluation of Nitroreductase and Acetyltransferase Participation in N-Nitrosodiethylamine Genotoxicity, Chem.-Biol. Interact. 161 (2006), 146 – 154. 4. Einisto, P., M. Watanabe, M. Ishidate Jr., T. Nohmi, Mutagenicity of 30 Chemicals in Salmonella typhimurium Strains Possessing Different Nitroreductase or O-Acetyltransferase Activities, Mutat. Res. 259 (1991), 95 – 102. 5. Kovacic, P., J. D. Jacintho, Mechanisms of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer, Current Med.

	Chem. 8, (2001), 773 – 796. 6. Witherell, H. L., R. A. Hiatt, M. Replogle, J. Parsonnet, Helicobacter pylori Infection and Urinary Excretion of 8-Hydroxy-2-deoxyguanosine, an Oxidative DNA Adduct, Canc. Epidemiol. Biomarkers & Prevention 7 (1998), 91 – 96. 7. Wiseman, H., B. Halliwell, Damage to DNA by Reactive Oxygen and Nitrogen Species: Role in Inflammatory Disease and Progression to Cancer, Biochem. J. 313 (1996), 17 – 29. 8. Purohit, V., A. K. Basu, Mutagenicity of Nitroaromatic Compounds, Chem. Res. Toxicol. 13(8) (2000), 673 – 692. 9. 2-Chloronitrobenzene, 3-Chloronitrobenzene and 4-Chloronitrobenzene, IARC Monographs Vol. 65 (1997); http://monographs.iarc.fr/ENG/Monographs/vol65/volume65.pdf. ISBN-13 (PDF): 978-92-832-1565-3. Last visited: June, 2021. 10. Shimizu, M., E. Yano, Mutagenicity of Mono-Nitrobenzene Derivatives in the Ames Test and Rec Assay, Mutat. Res. 170 (1986), 11 – 22. 11. Chemical Carcinogenesis Research Information System, TOXNET, US National Library of Medicine; https://chem.nlm.nih.gov/chemidplus/, last visited 06.2021. 12. Hemminki, K., K. Falck, K. Linnainmaa, Reactivity, SCE Induction and Mutagenicity of Benzyl Chloride Derivatives, J. Appl. Toxicol. 3(4) (1983), 203 – 207. 13. Fall, M., H. Haddouk, J. P. Morin, R. Forster, Mutagenicity of Benzyl Chloride in the Salmomella/Microsome Mutagenesis Assay Depends on Exposure Conditions, Mutat. Res. 633(1) (2007), 13 – 20; http://www.ncbi.nlm.nih.gov/pubmed/17631040. DOI: 10.1016/j.mrgentox.2007.04.017. 14. Lawson, T., P. M. Gannett, W. M. Yau, N. S. Dalal, B. Toth, Different Patterns of Mutagenicity of Arenediazonium Ions in V79 Cells and Salmonella typhimurium TA102: Evidence for Different Mechanisms of Action, J. Agric. Food Chem. 43 (1995), 2627 – 2635. 15. Marchesi, Fr., M. Turriziani, Gr. Tortorelli, G. Avvisati, Fr. Torino, L. De Vecchis, Triazene Compounds: Mechanism of Action and Related DNA Repair Systems, Pharmacol. Res. 56 (2007), 275 – 287.

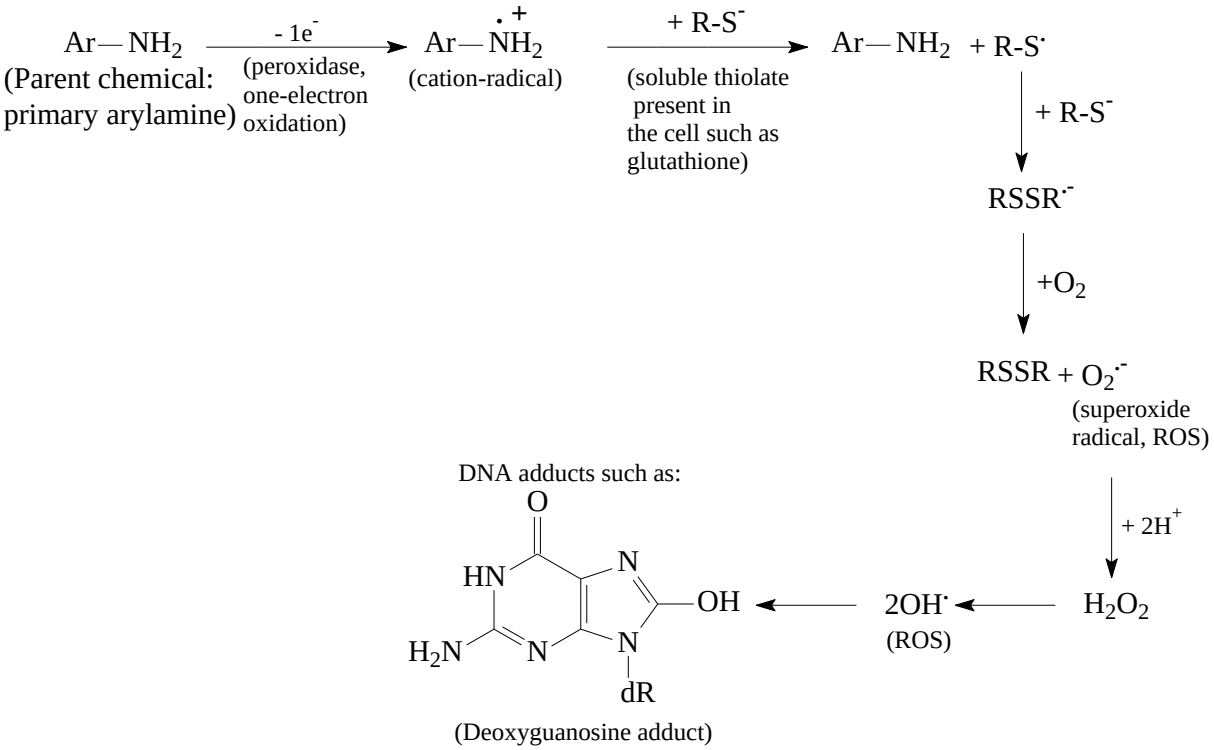
Individual profile/alert	
Name	Nitro Azoarenes and p-Monosubstituted Azobenzene Derivatives
Type of profile	Structural alert
Description/applicability domain	 R = any carbon or nitrogen, single arene ring only, no fused ring

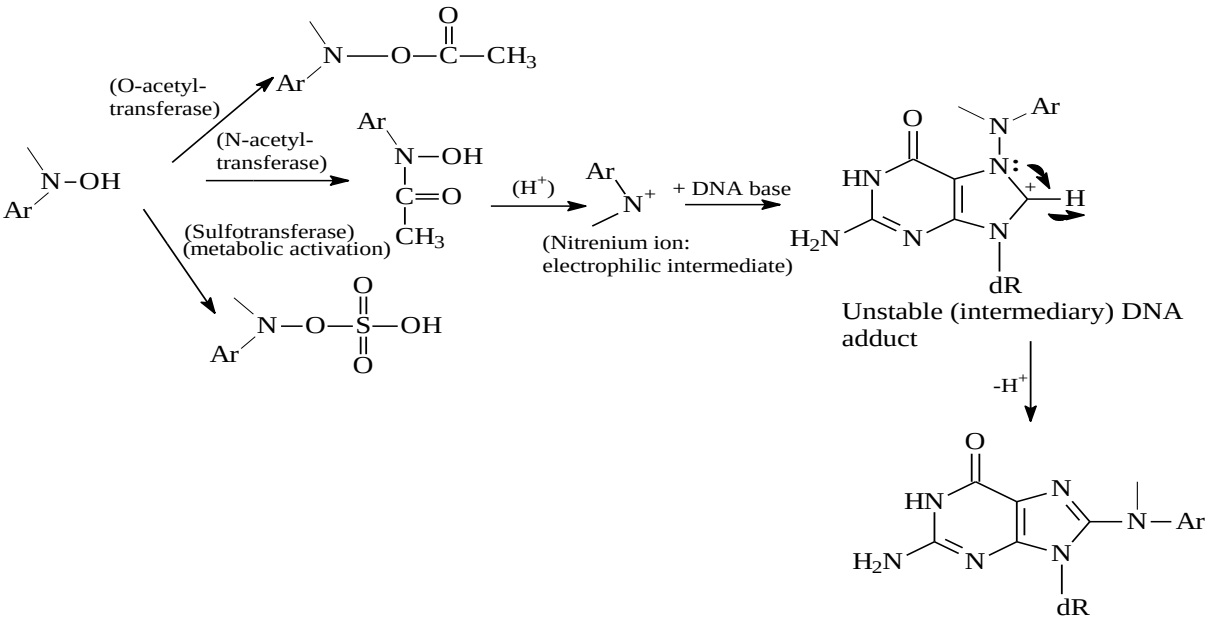
	fragments in the molecular structure Nitroazoarenes  Y= NH_2 or -NHOH
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases. (**Nucleophilic attack after reduction and nitrenium ion formation)** **Radical (Homolytic) Mechanism.** This is one of the mechanisms (but not the most important) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks) **(Radical mechanism *via* ROS formation (indirect)**
I. Nitroazoarenes  	



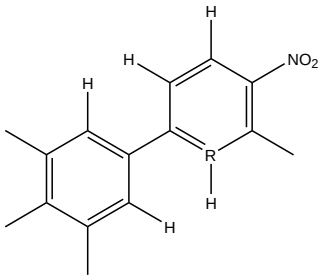
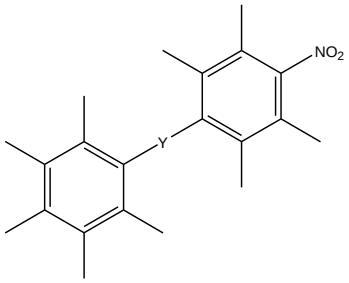


II. p-Monosubstituted Azobenzene Derivatives

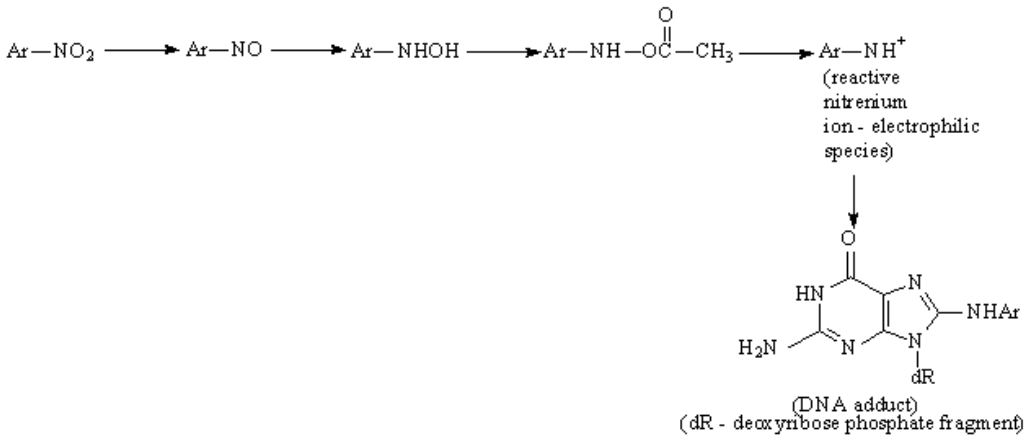




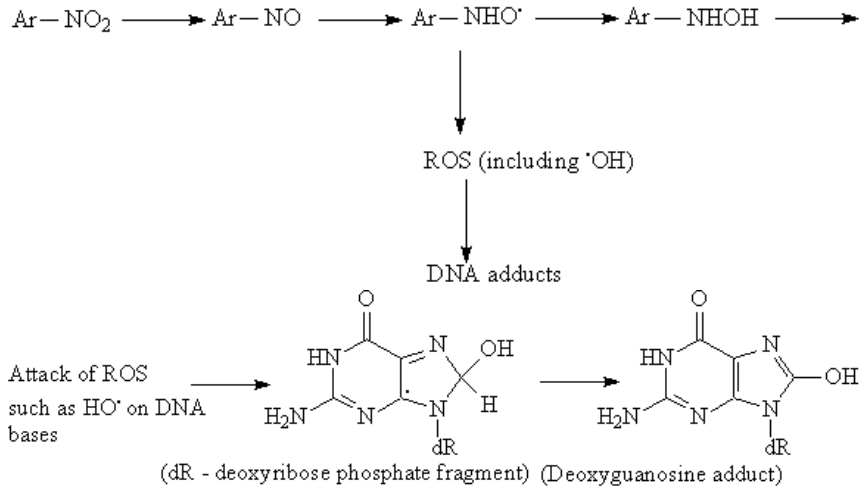
Set of chemicals used for profile development	Nitro Azoarenes and p-Monosubstituted Azobenzene Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2.Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3.Aiub, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4.Einisto, Mutat. Res. **259** (1991), 95 – 102. 5.Kovacic, Current Med. Chem. **8**, (2001), 773 – 796. 6.Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7.Wiseman, Biochem. J. **313** (1996), 17 – 29. 8.Purohit, Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9.Zbaida, S., J. Pharmacol. Exp. Ther. **260**(2) (1992), 554 – 561 10.*4-Nitroazobenzene*, GENE-TOX; https://pubchem.ncbi.nlm.nih.gov/substance/?source=gene-tox&sourceid=2491-52-3 Last visited: June, 2021. 11.Chung, Mutat. Res. **277** (1992), 201 – 220. 12.Gunkel, A. M., *Evaluation of the Mutagenicity and Toxicity of Monoazo Dyes in Wastewater Effluents and Sludge Supernatans* (Abstract); 13.Bakshi, J. Environ. Pathol. Toxicol. Oncol. 22(2) (2003), 101 – 109; http://www.ncbi.nlm.nih.gov/pubmed/14533873. Last visited: June, 2021. 14. Morita, T., Mutat. Res. 802 (2016), 1 – 29. 15. Mori, H., Cancer Res. **46**, 1986, 1654 - 1658. 16. Hashimoto, Y., Gan. 72(6) (1981), 921 – 929 (Abstract); https://www.ncbi.nlm.nih.gov/pubmed/7042447. Last visited: June, 2021. 17. Lang, B., Mutat. Res. **191** (1987), 139 – 143. 18. Shamovsky, I., JACS **133** (2011), 16168 – 16185.

Individual profile/alert	
Name	Nitrobiphenyls and Bridged Nitrobiphenyls
Type of profile	Structural alert
Description/applicability domain	Nitrobiphenyl  R= C or N(aromatic) o-distributed nitrobiphenyl are excluded Bridged Nitrobiphenyl  (Y can be O, S{V2}, -S{V4}=O, -S{V6}=$(O)_2$, -CH-CH-, -CH=CH)
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases. (**Nucleophilic attack after reduction and nitrenium ion formation)** **Radical (Homolytic) Mechanism.** This is one of the mechanisms (but not the most important) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks) **(Radical mechanism *via* ROS formation (indirect)**

Heterolytic

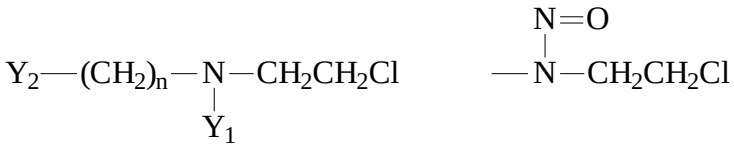
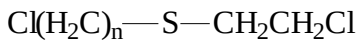
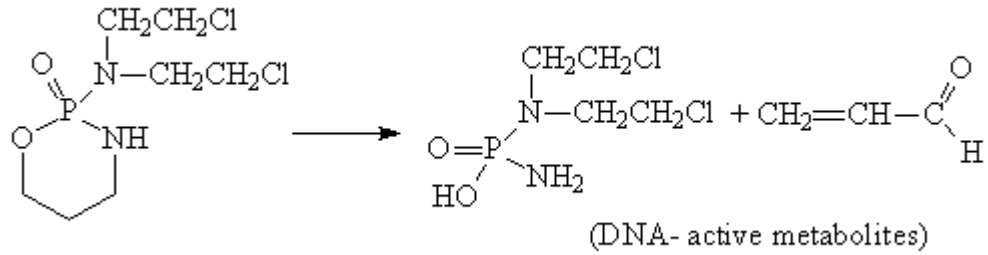


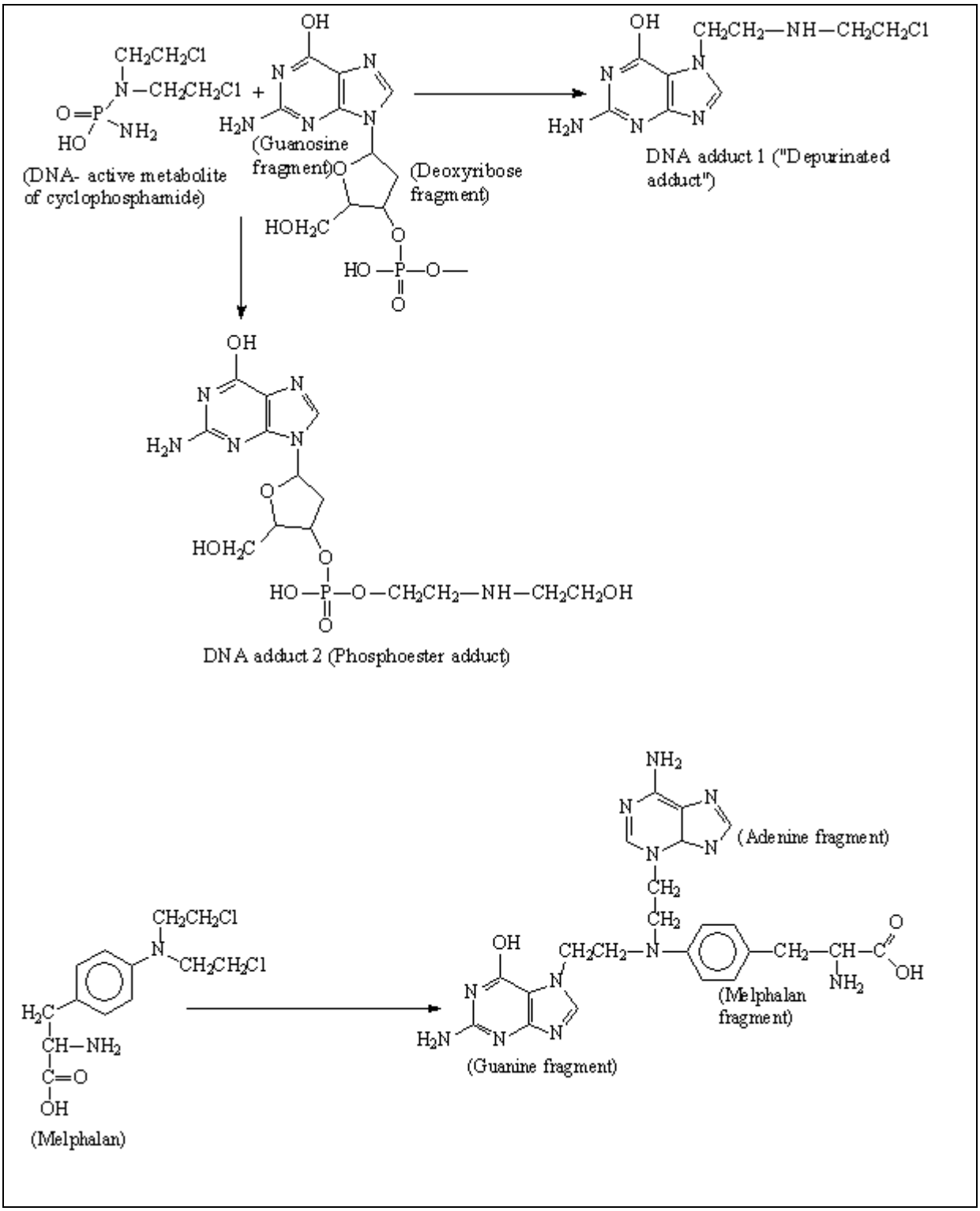
Homolytic

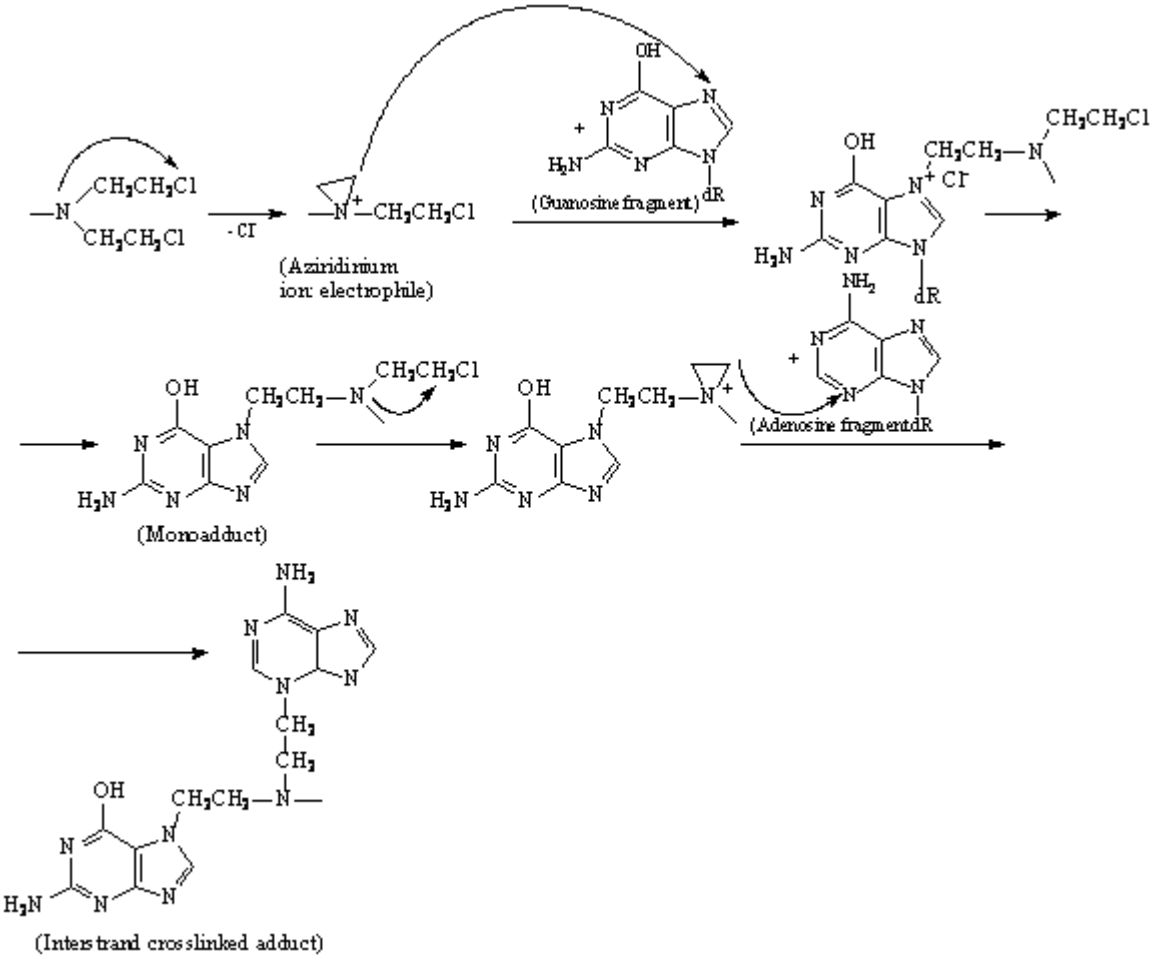


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2.Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3.Aiub, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4.Einisto, Mutat. Res. **259** (1991), 95 – 102. 5.Kovacic, Current Med. Chem. **8**, (2001), 773 – 796. 6.Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7.Wiseman, Biochem. J. **313** (1996), 17 – 29. 8.Purohit, Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9. El-Bayoumy, Mutat. Res. **81** (1981), 143 – 153. 10. Vance, Environ. Mutagen, **6** (6) (1984), 797 – 811). 11. *Chemical Carcinogenesis Research Information System (CCRIS)* https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=620-88-2. Last visited: June, 2021.

	12. Juneja, Mutat. Res. **263 (**9) (1991), 13 – 19. 13. Hooberman, Mutat. Res. **341** (1994), 57 – 69.

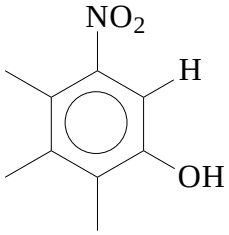
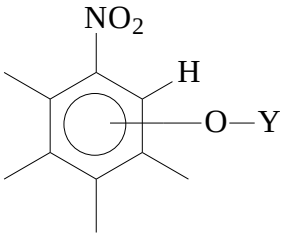
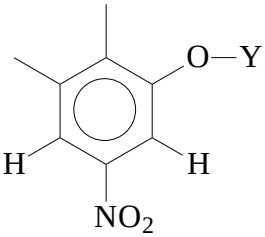
Individual profile/alert	
Name	Nitrogen and Sulfur Mustards
Type of profile	Structural alert
Description/applicability domain	 (Y_1 can be -H or C{sp3} or P{acy}V5}=O Y_2 can be O, NH, Cl; n = 2 or 3)  (n = 2 or 3)
Mechanism	S_N2 Alkylation, direct acting epoxides and related after cyclization
 (DNA- active metabolites)	

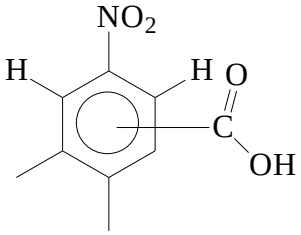
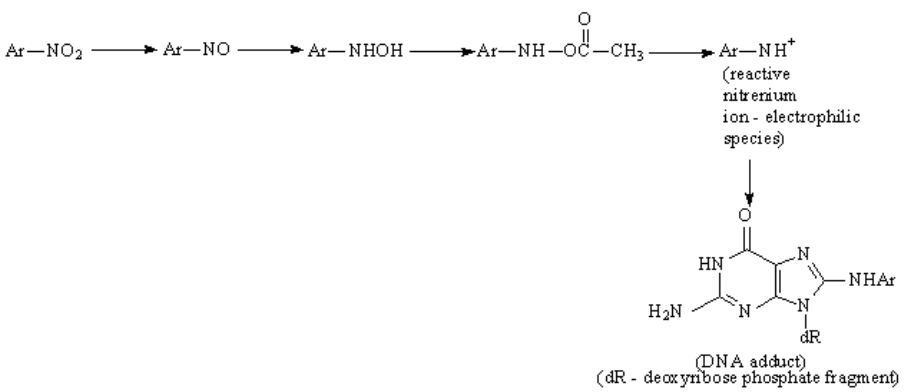

(DNA- active metabolite of cyclophosphamide)
(Guanosine fragment)
(Deoxyribose fragment)
DNA adduct 1 ("Depurinated adduct")
DNA adduct 2 (Phosphoester adduct)
(Melphalan)
(Adenine fragment)
(Melphalan fragment)
(Guanine fragment)



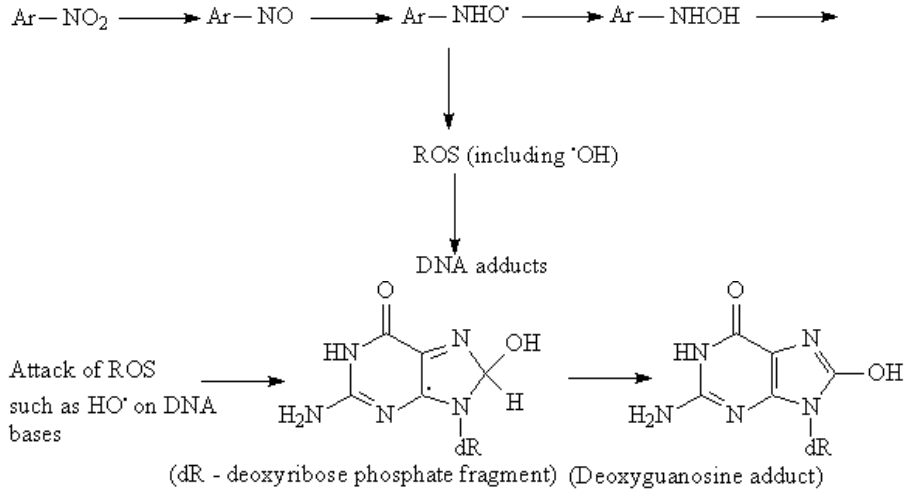
Set of chemicals used for profile development	Nitrogen and Sulfur Mustards
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kovacic, P., J. D. Jacinto, *Mechanism of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer*, Current Med. Chem. **8** (2001), 773 – 796. 2. Hartley, J. A., J. P. Bingham, R. L. Souhami, DNA *Sequence Selectivity of Guanine-N7 Alkylation by Nitrogen Mustards is Preserved in Intact Cells*, Nucl. Acids Res. **20**(12), (1990), 3175 - 3178. 3. *Nitrogen Mustard*; http://en.wikipedia.org/wiki/Nitrogen_mustard. Last visited: June, 2021. 4. Benedict, W. F., M. S. Baker, L. Haroun, *Mutagenicity of Cancer Chemotheraupetic Agents in the Salmonella/Microsome Test*, Canc. Res. **37** (1977), 2209 – 2213. 5. Alarcon, R. A., J. Meienhofer, E. Atherton, *Isophosphamide as a New Acrolein-Producing Antineoplastic Isomer of Cyclophosphamide*, Canc. Res. **32** (1972), 2519 – 2523. 6. DeMarini, D. M., H. N. Pham, A. J. Katz, H. E. Brockmann, *Relationship Between Structures and Mutagenic Potencies of 16 heterocyclic Nitrogen Mustards (ICR Compounds) in Salmonella typhimurium*, Mutat. Res. **136** (1984), 185 – 199. 7. Povirk, L. F., D. E. Shuker, DNA *Damage and Mutagenesis Induced by Nitrogen Mustards*, Mutat. Res. **318** (1994), 205 – 226. 8. Cahill, P. A., A. W. Knight, N. Billinton, M. G. Barker, L. Walsh, P. O. Keenan, C. V. Williams, D. J. Tweats, R. M. Walmsley, *The GreenScreen*

Genotoxicity Assay: A Screening Validation Programme, Mutag. **19**(2) (2004), 105 – 119.
9. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/. Last visited: June, 2021.
10. Stewart, D., E. Sass, L. Fritz, L. Sasser, *Toxicology Studies on Lewisite and Sulfur Mustard Agents: Mutagenicity of Lewisite in the Salmonella Histidine Reversion Assay*, U.S. Army Medical Research and Development Command, Ntis AD-A213102, 1989; http://www.osti.gov/scitech/servlets/purl/1086509. Last visited: June, 2021.
11. Ashby, J., H. Tinwell, R. D. Callander, N. Clare, *Genetic Activity of the Human Carcinogen Sulphur Mustard Towards Salmonella and the Mouse Bone Marrow*, Mutat. Res., **257**(3) (1991**),** 307 - 311.
12. CCRIS: Sulfur Mustard, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=505-60-2. Last visited: June, 2021.
13. Wattana, M., T. Bey, *Mustard Gas or Sulfur Mustard: An Old Chemical Agent as a New Terrorist Threat*, Prehospital and Disaster Medicine **24**(1) (2009), 19 – 29.

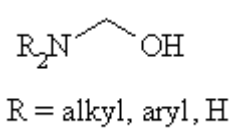
Individual profile/alert	
Name	Nitrophenols, Nitrophenyl Ethers and Nitrobenzoic Acids
Type of profile	Structural alert
Description/applicability domain	Nitrophenols  (No more than three substituents -SO_3H and -COO- excluded) Nitrophenyl ethers  Y is -CH_3 or -CH_2CH_3; no more than three substituents; no -SO_3H or -COO- "Mask": 

	Nitrobenzoic Acids  (No more than three substituents; no -SO_3H or additional -COO- groups) No more than three substituents No –SO_3H and –COO-
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases. (**Nucleophilic attack after reduction and nitrenium ion formation)** **Radical (Homolytic) Mechanism.** This is one of the mechanisms (but not the most important) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks) **(Radical mechanism *via* ROS formation (indirect)**
Heterolytic  	

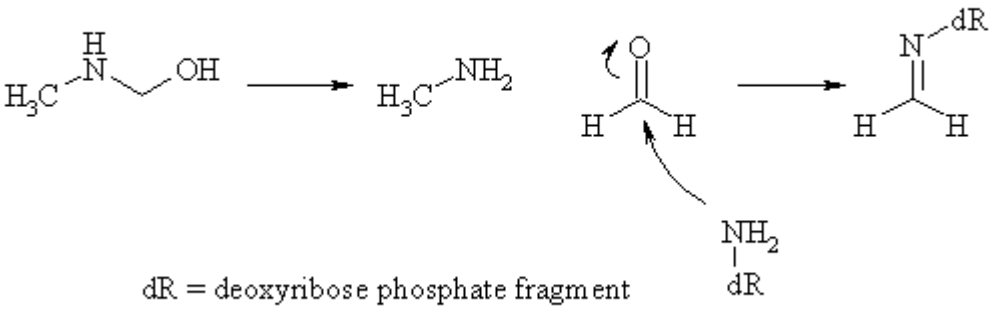
Homolytic



Set of chemicals used for profile development	Nitrophenols, Nitrophenyl Ethers and Nitrobenzoic Acids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2.Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3.Aiub, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4.Einisto, Mutat. Res. **259** (1991), 95 – 102. 5.Kovacic, Current Med. Chem. **8**, (2001), 773 – 796. 6.Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7.Wiseman, Biochem. J. **313** (1996), 17 – 29. 8.Purohit, Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9. Shimizu, Mutat. Res. **170** (1986), 11 – 22. 10. Sundvall, Mutat. Res. **137** (1984), 71 – 78. 11. Mononitrophenols, Concise International Chemical Assessment Document 20, World Health Organization, Geneva 2000.

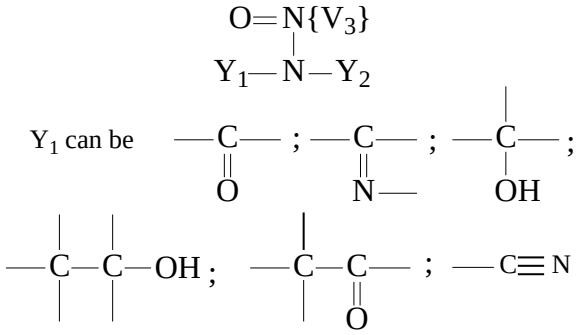
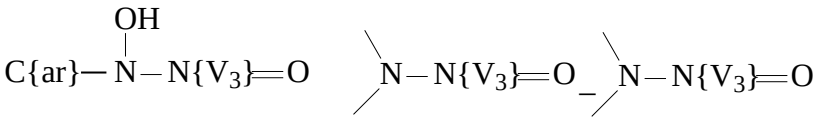
Individual profile/alert	
Name	N-Methylol Derivatives
Type of profile	Structural alert
Description/applicability domain	 R = alkyl, aryl, H
Mechanism	Schiff base formation **Chemicals Activated by P450 to Mono-aldehydes**

N-methylol derivatives have been suggested to be genotoxic via hydrolysis into formaldehyde (Ashby et al 1985). Formaldehyde then undergoes DNA binding via a Schiff base reaction (Cheng et al 2003).

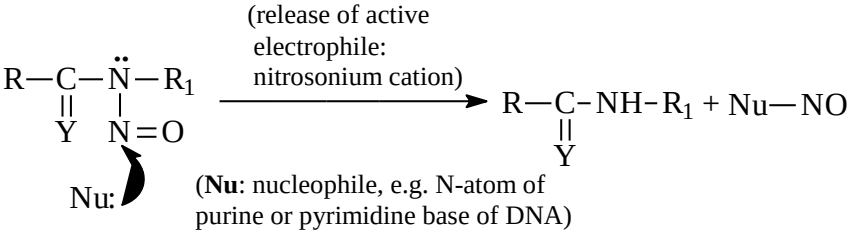


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Ashby et al (1985) Mutation Research, 156, 19-32 2. Cheng et al (2003) Chemical Research in Toxicology, 16, 145-152

Individual profile/alert	
Name	N-Nitroso Compounds
Type of profile	Structural alert

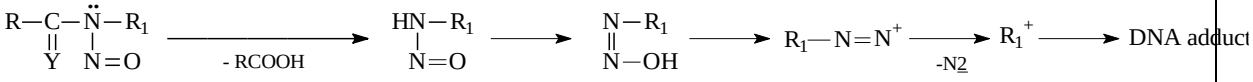
Description/applicability domain	 (-OH or C=O groups attached at *beta*-position towards -N-N=O functionality) Y_2 can be C or H or $-NO_2$ **(I)**  **(II)** (Two N-nitroso-groups within the same molecule) **(III)**
Mechanism	S_N1 Nucleophilic attack after carbenium ion formation & S_N1 Nucleophilic attack after nitrosonium cation formation

1. Mutagenicity without metabolic activation.



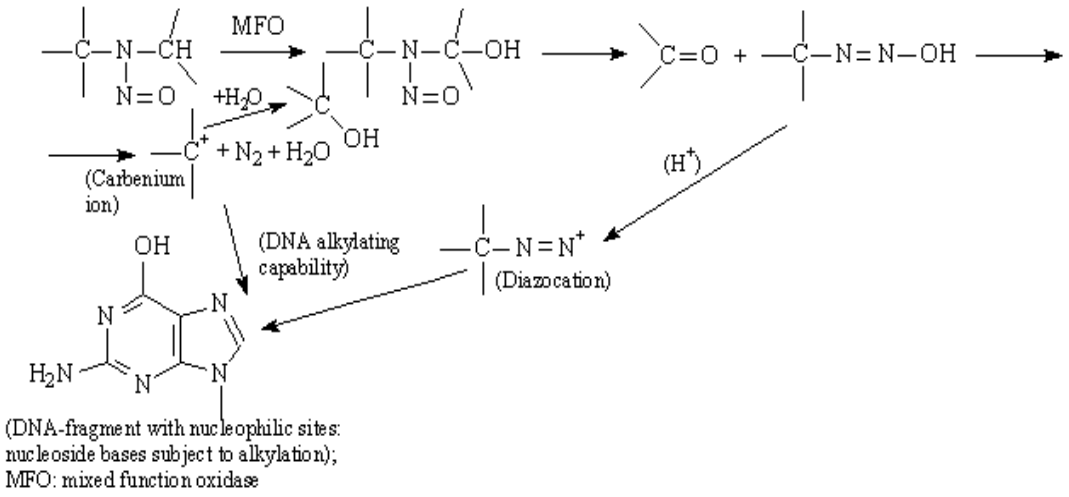
(**Nu**: nucleophile, e.g. N-atom of purine or pyrimidine base of DNA)

(Y can be O or NH)



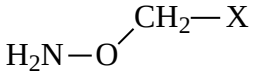
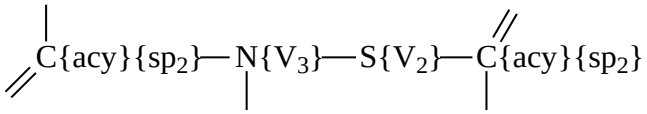
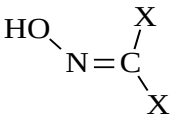
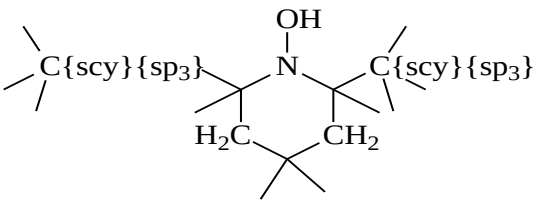
(Y can be O or NH)

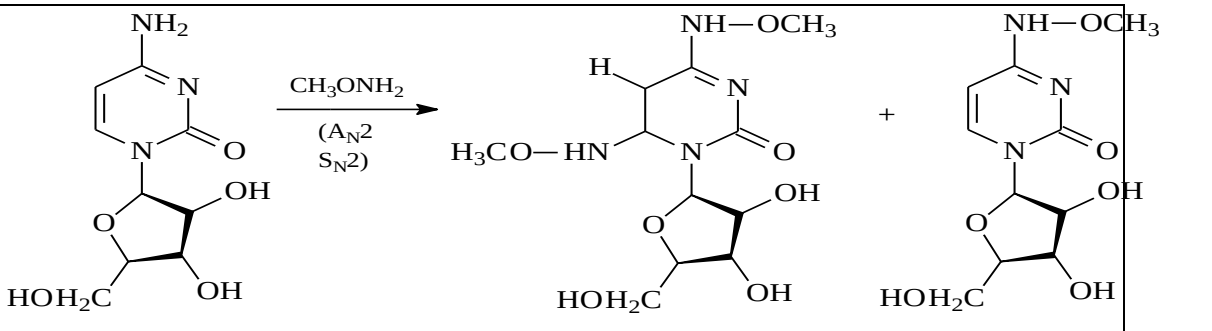
2. Mutagenicity with metabolic activation.



Set of chemicals used for profile development	N-Nitroso Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Toxicological Profile for N-Nitrosodiphenylamine*, US Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Sisease Registry, April 1993; http://www.atsdr.cdc.gov/ToxProfiles/tp16.pdf. Last visited: June, 2021. 2. Miura, M., Tetrahedron Lett. **41** (2000), 3637 – 3641. 3. Kovacic, P., Current Med. Chem. **8**, (2001), 773 – 796. 4. Wang, P. G., Chem. Rev. **102** (2002), 1091 – 1134. 5. Janczuk, *Nitric Oxide Donors: Chemical Activities and Biological Applications*, Chem. Rev. **102** (2002), 1091 – 1134. 6. Guttenplan, J. B., Mutat. Res. **186** (1987), 81 – 134. 7. Ethylnitrosocyanamide CASRN: 38434-77-4, GENE-TOX, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=gene-tox&sourceid=38434-77-4. Last visited: June, 2021. 8. Nakamura, S.-i., Mutat. Res. **192** (1987), 239 – 246. 9. Lee, K., Mutat. Res. **48** (1977), 131 – 138. 10. *Chemical Carcinogenesis Research Information System (CCRIS)*; https://chem.nlm.nih.gov/chemidplus/ Last visited: June, 2021 (for bacterial mutagenicity data for chemicals such as Dinitrosopentamethylenetetramine, N-nitroso phenylhydroxylamine and N-Nitrosodiethanolamine. 11. Kushida, H., Carcinogenesis **21**(6) (2000), 1227 – 1232. 12. Maertens, L. A., Drug Metabol. Dispos. **38** (2010), 752 – 760. 13. Peterson, L. A., Canc. Res. **61** (2001), 5757 – 5763. 14. *N-Nitrosomethylethylamine, Summaries & Evaluations*, IARC, Vol. 17 (1978), p. 221; http://www.inchem.org/documents/iarc/vol17/nitrosomethylethylamine.html. Last visited: June, 2021. 15. Farelly, J. G., Canc. Res. **42** (1982), 2106 – 2109. 16. Von Hofe, E., Canc. Res. **46** (1986), 1038 – 1042. 17. Rao, T.K., Mutat. Res. 89(1) (1981**)**, 35 – 43.

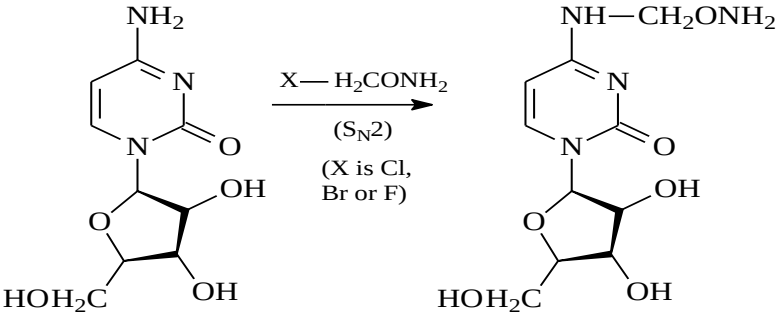
	18. Rao, T.K., Mutat. Res**. 67**(1) (1979), 21 - 26. 19. Padma, P.R., Cancer Lett. **46**(3) (1989), 173 - 180. 20. N-Nitroso-1,2,3,6-Tetrahydropyridine CASRN: 55556-92-8, GENE-TOX, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=gene-tox&sourceid=55556-92-8. Last visited: June, 2021.

Individual profile/alert	
Name	Non-Aromatic Hydroxylamine Derivatives
Type of profile	Structural alert
Description/applicability domain	 (X is F, Cl, Br, H)   (X can be F, Cl, Br) 
Mechanism	AN2 Nucleophilic addition to activated C=C bond SN2 Nucleophilic substitution on activated primary amino group Radical Radical mechanism via ROS formation
According to one publication, the reaction of hydroxylamine NH2OH or methoxyamine NH2OCH3 with pyrimidine bases of DNA and RNA such as uridine and cytidine under nearly physiological conditions occurs via an addition of the reagent to the 5,6-double bond, followed by replacement of the amino group by hydroxylamino- or methoxyamino one, respectively. Thus the following mechanistic schemes can be inferred:	



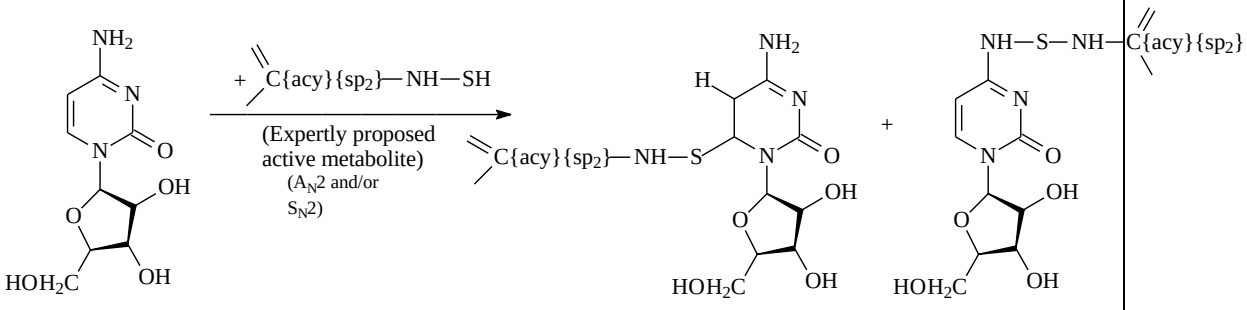
(Cytidine) (6-Methoxyamino-5,6-dihydro-N-4-methoxycytidine) (N-4-Methoxycytidine)

Another S_N2 scheme of formation of DNA-type adducts is also possible:



(Cytidine) (N-4-Aminoxymethylcytidine)

For some thiohydroxylamine derivatives, eliciting bacterial mutagenicity after metabolic activation only, the following mechanistic scheme, similar to Scheme 1a could be expertly suggested:



(Cytidine fragment of DNA) (Possible adducts)

Hydroxylamine and some of its N-alkyl derivatives were also reported to undergo autoxidation in the presence of traces of transition metals (Men). During autoxidation of hydroxylamine, superoxide radical-anion is formed which acts as reactive oxygen species (ROS), attacking DNA (Scheme 3):

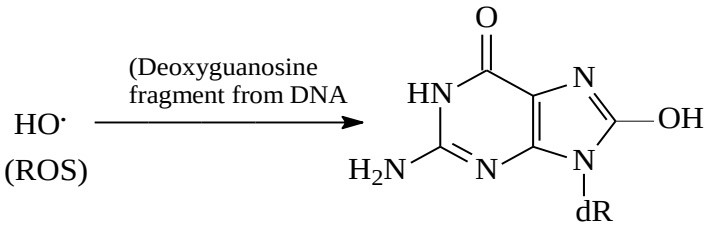
$$NH_2OH + Me^n \rightarrow NH_2O^{\cdot} + H^+ + Me^{n-1}$$

$$NH_2O^{\cdot} + O_2 \rightarrow NO^- + O_2^{\cdot -} \text{ (ROS)}$$

$$Me^{n-1} + O_2 \rightarrow Me^n + O_2^-$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

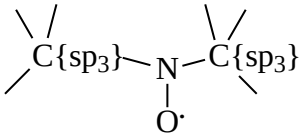
$H_2O_2 \rightarrow 2HO^{\cdot}$



DNA adduct

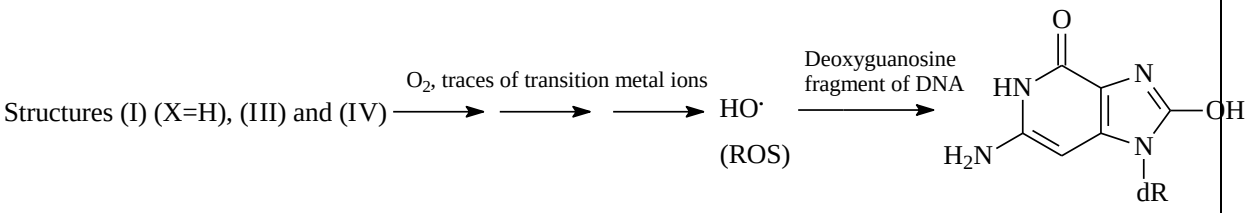
The oxidative potency of hydroxylamine and its O-derivatives such as O-methyl- and O-ethyl hydroxylamine was reported to be generally higher than the corresponding effects of the N-derivatives (N-methyl-, N-dimethyl-, and N,O-dimethyl hydroxylamine). The occurrence of cell-damaging products like superoxide and H2O2 was proved [4], which also causes toxicity, including mutagenicity.

Nitroxyl radicals of the type:



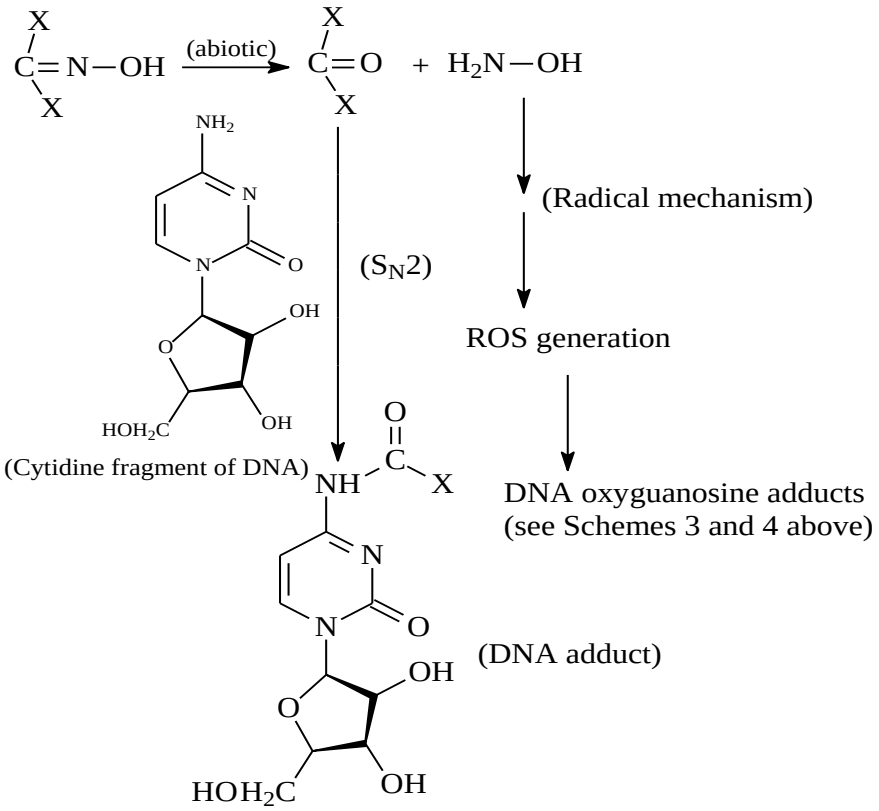
formed from N,N-dialkylhydroxylamines by mechanism, similar to that shown in Scheme 3 above (see the formation of the hydroxylamine-derived radical NH2O.) enhanced the formation of H2O2 and ROS, and were reported as mutagenic in Salmonella typhimurium strains TA104 and TA100 [5].

Principal mechanistic scheme of the ROS formation, and the resulting oxidative DNA nucleoside adducts is shown below:



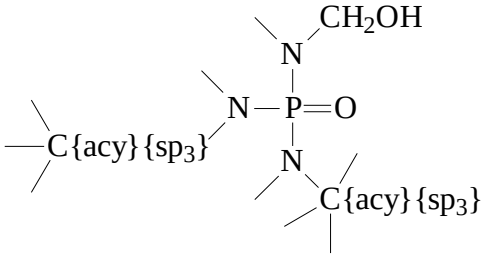
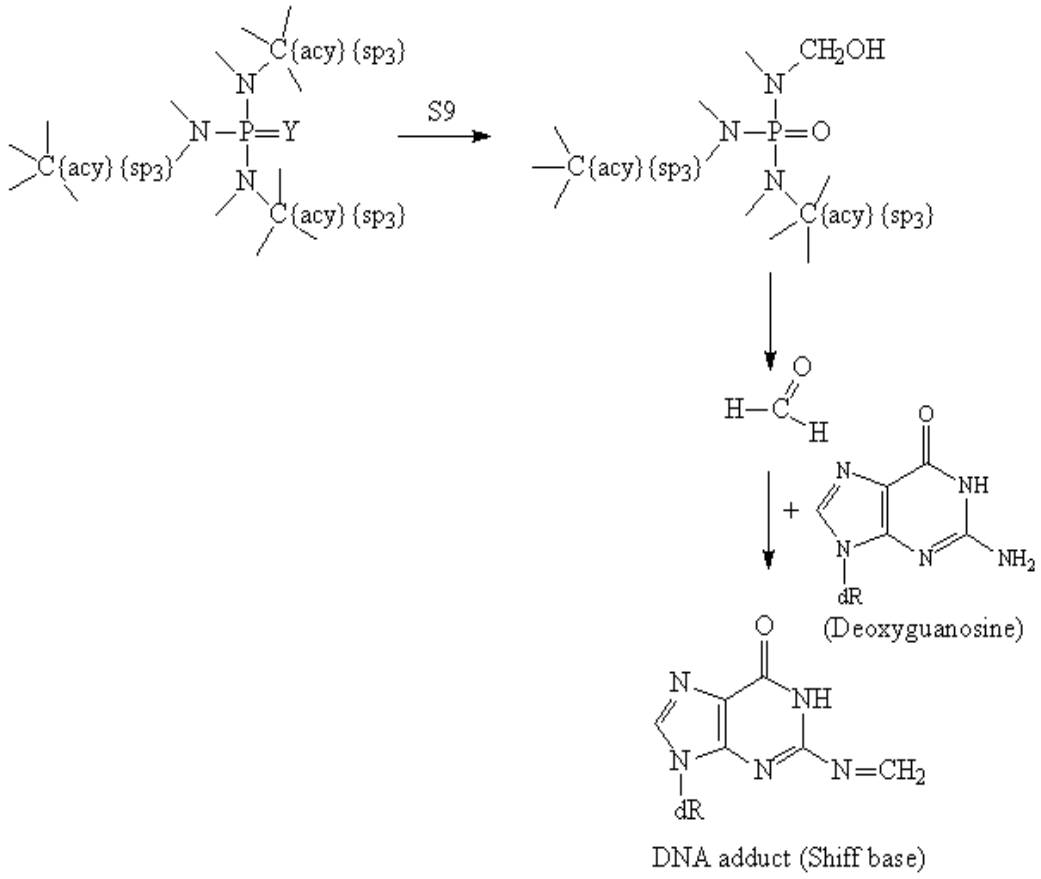
(DNA adduct)

Finally, for chemicals with structures similar to (III) above, the following mechanistic scheme is also possible:

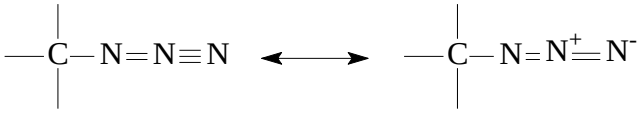
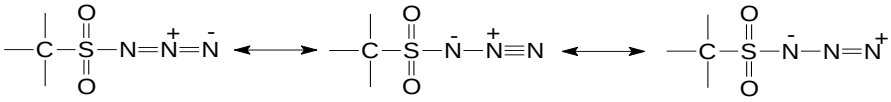
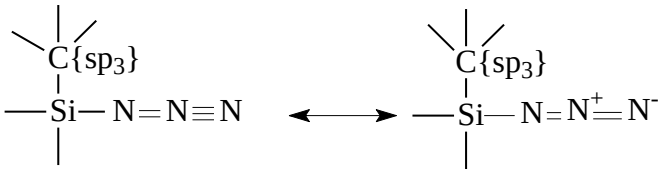


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Fraenkel-Conrat, H., B. Singer, The chemical basis for the mutagenicity of hydroxylamine and methoxyamine, Biochim. et Biophys. Acta 262 (1972), 264 – 268. 2. Studies in Environmental Science, Potential Industrial Carcinogens and Mutagens, Vol. 4, Ch. 16, Hydrazines, Hydroxylamines, Carbamates, Acetamides, Thioacetamides and Thioureas, 1979, 307 – 330; DOI: http://anonym.to/?http://doi.org/10.1016/S0166-1116%2808%2971327-X Last visited: June, 2021. 3. Kono, Y., Generation of Superoxide Radical during Autoxidation of Hydroxylamine and an Assay for Superoxide Dismutase, Arch. Biochem. Biophys. 186(1), 1978, 189 – 195. 4. Anita A., M. G. Spooren, Chr. T. A. Evelo, A Study on the Interaction between Hydroxylamine Analogues and Oxyhemoglobin in Intact Erythrocytes, Blood Cells, Molecules, and Diseases 26(4) (2000), 373–386. 5. Xiaoqing, G., R. A. Mittelstaedt, L. Guo, J. G. Shaddock, R. H. Heflich, A. H. Bigger, M. M. Moore, N.Mei, Nitroxide TEMPO: A genotoxic and oxidative stress inducer in cultured cells, Toxicology in Vitro 27 (2013) 1496–1502.

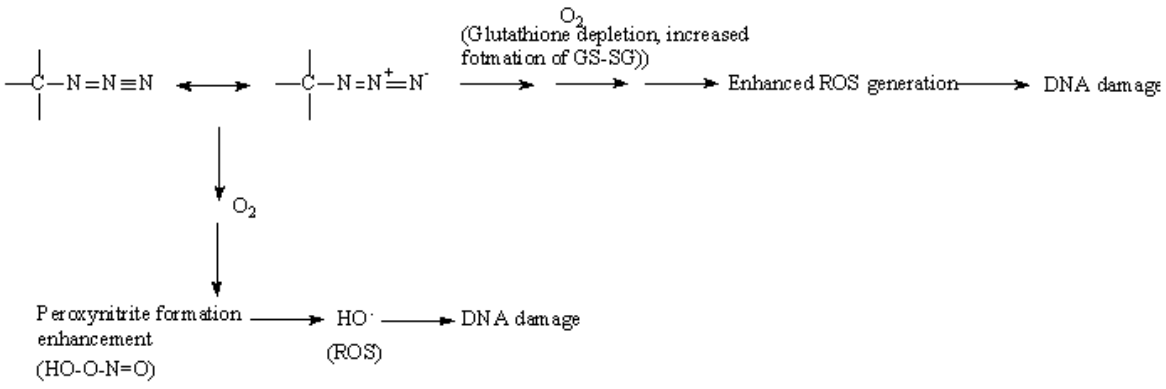
Individual profile/alert	
Name	Non-Cyclic Alkyl Phosphoramides and Thionophosphoramides

Type of profile	Structural alert
Description/applicability domain	 C{acy}{sp3} corresponds to -CH_3 or -C_2H_5 or -CH_2OH; no more than two -CH_2OH groups, should be bound to different N-atoms)
Mechanism	A_N2 Shiff base formation (after S9 metabolic activation only)
	
Set of chemicals used for profile development	Non-Cyclic Alkyl Phosphoramides CAS
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Anderson, D., Br. J. Cancer, **37**(6) (**1**978**)**, 924 – 930. 2. Sarrif, A.M., Mutat. Res., **380**(1-2) **(**1997), 167 - 177. 3. CCRIS: Hexamethylphosphoramide, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=6

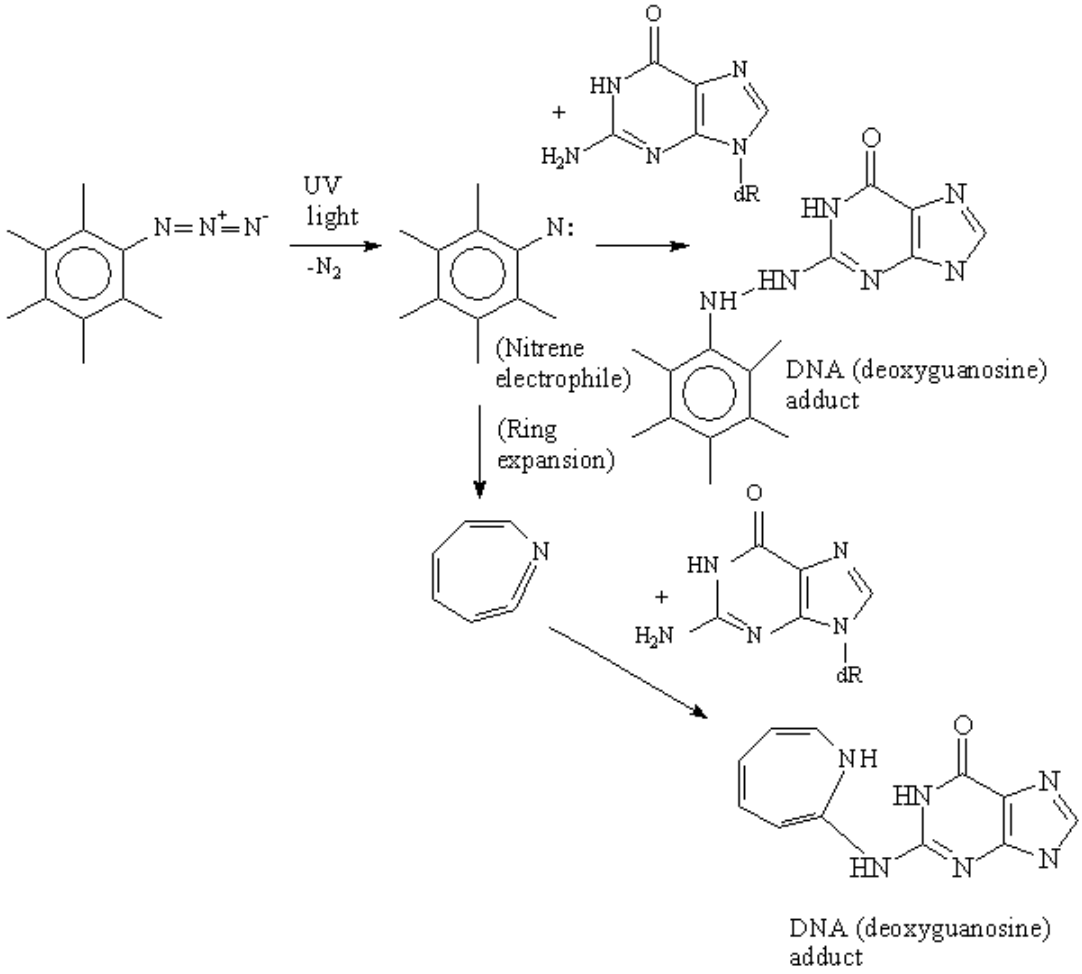
	80-31-9. Last visited: June, 2021. 4. Jones, A. R., Biochem. Pharmacol. **17** (1968), 2247 – 2252. 5. Ashby, J., Br. J. Cancer **38** (1978), 418 – 429. 6. Lu, K., J. Am. Chem. Soc. **132**(10) (2010), 3388 – 3399.

Individual profile/alert	
Name	Organic Azides
Type of profile	Structural alert
Description/applicability domain	 (Organic Hydrocarbon Azides: resonance structures)  (Organic Sulfonyl Azides: resonance structures)  (Organic Organosilicon Azides: resonance structures)
Mechanism	Radical ROS generation, S_N1 Nucleophilic attack after nitrene formation and Non-covalent interactions DNA intercalation; SN2 Nucleophilic attack on sulfonyl azide

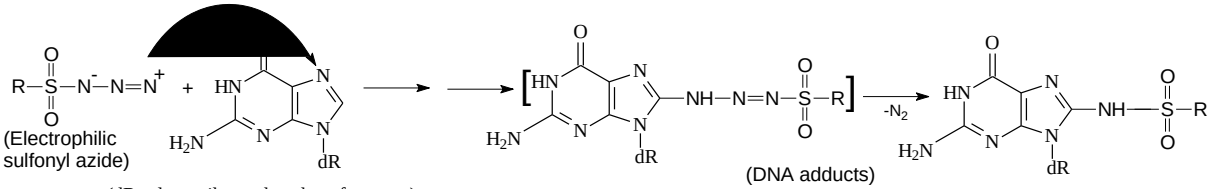
Two principal mechanisms of DNA damage, eliciting bacterial mutagenicity can be suggested. The first one is associated with the pro-oxidant properties of organic azides such as AZT, resulting in endogenous glutathione depletion and enhanced peroxynitrite and reactive oxygen species (ROS) formation [9, 10]. The following mechanistic scheme can be expertly outlined:



The second mechanism is mainly associated with arylazides, and the subsequent generation of electrophilic arylnitrene species, following light activation [11]. The following expertly assumed mechanistic scheme can be outlined:

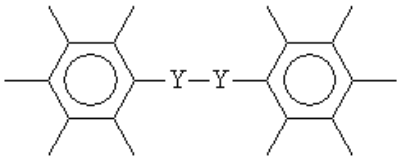
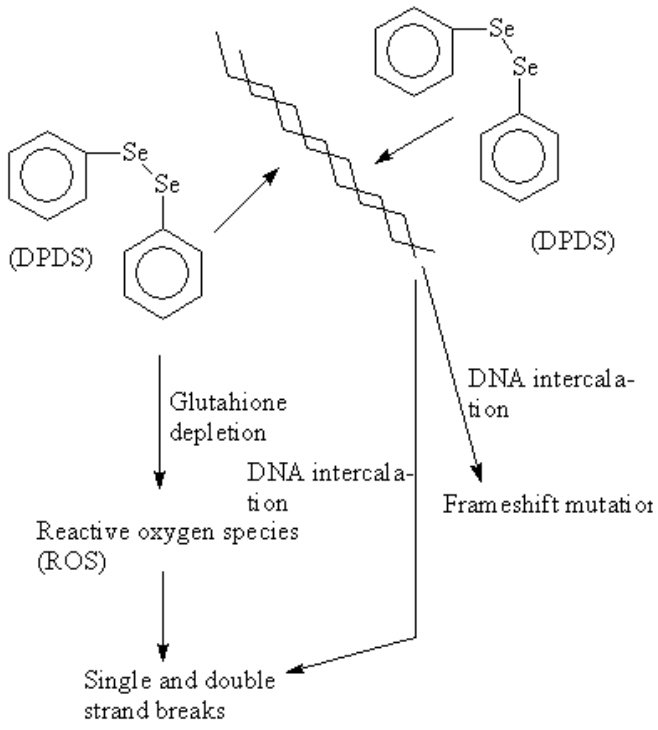


One additional mechanistic scheme is associated with eliciting cross-linking effects on biopolymer molecules or by forming DNA adducts. The mechanism below is only expertly assumed and is based on the electrophilicity of sulfonyl azides

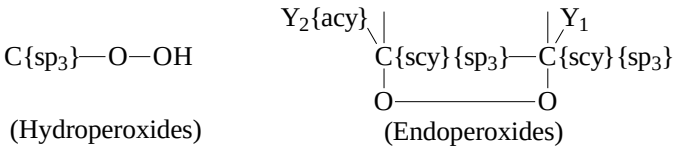
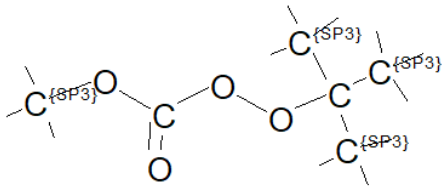


Set of chemicals used for profile development	Organic Azides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Zeller, Toxicol. Sci. **135**(2) (2013**)**, 317 - 327. 2. Ayers, Fundam. Appl. Toxicol. **32**(2) (1996), 148 - 158. 3. Ballardin, Ann. N.Y. Acad. Sci. **1056** (2005**)**, 303 - 310. 4. Gao, Mol. Med. Report **4**(1) (2011), 151 – 155. 5. Bialkowska, Carcinog. **21**(5) (2000), 1059 – 1062.

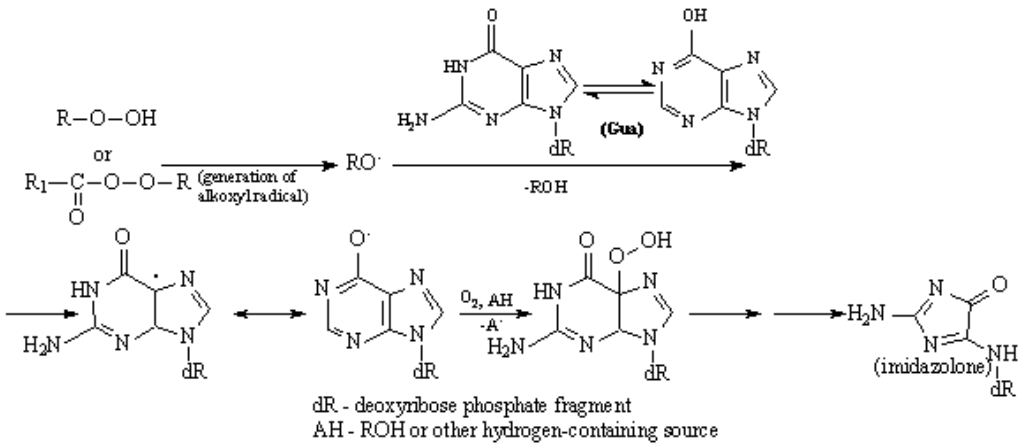
	6. Olivero, Environ. Molec. Mutagen. **48** (2007), 215 – 223. 7. Owais, Mutat. Res. **118** (1983), 229 – 239. 8. Owais, Mutat. Res. **197** (1988), 313 – 323. 9. Osborne, J. AIDS Clin. Res. 6(4) (2015); DOI: 10.4172/2155-6113.1000441. 10. Mak, Cardiovasc. Toxicol**. 04** (2004), 109 – 115). 11. Photoreactive Crosslinker Chemistry, Transfection & Genome Engineering Handbook; http://www.lifetechnologies.com/bg/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/photoreactive-crosslinker-chemistry.html#, last visited 06.2021.

Individual profile/alert	
Name	Organic Diselenides and Ditellurides
Type of profile	Structural alert
Description/applicability domain	 (Y is Se or Te)
Mechanism	Non-covalent interactions DNA intercalation and Radical ROS generation
	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Rosa, Mutat. Res. 563(2) (2004**)**, 107 - 115.

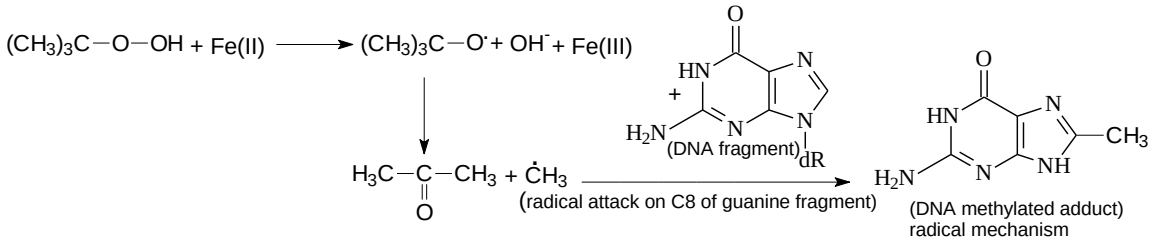
	2. Degrandi, Mutagen. **25**(3) (2010), 257 – 269. 3. Rosa, Braz. J. Med. Biol. Research **40** (2007), 1287 – 1304. 4. Brito, Acta Biochim. Pol. **56**(1) (2009), 125 – 134. 5. Prigol, Chem. Biol. Interact. **200** (2012) 65 – 72.

Individual profile/alert	
Name	Organic Peroxy Compounds
Type of profile	Structural alert
Description/applicability domain	 (Y_1 can be -CH_3; Y_2{acy}can be -H, -CH_3, -OCH_3, -CH_2O (not -CH_2OH)) 
Mechanism	**Radical ROS generation (indirect) or direct radical attack on DNA**

Alkoxyl radicals have been detected during the photolysis of water-soluble peroxyester, and, in the presence of DNA, oxidative damage of the latter was demonstrated *via* the formation of guanidine-releasing products by alkoxyl radicals, according to the following mechanistic scheme 1 [2]:

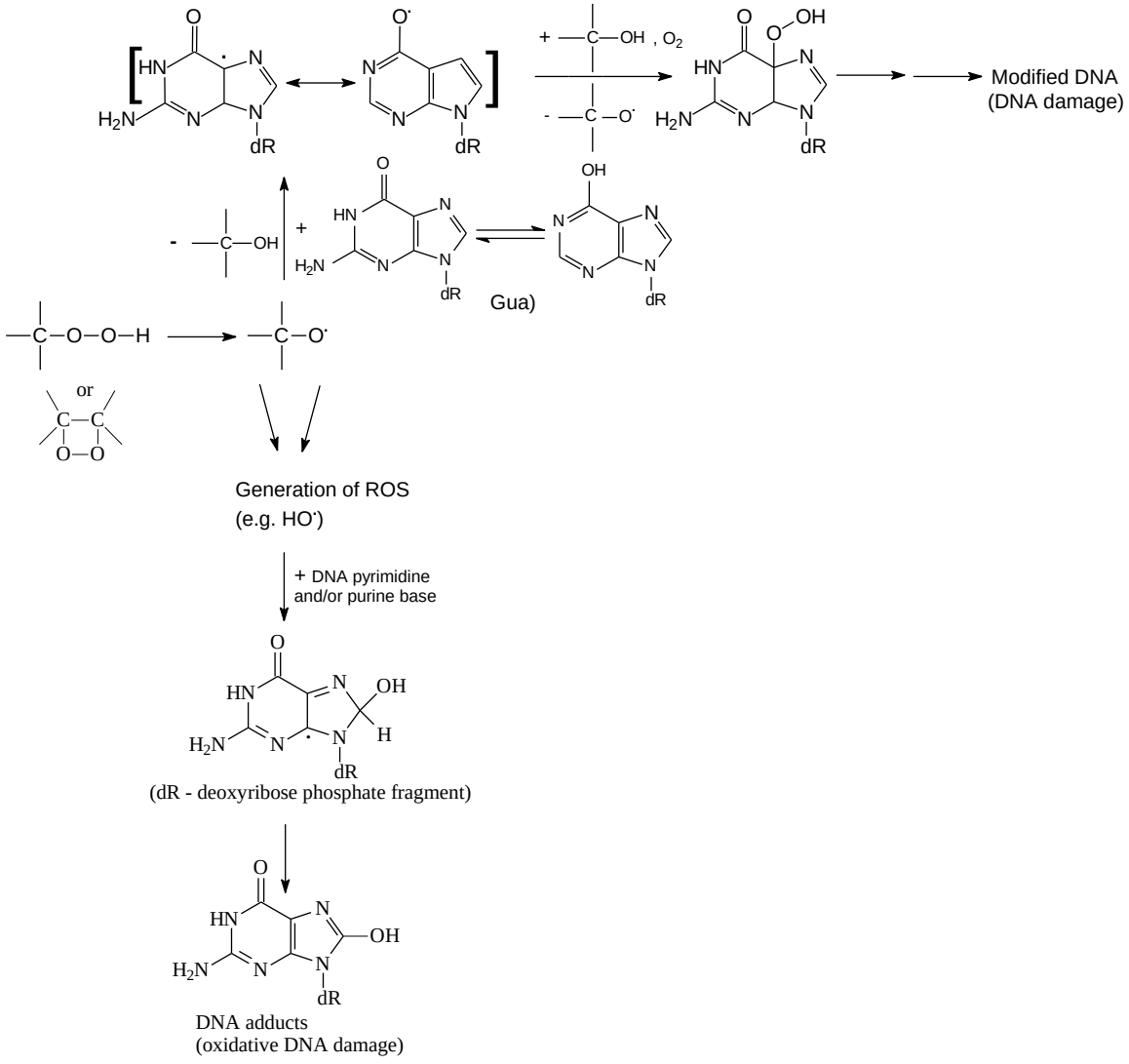


Such radicals, similarly to the hydroxyl ones are also involved in the oxidative stress [2].
Mutagenicity of various organic peroxy compounds, including TBHP, cumene hydroperoxide, 1,2,3,4-tetrahydronaphtyhalene hydroperoxide, etc. has been observed.
Based on the above assumptions and other considerations proving the DNA damage by TBHP mediated by Fe(II)-catalyzed generation of oxygen radicals [9], the following radical mechanism of DNA attack by TBHP has been proposed [10]:



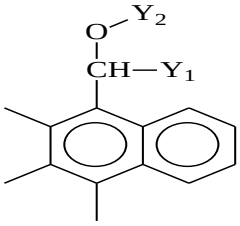
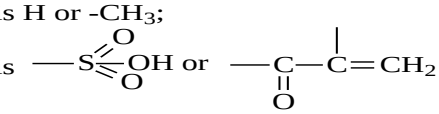
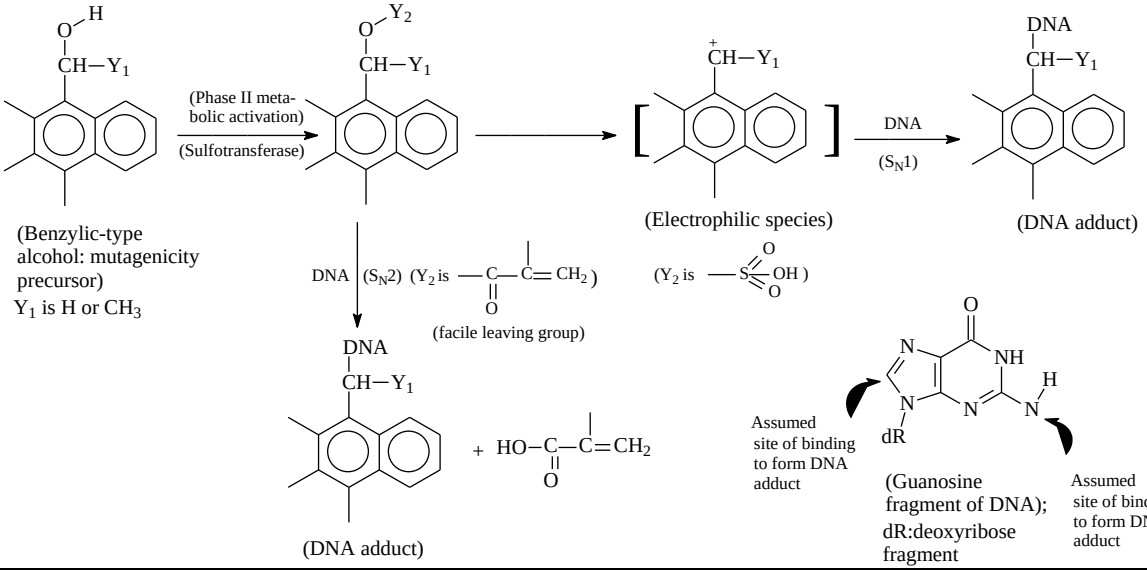
Endoperoxides have also been suggested to induce generation of reactive oxygen species [11]. However, with endoperoxides, it is assumed that hydrophobicity and the lack of bulky substitients on the carbon atoms, which could cause steric hindrance are important factors contributing to mutagenicity.

Therefore, a more general mechanistic scheme should involve the generation of ROS, more specifically, hydroxyl radicals HO. which are DNA-reactive, and thus adducts, involving pyrimidine and purine nucleoside bases can be formed. Such adducts are therefore formed when a DNA molecule is exposed to pro-oxidant species. On the basis of the above discussions, the following hypothetical mechanistic scheme for eliciting mutagenicity of hydroperoxides and endoperoxides can be assumed:

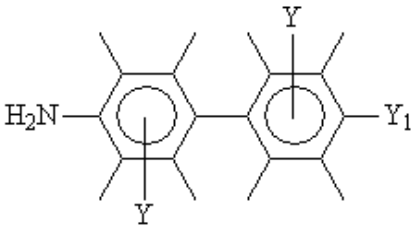
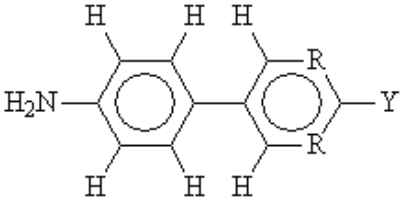


Set of chemicals used for profile development	Organic Peroxy Compounds

Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.O' Donnel, Biochem. J. **304** (1994), 707 - 713. 2. Adam, Chem. Res. Toxicol. **11** (1998), 1089 - 1097. 3. Stock, S., Arch. Toxicol. **72**(6) (1998), 342 - 346. 4. Dillon, Mutagenesis **13**(1) (1998), 19 - 26. 5. Edenharder, Mutat. Res. **540**(1) (2003), 1 - 18. 6. Kovacic, Current Med. Chem. **8** (2001), 773 – 796. 7. Aust, Proc. Soc. Exp. Biol. Med. **222**(3) (1999), 246 – 252. 8. Valko, Chem. Biol. Interact. **160** (2006), 1 – 40. 9. Epe, Environ. Health Persp. **88** (1990), 111 – 115. 10. Hix, Chem.-Biol. Interact. **118** (1999), 141 – 149. 11. Mercer, J. Biol. Chem. **286**(2) (2011), 987 – 996.

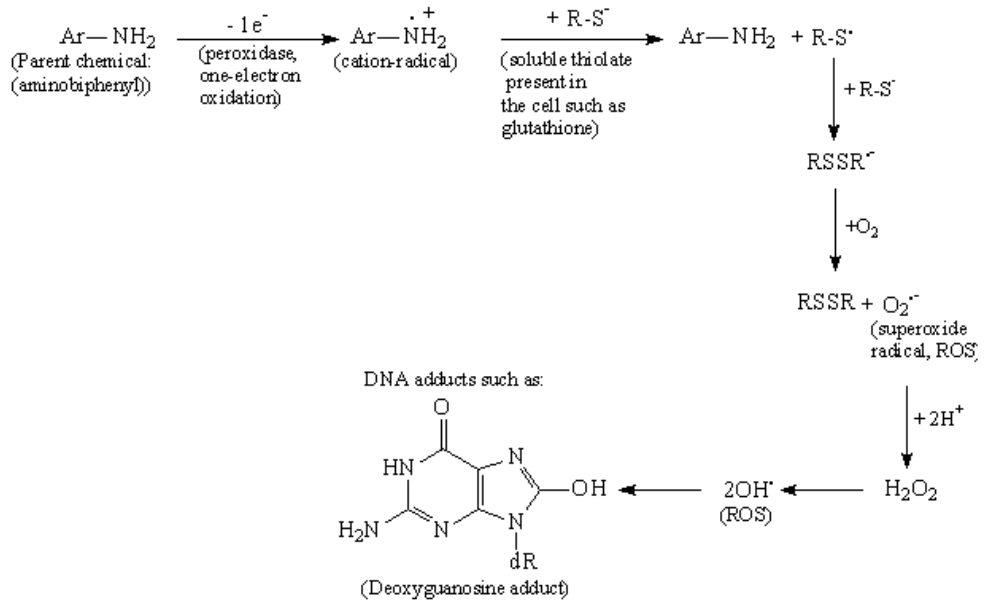
Individual profile/alert	
Name	PAH Benzylic Alcohol Esters
Type of profile	Structural alert
Description/applicability domain	 Y_1 is H or -CH_3; Y_2 is 
Mechanism	SN1 Electrophilic species generation followed by nucleophilic attack of DNA fragment SN2 Nucleophilic replacement of facile leaving group by DNA fragment
	
Set of chemicals used for profile development	No chemicals in the local training set

Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Glatt, H., Bioactivation of Mutagens via Sulfation, The FASEB Journal 11 (1997), 314 – 321; https://faseb.onlinelibrary.wiley.com/doi/pdf/10.1096/fasebj.11.5.9141497. Last visited: June, 2021. 2. Ravi Kumar, M. N. V., M. V. Vadhanam, J. Horn, J. W. Flesher, R. G. Gupta, Formation of Benzylic-DNA Adducts Resulting from 7,12-Dimethylbenz[a]anthracene in Vivo, Chem. Res. Toxicol 18 (2005), 686 - 691. 3. Jeurissen, S. M. F., Bioactivation and Genotoxicity of the Herbal Constituents Safrole, Estragole and Methyleugenol, PhD thesis, 2007; https://edepot.wur.nl/121896. Last visited: June, 2021.

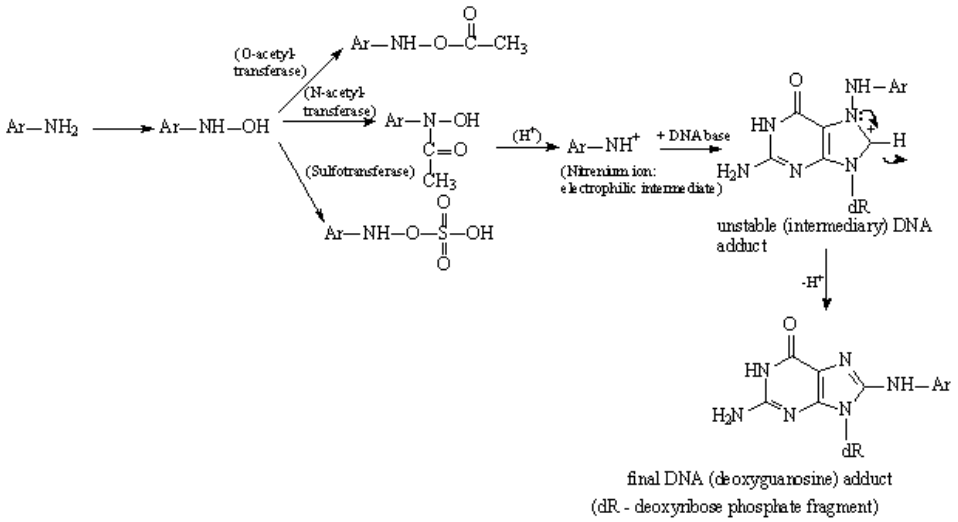
Individual profile/alert	
Name	p-Aminobiphenyl Analogs
Type of profile	Structural alert
Description/applicability domain	 (Y can be F, Cl, Br, or -OCH_3, or -CH_3 or -NO_2; no other types of substituents; Y_1 can be -NH_2 or *p*-$C_6H_4NH_2$; no more than totally three substituents on each benzene ring; single (non-fused) benzene rings only)  (Y can be -NH_2 or *p*-$C_6H_4NH_2$; R can be C and N or both N)

Mechanism	S_N1 Nucleophilic attack after nitrenium ion formation & Radical ROS generation (indirect)

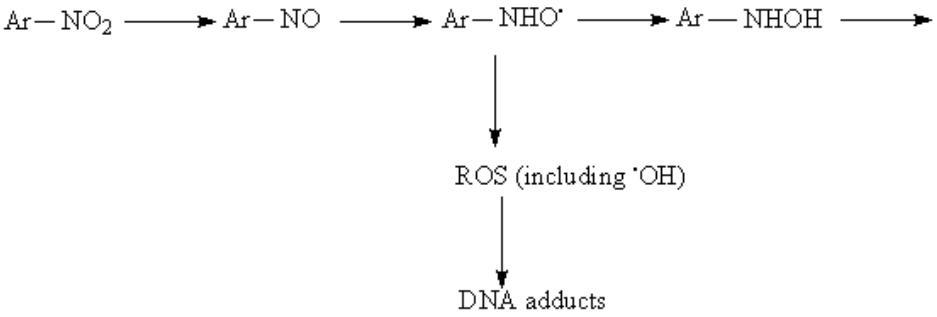
If the presence of endogenous peroxidase enzymes in the "classical" *Salmonella typhimurium* strains is assumed, the following mechanistic scheme involving the formation of reactive oxygen species (ROS) could explain the observed positive *in vitro* bacterial mutagenicity results for aminobiphenyls as parent chemicals shown below in Scheme 1:



However, there is strong evidence that aromatic amines, including aminobiphenyls in most cases require metabolic activation with the external microsomal S9 system for eliciting mutagenicity and carcinogenicity. According to an excellent review on the bioactivation pathways of organic functional groups, the obligatory step in the bioactivation of all aniline derivatives involves enzymatic N-hydroxylation on the primary amine nitrogen, leading to the formation of *N-hydroxylamine* intermediate. These reactive N-hydroxylamine derivatives (metabolites) can undergo phase II conjugation, to generate the more reactive N-O sulfate and/or N-O acetyl conjugates. The excellent leaving group capability of sulfonyloxy- and acetoxy-functionalities in these conjugates is believed to lead to a highly reactive *nitrenium ion*. The nitrenium ion electrophilic species may readily bind covalently to cellular DNA and RNA [5]. The principal *in vitro* metabolic pathway causing mutagenicity of aromatic amines is thefefore associated with metabolic activation induced by interactions with the CYP450 isoenzyme CYP1A2, and can be outlined as follows shown below in Scheme 2 [6]:



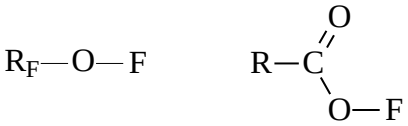
Reduction of the nitro group to nitroso intermediate is followed by formation of N-hydroxylamine species, and may occur endogenously by the bacterial nitroreductase in the prokaryotic *Salmonella typhimurium* cell. As a result, from the generation of reactive radical species such as $ArNHO^{\cdot}$, an additional formation of ROS such as $O_2^{\cdot-}$ and/or $HO^{\cdot}$ occurs. The hydroxyl radical, for example, is DNA-reactive and adducts, involving pyrimidine and purine nucleoside bases can be formed. The 8-hydroxyguanine adduct is one of the most mutagenic lesions so far discovered, which can induce DNA strands breaks, etc. Shown below in Scheme 3 [15, 16]:



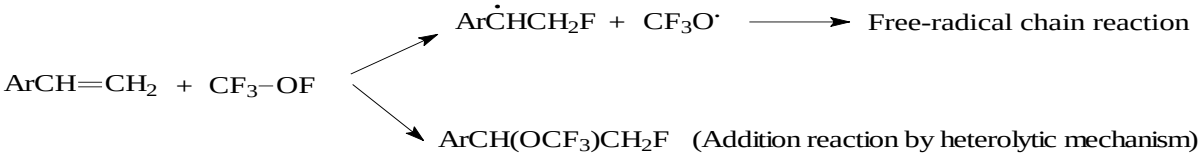
Scheme 3

Set of chemicals used for profile development	p-Aminobiphenyl Analogs
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Savard, Carcinnog. **7** (1986), 1239 – 1241. 2. Lang, Mutat. Res. **191** (1987), 139 – 143. 3. Subrahmany, Chem.-Biol. Interactions **56** (1985), 185 – 199. 4. Makena, Environ. Molec. Mutagenesis **48** (2007), 404 – 413. 5. Kalgutkar, Curr. Drug Metabol. **6**(3), 2005, 161 – 225. 6. Shamovsky, JACS **133** (2011), 16168 – 16185. 7. Humphreys, Proc. Natl. Acad. Sci USA, **89** (1992), 8278 – 8282. 8. Reid, Environ. Mutag. **6** (1984), 145 – 151. 9. Ashby, Mutat. Res. **257** (1991), 229 – 306. 10. Sokolowska, Dyes and Pigments **48** (2001), 15 – 27.

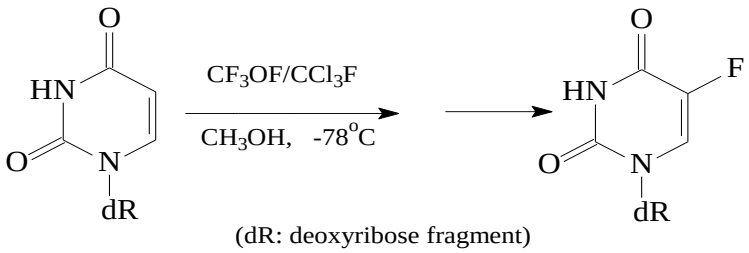
	11. El-Bayoumy, Mutat. Res. 90 (1981), 345 – 354. 12. Sinsheimer, Mutat. Res. **268** (1992), 255 – 264. 13. Chung, Toxicol. Sci **56** (2000), 351 – 356. 14. Ioannides, Carcinog. **10**(8) (1989), 1403 – 1407 (Abstract); http://www.ncbi.nlm.nih.gov/pubmed/2665965. Last visited: June, 2021. 15. Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 16. Wiseman, Biochem. J. 313 (1996), 17 – 29. 17. You, Mutat. Res. 319 (1993), 19 – 30.

Individual profile/alert	
Name	Perfluorinated Hypofluorites – Potential DNA Reactivity
Type of profile	Structural alert
Description/applicability domain	 (R_F is C_nF_{2n+1} (perfluorinated alkyl chain); R is C_nH_{2n+1} or C_nF_{2n+1}, n = 1 - 5)
Mechanism	S_E2: Electrophilic substitution at sp3 and sp2-carbon atoms A_E2: Electrophilc addition to C=C double bond

The following generalized mechanistic scheme involving radical and/or heterolytic mechanism of interactions of perfluoroalkyl hypofluorites with alkenes has been assumed [2]:



Direct fluorination of uracil and cytosine bases and nucleosides by using trifluoromethyl hypofluorite has been reported. The formation of DNA fluorinated adduct(s) would occur, according to the following general scheme [3]:



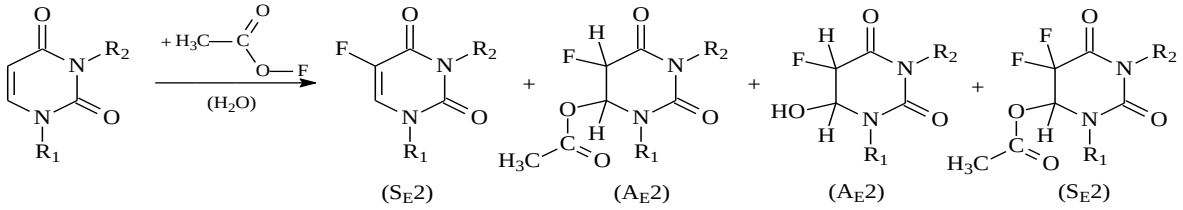
(dR: deoxyribose fragment)

(Scheme 1)

The reaction of acetyl hypofluorite with DNA bases such as uracil, cytosine and some of their N-substituted derivatives dissolved in water has been studied. Cytosine adducts readily underwent deamination in water to the corresponding uracil analogues. The following schemes for interaction, occurring by electrophilc attacks of fluorine on DNA bases have

been suggested [5]:

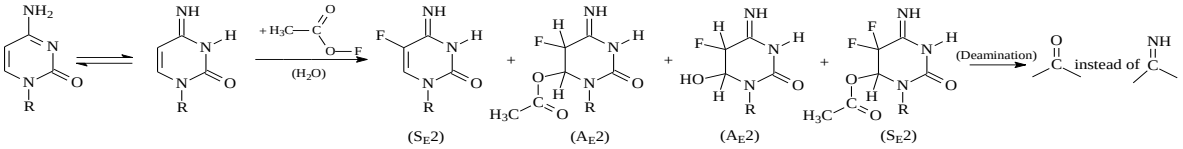
Uracil and Its Derivatives (Scheme 2):



(R_1, R_2 are H (both) or -CH_3 (both) or H and -CH_3) (

Scheme 2)

Cytosine and Its Derivatives (Scheme 3):



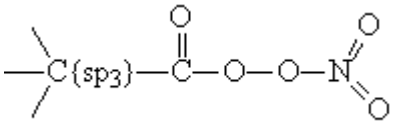
(R is H or -CH_3) (

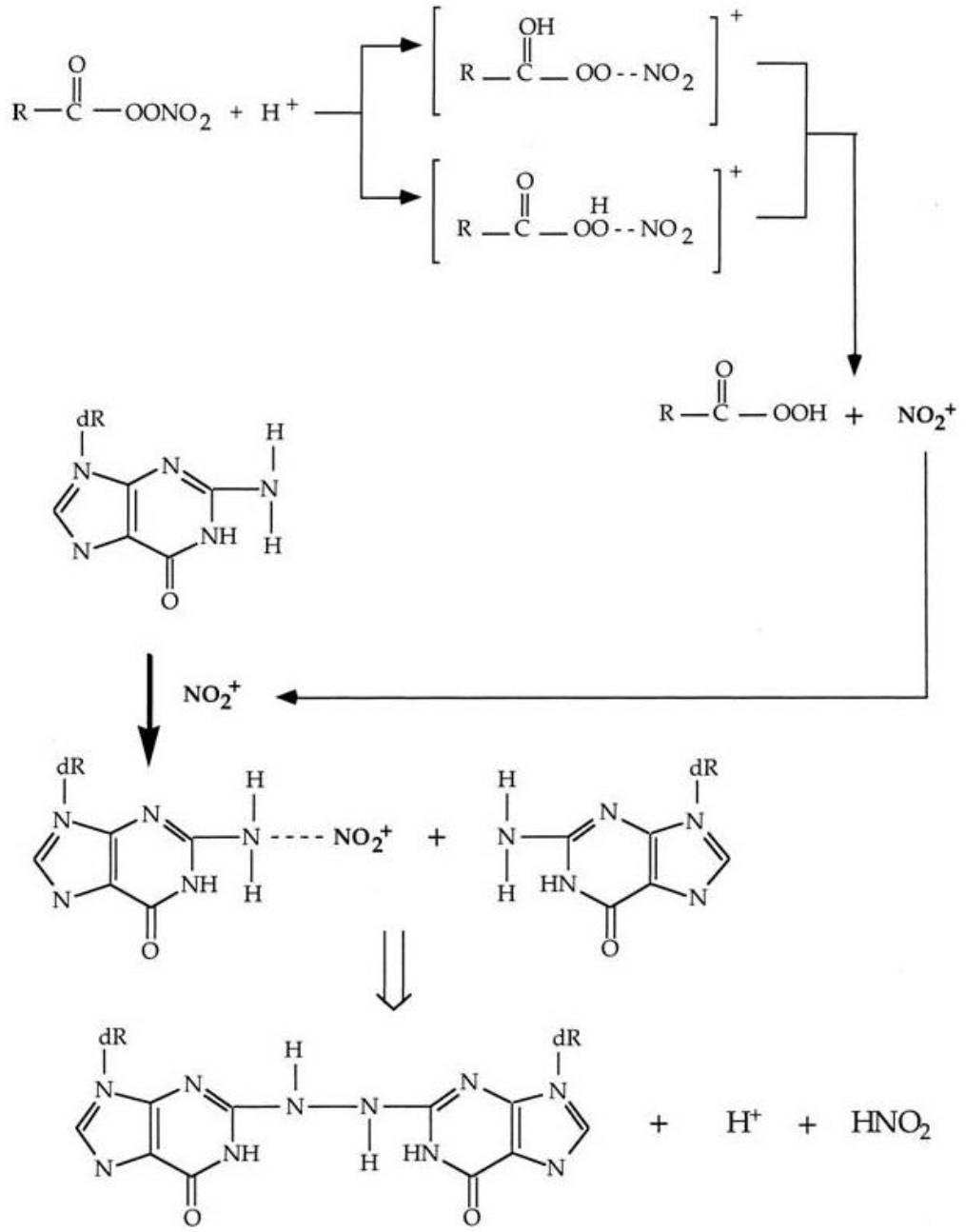
Scheme 3)

Conclusion: Chemicals from the sub-class discussed above are assumed to be DNA-reactive and, despite of lack of any relevant data, are likely to exert positive in vitro genotoxicity effects.

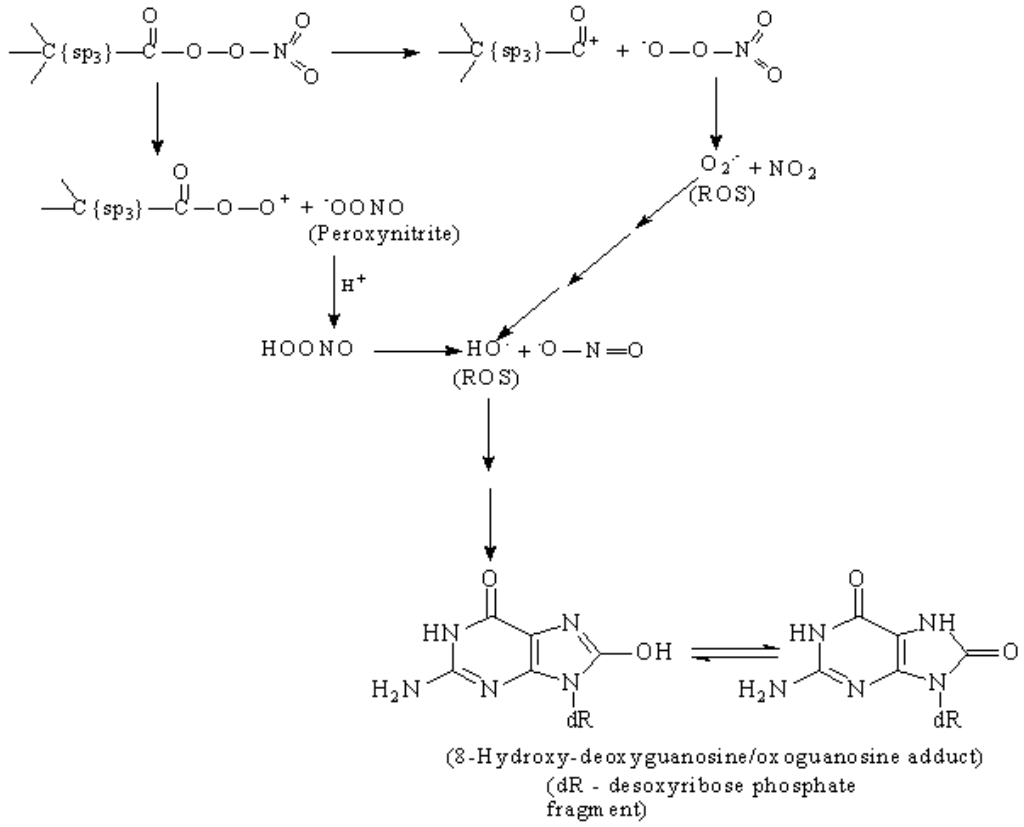
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Navarrini, W., FR. Venturini, M. Sansotera, M. Ursini, P. Metrangolo, G. Resnati, M. Galimberti, E. Barchiei, P. Dardani, The use of perfluoroalkyl hypofluorites for an efficient synthesis of perfluorinated ethers characterized by low Ostwald coefficient, J. Fluor. Chem. 129 (2008), 680 – 685. 2. Navarini, W., V. Tortelli, A. Russo, S. Corti, Organic Hypofluorites and Their New Role in Industrial Fluorine Chemistry, J. Fluor. Chem. 95 (1999), 27 – 39. 3. Robins, M. J., M. MacCoss, S. R. Naik, G. Ramani, Nucleic Acid Related Compounds. 21. Direct Fluorination of Uracil and Cytosine Bases and Nucleosides Using Trifluoromethyl Hypofluorite. Mechanism, Stereochemistry,

	and Synthetic Applications, J. Am. Chem. Soc. 98:23 (1976), 7381 – 7389. 4. Acetyl Hypofluorite; http://reag.paperplane.io/00000028.htm, last visited 09.2019.. 5. Visser, G. W. M., R. E. Herder, F. J. J. deKanter, D. M. Jacobus, Fluorination of Pyrimidines. Part 2. Mechanistic Aspects of the Reaction of Acetyl Hypofluorite with Uracil and Cytosine Derivatives, J. Chem. Soc. Perkin Trans. I, 1988, 1203 – 1207.

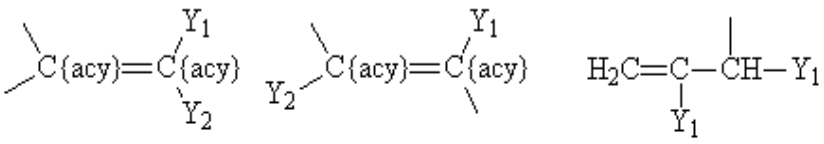
Individual profile/alert	
Name	Peroxyacyl Nitrates
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS generation and S_N1 or S_N2 Nitrosation
The following mechanistic scheme for the generation of active electrophilic species and interaction with DNA (deoxyguanosine fragment) has been suggested [3]:	



According to another mechanistic hypothesis, PAN may release peroxyacetyl nitrite and other reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxide radical, which may cause mutagenicity and cell apoptosis. The corresponding scheme of ROS generation and formation of DNA adduct can be outlined as follows [5]:

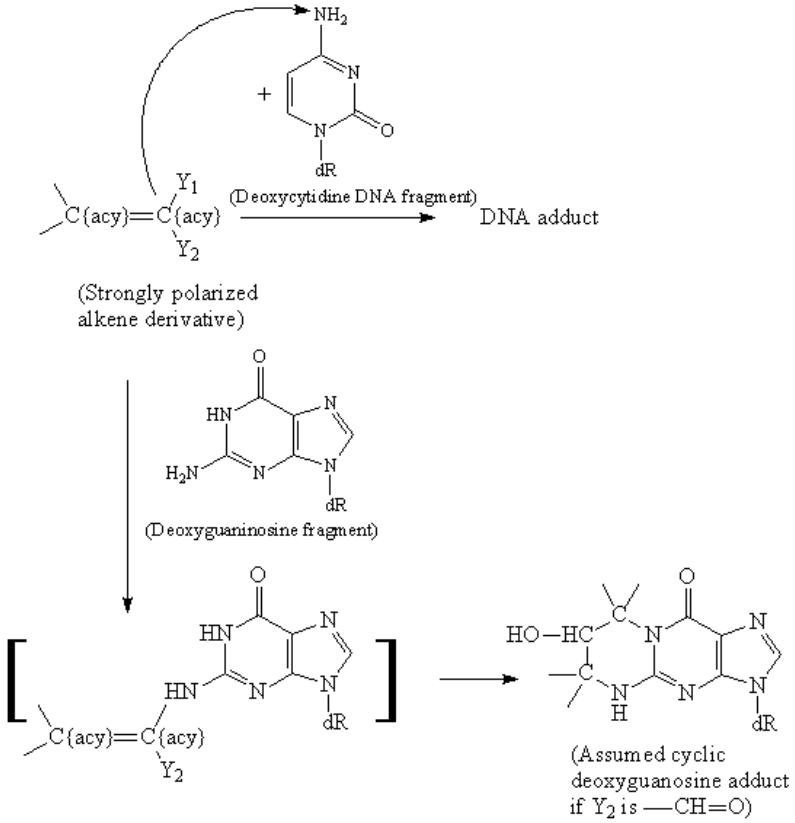


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kleindienst, Mutat. Res. **157**(2-3) (1985), 123 - 128. 2. Kleindienst, Environ. Mol. Mutagen. **16**(2) (1990), 70 - 80. 3. DeMarini, Mutat. Res. **457**(1-2) (2000**)**, 41 – 55. 4. CCRIS: *Peroxyacetylnitrate, Toxicology Data Network*, U.S. https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=2278-22-0. Last visited: June, 2021. 5. Liu, Mol. Carcinog. **25** (1999), 196 – 206.

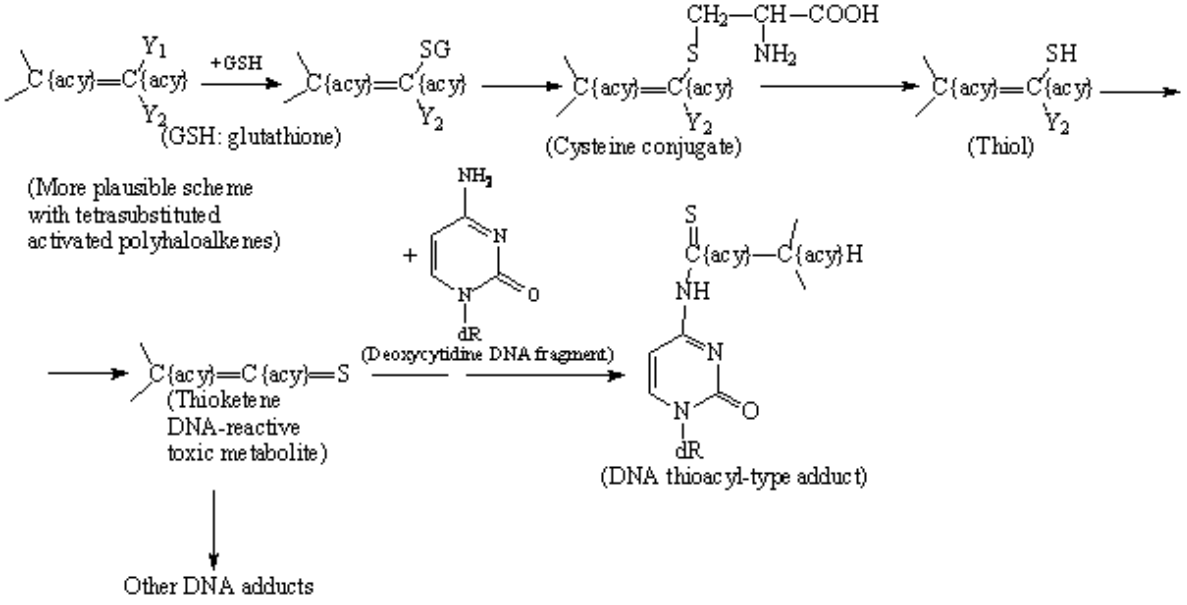
Individual profile/alert	
Name	Polarized Haloalkene Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y_1 is -Cl, -Br, -I; Y_2 is C(O) (carbonyl), -CN, -C-Cl, -C-Br, -C-I -OP(O)O- (phosphate group), -NO_2)
Mechanism	S_N2 Alkylation, direct acting epoxides and related after P450-mediated

	metabolic activation, S_N2-type alkylation at sp3 and activated sp2 carbon atom, A_N2 Thioacylation *via* nucleophilic addition after thioketene formation and A_N2 Shiff base formation

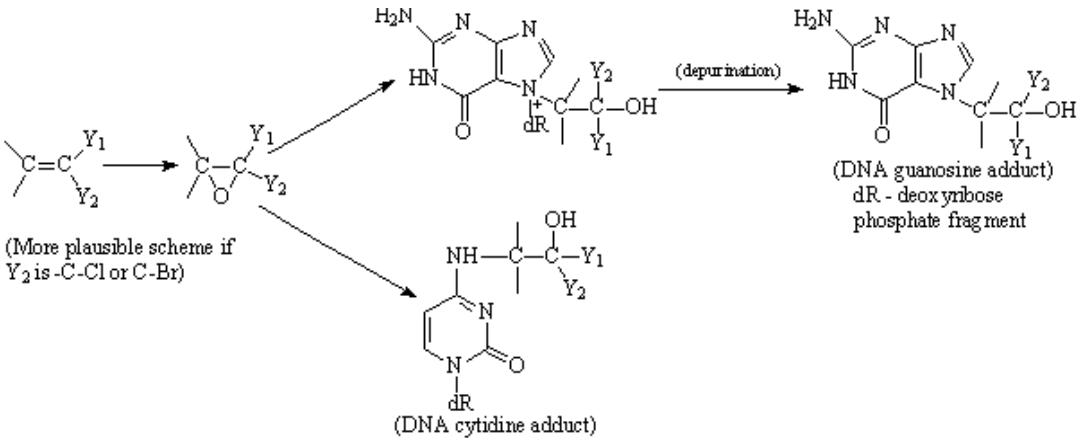
Direct alkylation (expertly assumed) – geminally bound halogen (Y_1) and strong electron-withdrawing substituent (Y_2) could make the former more labile, eliciting alkylating capability towards DNA pyrimidine and/or purine bases shown in Scheme 1:



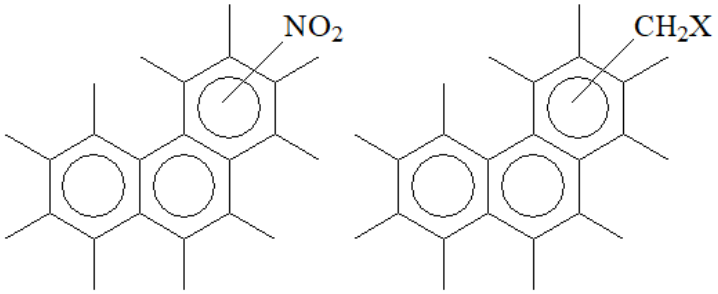
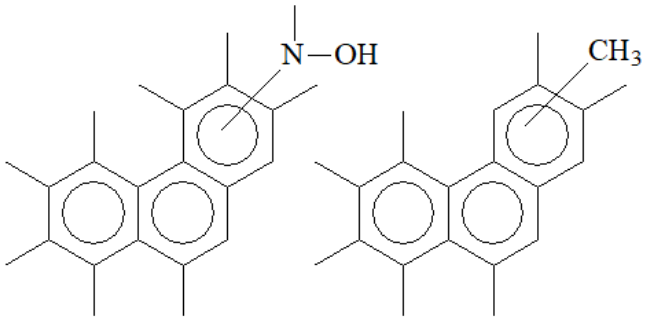
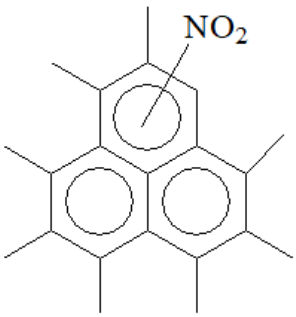
Bearing in mind the structural similarity of compounds such as trichloropropenenitrile and 2-chloropentene-2-nitrile with other haloalkenes such as trichloroethylene, terachloroethylene, trichlorotrifluoropropene, etc., glutathione-dependent enzymatic metabolic bioactivation with the formation of active thioketene metabolite, catalyzed by phase II glutathione transferase and beta-lyase can be suggested for this class of chemicals [6, 7]. 3,N^4-Thioacetylcytosine has been, for example, identified as one of the DNA adducts with thioketene intermediates [8]. Therefore, by analogy, one of the possible mechanistic schemes that could be applied to this class of chemicals could be expertly suggested as follows shown in Scheme 2:

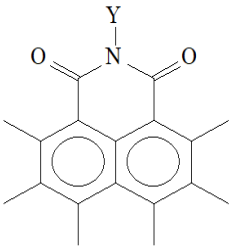
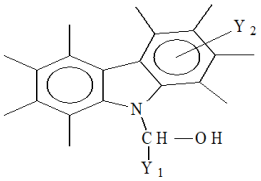


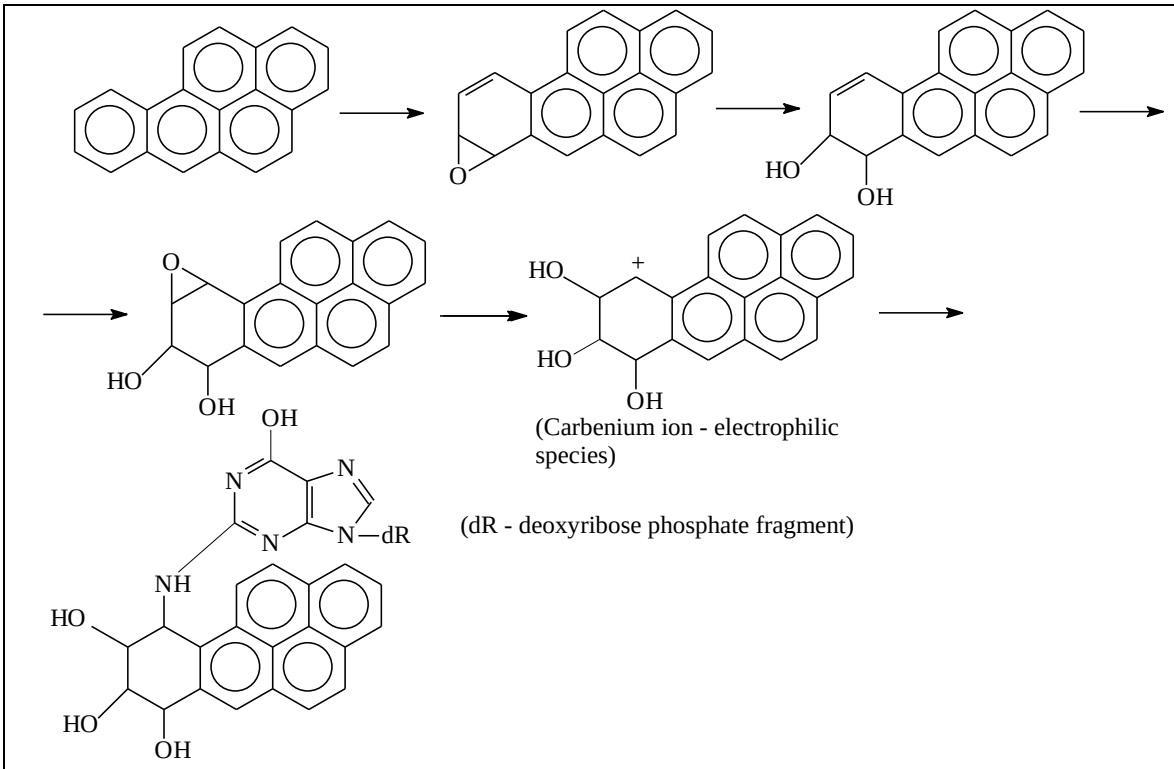
Scheme III: Metabolic activation *via* epoxidation shown in Scheme 3:

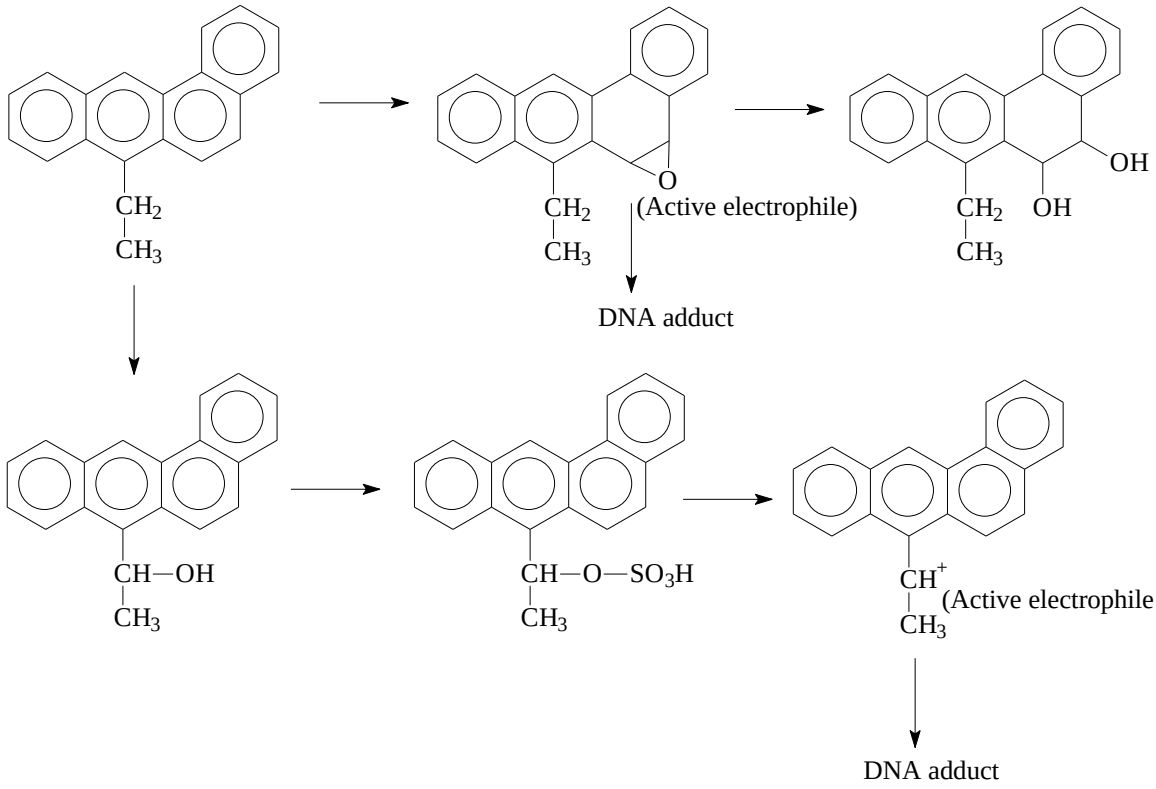


Set of chemicals used for profile development	Polarized Haloalkene Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Woo, Environ. Health Persp. **110** (Suppl. 1) (2002), 75 - 87. 2. Bull, Toxicol. **286** (2011), 1 – 19. 3. *Beta-Bromo-Beta-Nitrostyrene (CAS No. 7166-19-0) Administered by Gavage to F344/N rats and B6C3F1 Mice* (Prepared by J. R. Bucher), NTP, NIH Publication 94-3389, US Department of Health and Human Services, NIH, August 1994. 4. Eder, Mutat. Res. **322** (1994), 321 – 328. 5. Neudecker, Mutat. Res. **170** (1986), 1 – 9. 6. Kim, D., Drug Metab. Dispos. **34**, 2006, 2020 – 2027. 7. Decant, Environ. Health Persp. **88** (1990), 107 – 110. 8. Muller, Toxicol. **11**(5) (1998), 464 – 470; http://pubs.acs.org/doi/abs/10.1021/tx9701440. Last visited: June, 2021.

Individual profile/alert	
Name	Polycyclic Aromatic Hydrocarbon, Naphthaleneimide and Carbazole Derivatives
Type of profile	Structural alert
Description/applicability domain	 (X is Cl, Br)   (The substituents can be attached anywhere) **Typical PAH derivatives**

	 (Y is —(CH$_2$)$_n$—N{V$_3$}— or —N{V$_3$} (n = 2 or 3) No more than two fused benzene rings; No -C(O)O-, -C(O)NH- or -SO$_3$H groups attached **Naphthaleneimide derivatives**  (Y$_1$ is –H or –CH$_3$; Y$_2$ is –H or –CH$_3$ (number of –CH$_3$ groups 1 or 2, can be attached anywhere); or –H (all); No other substituents) **Carbazole derivatives**
Mechanism	S_N2 Alkylation, direct acting epoxides and related after P450-mediated metabolic activation, S_N1Alkylation after metabolically formed carbenium ion species and Non-covalent interactions DNA intercalation S_N1 Nucleophilic attack after metabolic N-hydroxylation and nitrenium ion formation

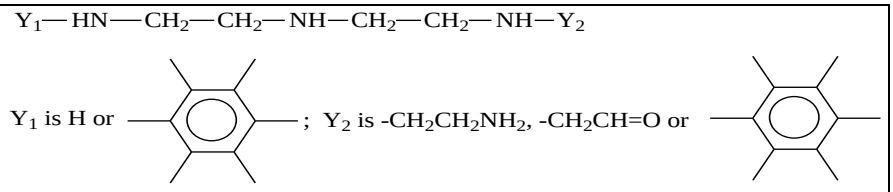




Set of chemicals used for profile development	Polycyclic Aromatic Hydrocarbon, Naphthaleneimide and Carbazole Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Low, L. K., N. Castagnoli, Jr., *Drug Biotransformations* (In Burger's

	Medicinal Chemistry, 4th Ed., Part I (The Basis of Medicinal Chemistry, John Wiley&Sons, Inc. 1979), pp. 107 - 226. 2. Weston, A., C. C. Harris, *Chemical Carcinogenesis* (Ch. 12 from Cancer Medicine, 5th Edition, Ed. By R. C. Bast, D. W. Kufe, R. E. Pollock, R. R. Weichselbaum, J. F. Holland, E. Frei, 2000); http://www.ncbi.nlm.nih.gov/books/NBK20839/ Last visited: June, 2021. 3. Boroski, G. L., *Theoretical Study Related to the Carcinogenic Activity of Polycyclic Aromatic Hydrocarbon Derivatives*, J. Org. Chem. **64** (1999), 7738 – 7744. 4. Nagao, M., T. Yahagi, Y. Seino, T. Sugimura, N. Ito, *Mutagenicity of Quinoline and Its Derivatives*, Mutat. Res. **42** (1977), 335 – 342. 5. McKay, S., P. B. Farmer, P. D. Cary, P. L. Grover, *The Metabolism of 7-Etylbenz[A]anthracene by Rat Liver Microsomal Preparations*, Drug Metabol. Dispos. **15** (1987), 682. 6. Rinderie, St. J., S. D. Black, P. K. Sharma, *Comparative Metabolism In Vitro of a Novel Carcinogenic Polycyclic Aromatic Hydrocarbon, 1,2,3,4-Tetrahydro-7,12-Dimethylbenz[a]anthracene, and Its Two Regioisomeric B-Ring Fluoro Analogues*, Canc. Res. **52** (1992), 3035 – 3042. 7. Guengerich, F. P., J. B. Wheeler, Y. J. Chun, D. Kim, T. Shimada, P. Aryal, Y. Oda, E. M. Gilliam, *Use of Heterologously-Expressed Cytochrome P450 and Glutathione Transferase Enxymes in Toxicity Assays*, Toxicology **181 – 182** (2002), 261 – 264. 8. McKnight, R. E., *Insights Into the Relative DNA Binding and Preferred Binding Mode of Homologous Compounds Using Isothermal Titration Calorimetry (ITC)* (Ch. 6 in *Applications of Calorimetry in a Wide Context – Differential Scanning Calorimetry, Isothermal Titration Calorimetry and Microcalorimetry*), January 23, 2013; http://www.intechopen.com/books/applications-of-calorimetry-in-a-wide-context-differential-scanning-calorimetry-isothermal-titration-calorimetry-and-microcalorimetry/insights-into-the-relative-dna-binding-affinity-and-preferred-binding-mode-of-homologous-compounds-u). 9. Czerwinska, I., Sh. Sato, B. Juskowiak, Sh. Takenaka, *Interactions of Cyclic and Non-Cyclic Naphthalene Diimide Derivatives with Different Nucleic Acids*, Bioorg. & Med. Chem. **22** (2014), 2593 – 2601. 10. Liu, Z. R., K. H. Hecker, R. L. Rill, *Selective DNA Binding of (N-Alkylamine)-Substituted Naphthalene Imides and Diimides to G+C-Rich DNA*, J. Biomolec. Struct. And Dynamics **14**(3) (1996), 331 – 339 (Abstract); http://www.ncbi.nlm.nih.gov/pubmed/9016410. 11. LaVoie, E. J., G. Briggs, V. Bedenko, D. Hoffmann, *Mutagenicity of Substituted Carbazoles in Salmonella typhimurium*, Mutat. Res. **101** (1982), 141 – 150.

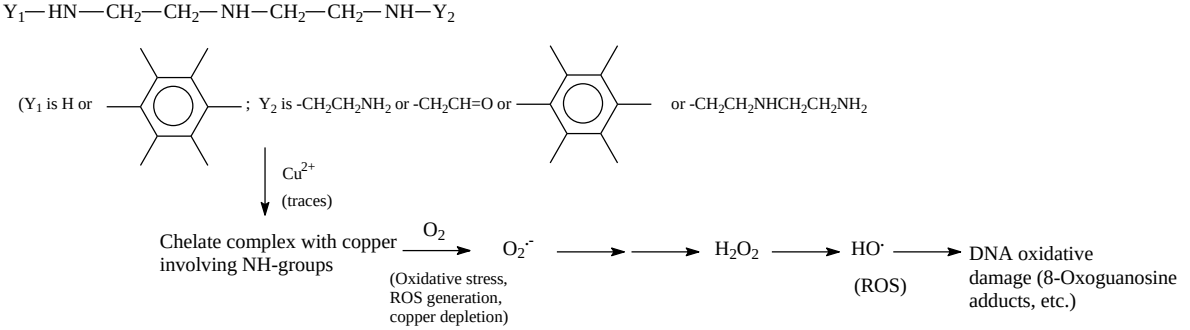
Individual profile/alert	
Name	Polyethylene Polyamines
Type of profile	Structural alert

Description/applicability domain	
Mechanism	Radical ROS generation

Trientine hydrochloride (TETA) showed mutagenicity as it was tested positive in the Ames Salmonella assay. As far as the in vitro mutagenic activity is concerned, the lower linear alkyleneamines such as some diamines and triamines were found to be devoid of genotoxic potential. However, positive mutagenic activity appears to be associated with the higher alkyleneamines (TETA, a tetramine and TEPA, a pentamine) [1].

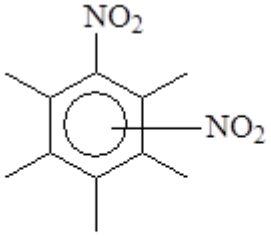
According to another report, trientine possesses strong chelating properties with respect to copper, due to the specific structure of linear tetramine with nitrogen-containing moieties as ligands. Trientine showed mutagenicity in bacterial cells with and without S9 metabolic activation in a broad range of bacterial strains, which indicated that this could be caused by oxidative stress, triggered as a consequence of copper depletion [2].

Based on the above discussions, and the presence of traces of transition metals such as coper in the incubation medium, the following, rather simplified mechanistic scheme can be expertly proposed:

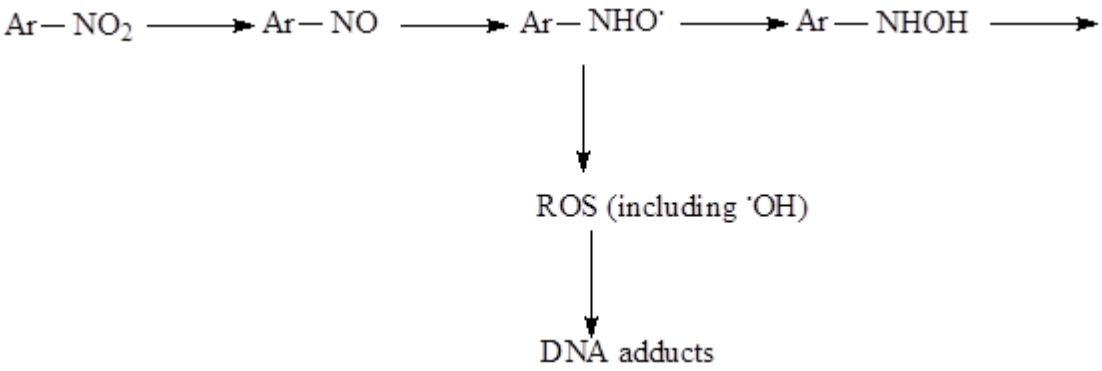


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Leung, H.-W., Evaluation of the genotoxic potential of trientine, Mutat. Res. 320 (1994), 31 – 43. 2. Assessment Report, Cufence (International non-proprietary name: Trientine hydrochloride), Procedure No. EMEA/H/C/004111/0000, European Medical Agency, Committee for Medicinal Products for Human Use (CHMP), 29 May 2019.

Individual profile/alert	
Name	Polynitroarenes
Type of profile	Structural alert

Description/applicability domain	 (Single arene ring in the whole molecular structure only; number of -NO_2 groups 2 or 3; number of substituents: no more than 4)
Mechanism	SN1: Nucleophilic attack after reduction and nitrenium ion formation and radical: ROS generation

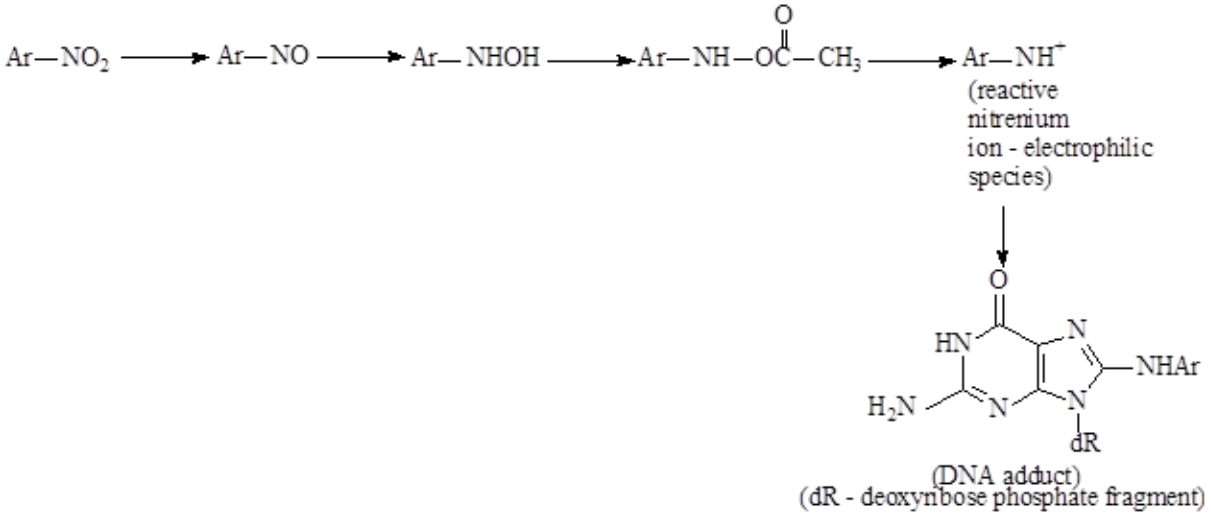
Radical (Homolytic) Mechanism**.** This is one of the mechanisms (*but not the most important*) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds ($ArNO_2$) are implicated in carcinogenesis [5]. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic *Salmonella typhimurium* cell. Several transient *radical intermediates*, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks)**:**



As a result from the generation of reactive radical species such as $ArNHO^{\cdot}$, an additional formation of ROS such as $O_2^{\cdot-}$ and/or $HO^{\cdot}$ occurs. The hydroxyl radical, for example, is DNA-reactive and adducts, involving pyrimidine and purine nucleoside bases can be formed. The 8-hydroxyguanine adduct is one of the most mutagenic lesions so far discovered, which can induce DNA strands breaks, etc. [6, 7]:

(dR - deoxyribose phosphate fragment) (Deoxyguanosine adduct)

Heterolytic Mechanism. This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases [1, 2, 8]:



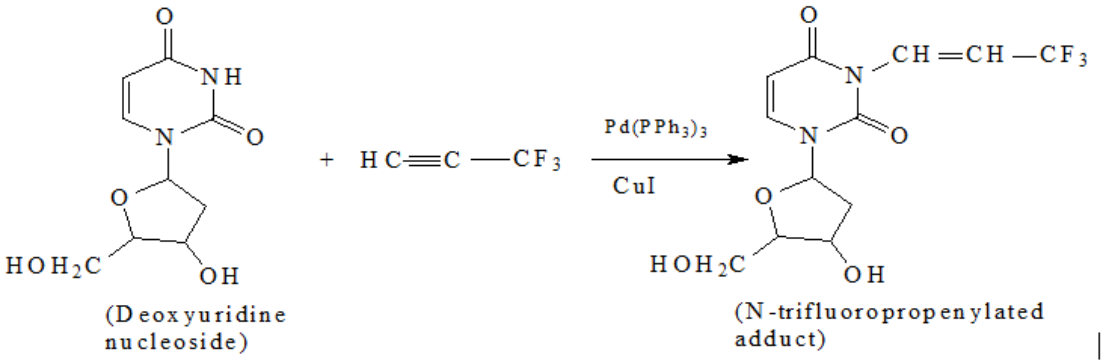
Chemicals such as 2,6-dinitrotoluene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene, etc., containing more than one nitro group were found to be bacterial mutagens both in the presence and the absence of S9 mix [4].

Set of chemicals used for profile development	Polynitroarenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Sabbioni, G., Hemoglobin Binding of Arylamines and Nitroarenes: Molecular Dosimetry and Quantitative Structure-Activity Relationships, Envir. Health Persp. 102, Suppl. 6 (1994), 61 – 67. 2. Kalgutkar, A. S., I. Gardner, R. S. Obach, C. L. Shaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O, Donnell, J. Boer, S. P. Harriman, A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups, Current Drug Metabol. 6 (2005), 161 – 225. 3. Aiub, Cl. A. Fortes, J. L. Mazzei, L. F. R. Pinto, I. Felzenszwalb, Evaluation of Nitroreductase and Acetyltransferase Participation in N-Nitrosodiethylamine Genotoxicity, Chem.-Biol. Interact. 161 (2006), 146 – 154. 4. Einisto, P., M. Watanabe, M. Ishidate Jr., T. Nohmi, Mutagenicity of 30 Chemicals in Salmonella typhimurium Strains Possessing Different Nitroreductase or O-Acetyltransferase Activities, Mutat. Res. 259 (1991), 95 – 102. 5. Kovacic, P., J. D. Jacintho, Mechanisms of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer, Current Med. Chem. 8, (2001), 773 – 796. 6. Witherell, H. L., R. A. Hiatt, M. Replogle, J. Parsonnet, Helicobacter pylori Infection and Urinary Excretion of 8-Hydroxy-2-deoxyguanosine, an Oxidative DNA Adduct, Canc. Epidemiol. Biomarkers & Prevention 7 (1998), 91 – 96. 7. Wiseman, H., B. Halliwell, Damage to DNA by Reactive Oxygen and Nitrogen Species: Role in Inflammatory Disease and

	Progression to Cancer, Biochem. J. 313 (1996), 17 – 29. 8. Purohit, V., A. K. Basu, Mutagenicity of Nitroaromatic Compounds, Chem. Res. Toxicol. 13(8) (2000), 673 – 692. 9. Grummt, T., H. G. Wunderlich, A. Chakraborty, M. Kundi, B. Majer, Fr. Ferk, A. K. Nersesyan, W. Parzefall, S. Knasmuller, Genotoxicity of Nitrosulfonic Acids, Nitrobenzoic Acids and Nitrobenzylalcohols, Pollutants Commonly Found in Ground Water Near Ammunition Facilities, Environ. Molec. Mutag. 47 (2006), 95 – 106.

Individual profile/alert	
Name	Propyne Derivatives – Potential DNA Reactivity
Type of profile	Structural alert
Description/applicability domain	$HC\equiv C-Y$ (Y are electron-withdrawing groups such as $-CF_3$, $-CHF_2$, $-CH_2F$, $-CH_2Cl$, $-CH_2Br$ or –CH=O)
Mechanism	SN_2: Alkylation, nucleophilic substitution at sp3-carbon atom AN_2: Nucleophilic addition to α,β-unsaturated carbonyl compounds

The reaction of 3,3,3-trifluoropropyne (CAS No. 661-54-1) with 2'-deoxyuridine to give N-propenylated nucleoside (N3-alkylation) was reported to occur, according to the following scheme:

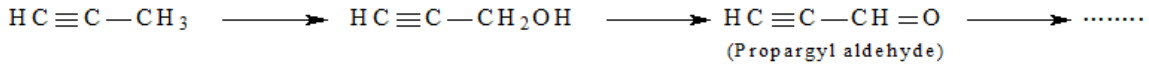


(Scheme 1)

In some separate experiments, however, it was shown that the catalyst was not required for the adduct formation. The mechanism of N-trifluoropropenylation was considered to be similar to the Michael-type addition. Here the N3 atom of pyrimidine fragment adds as a nucleophile to the terminal carbon atom of trifluoropropyne, which is electrophilic, due to the presence of strong electron-withdrawing –CF3 group (Scheme 1) [1]:

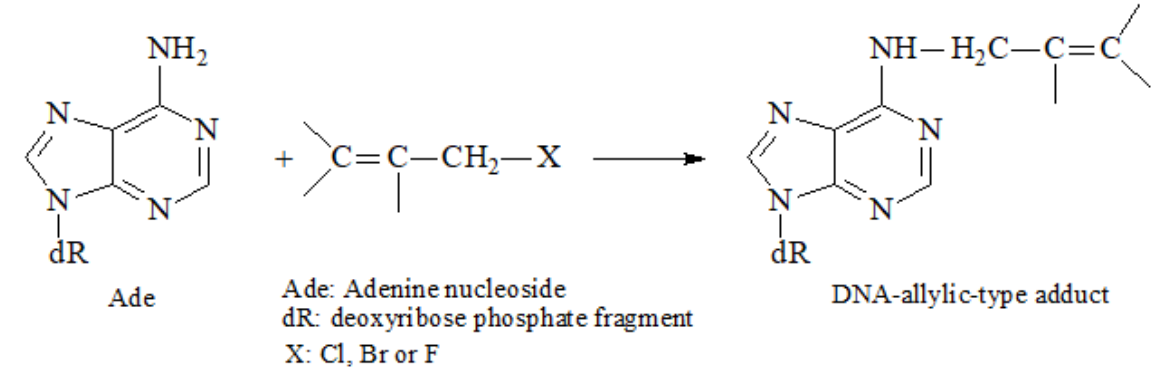
Therefore, despite the lack of relevant data on the in vitro genotoxicity of trihalopropynes such as 3,3,3-trifluoropropyne, potential DNA reactivity of this chemical is assumed.

After microsomal/S9 metabolic activation, propyne may be converted into propargyl aldehyde by the following scheme:

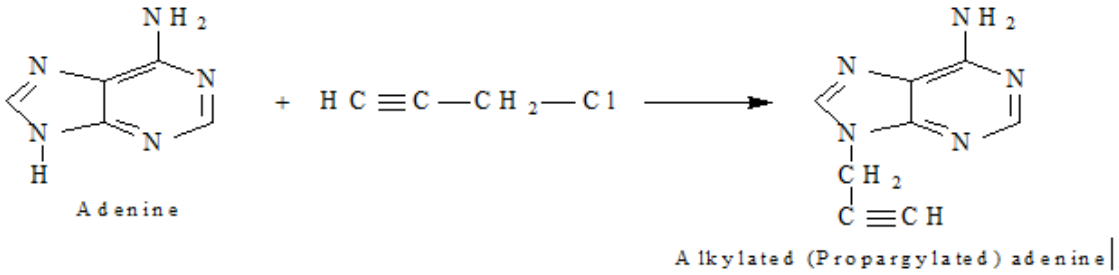


(Scheme 2)

Propargyl aldehyde has been reported to be strong bacterial mutagen [2]. It is likely to exert its DNA reactivity by a mechanism, similar to that depicted in Scheme 1 above.
Structurally close chemicals with electron-withdrawing -CH2Br or -CH2Cl groups attached to -C#CH fragment such as propargyl chloride and propargyl bromide, and positive bacterial mutagenicity data were found by read-across analysis. However, these chemicals are assumed to be DNA-reactive by different (SN) mechanism of DNA-alkylation (via heterolytic cleavage of the labile C-Hal bond), similarly to their allylic-type analogues (Schemes 3 and 4) [3, 4]:



(Scheme 3)

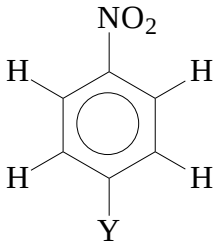


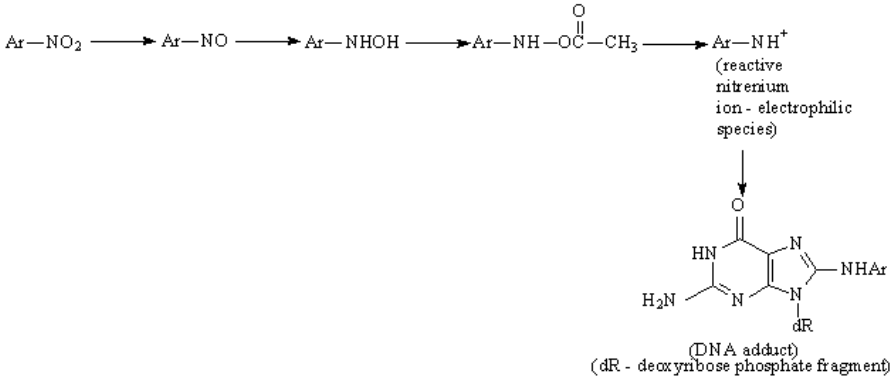
(Scheme 4)

Conclusion: Chemicals from the sub-class discussed above are assumed to be DNA-reactive and are likely to exert positive in vitro genotoxicity effects.

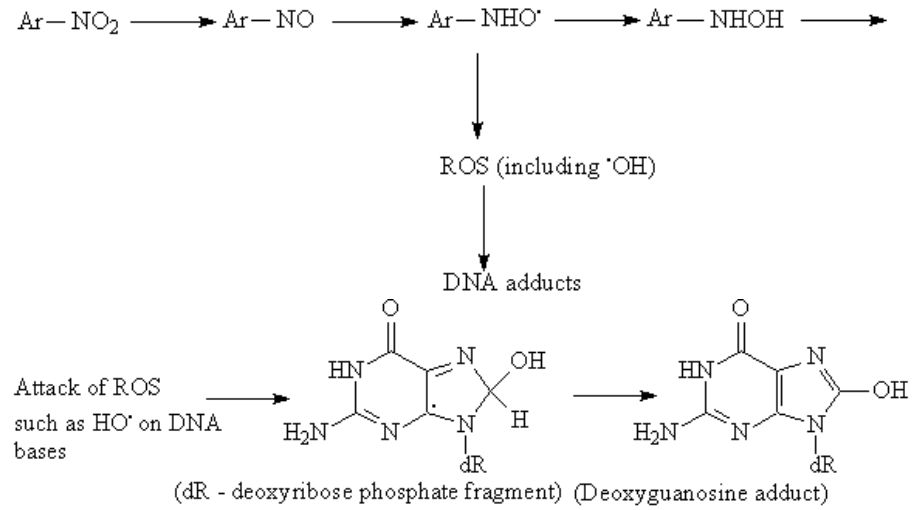
Set of chemicals used for profile development	Propyne Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Chirakul, P., S. Th. Sigurdsson, Unexpected Formation of 2'-Deoxy-N3-(3,3,3-Trifluoro-1-Propenyl) Uridine via a Michael-Type Addition to 3,3,3-Trifluoropropyne, Tetrahed. Lett. 44 (2003), 6899 – 6901. 2. Basu, A. K., L. J. Marnett, Molecular Requirements for the Mutagenicity of Malondialdehyde and Related Acroleins, Canc. Res.

	44 (1984), 2848 – 2854. 3. Eder, E., D. Henschler, T. Neudecker, Mutagenic Properties of Allylic and Alpha, beta-Unsaturated Compounds: Consideration of Alkylating Mechanisms Xenobiotica 12(12), 1982, 831-848. 4. Joshy, R. V., J. Zemlicka, Alkylation of Adenine with t-Propargyl Chlorides: Acetylene/Allene Ratio and N9/N1 Regioselectivity, Tetrahedron, 49 (12) (1993), 2353 – 2360.

Individual profile/alert	
Name	p-Subtituted Mononitrobenzenes
Type of profile	Structural alert
Description/applicability domain	 (Y can be C{sp3} or C{sp2 non-aromatic}
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, including the N-hydroxylamine (proximate mutagenic form) and its O-esterified derivative formed by phase II (O-acetylation, sulfation) enzymatic reaction with the subsequent generation of electrophilic nitrenium ion. The latter species may exert electrophilic attack on DNA bases. (**Nucleophilic attack after reduction and nitrenium ion formation)** **Radical (Homolytic) Mechanism.** This is one of the mechanisms (but not the most important) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds (ArNO2) are implicated in carcinogenesis. Reduction of the nitro to the nitroso intemediate is followed by formation of N-hydroxylamine species and may occur in the prokaryotic Salmonella typhimurium cell. Several transient radical intermediates, including reactive oxygen species (ROS) are formed during this process, and have been found to cause oxidative DNA damage (strand breaks) **(Radical mechanism *via* ROS formation (indirect)**
Heterolytic	

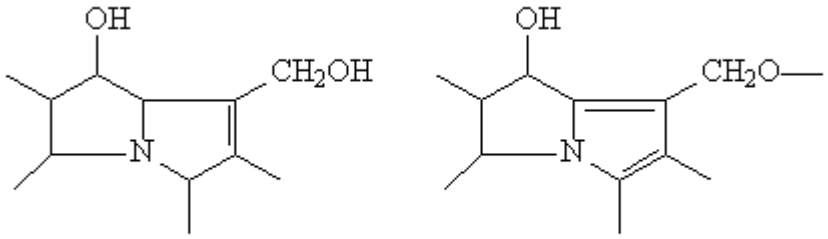


Homolytic

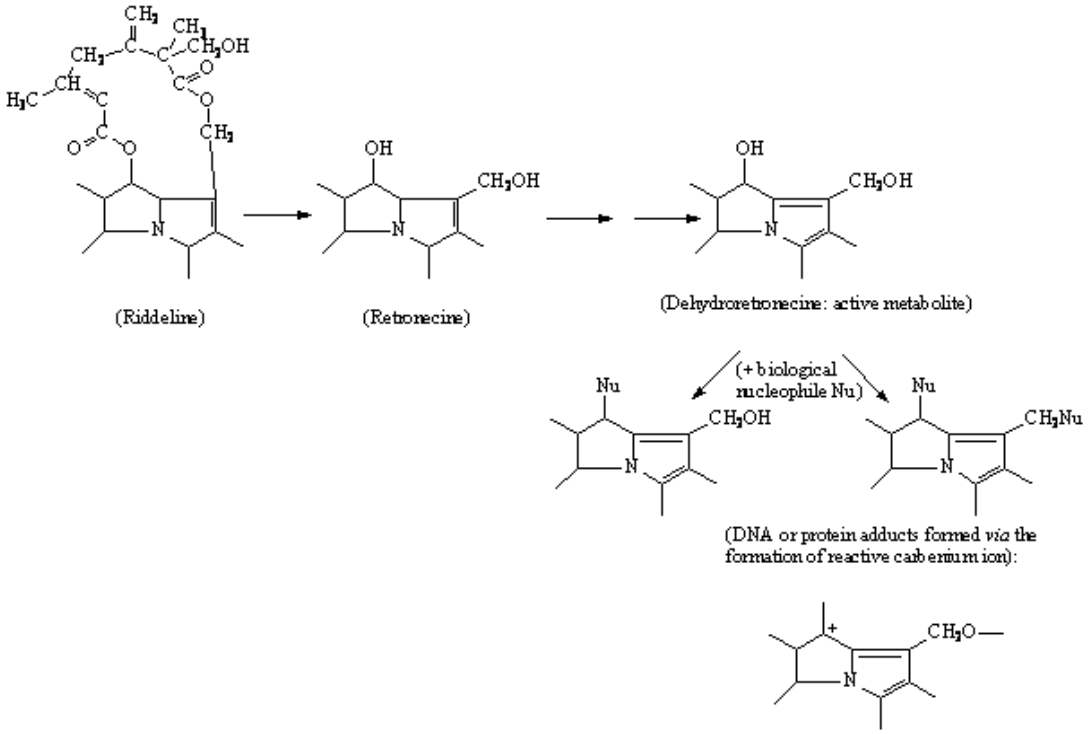


Set of chemicals used for profile development	p-Substituted Mononitrobenzenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, Envir. Health Persp. **102**, Suppl. 6 (1994), 61 – 67. 2.Kalgutkar, Current Drug Metabol. **6** (2005), 161 – 225. 3.Aiub, Chem.-Biol. Interact. **161** (2006), 146 – 154. 4.Einisto, Mutat. Res. **259** (1991), 95 – 102. 5.Kovacic, Current Med. Chem. **8**, (2001), 773 – 796. 6.Witherell, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96. 7.Wiseman, Biochem. J. **313** (1996), 17 – 29. 8.Purohit, Chem. Res. Toxicol. **13**(8) (2000), 673 – 692. 9. Shimizu, M., E. Yano, Mutat. Res. **170** (1986), 11 – 22; *Chemical Carcinogenesis Research Information System*, TOXNET, US National Library of Medicine.

Individual profile/alert	
Name	Pyrrolizidine Derivatives
Type of profile	Structural alert

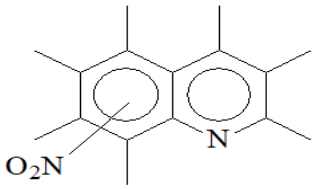
Description/applicability domain	
Mechanism	S_N1 Nucleophilic attack after carbenium ion formation

The following scheme of bioactivation and the formation of adducts with biological macromolecules has been proposed:

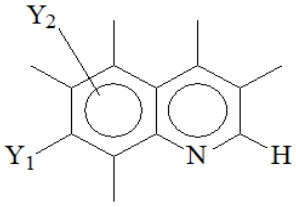


Set of chemicals used for profile development	Pyrrolizidine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Fu, Drug Metabol. Rev. **36**(1) (2004), 1 – 55. 2. Robertson, Canc. Res. **42** (1982), 8 – 14. 3. Reed, Carcinog. **9**(8) (1988), 1355 – 1361. 4. Yamanaka, Mutat. Res. **68** (1979), 211 – 216.

Individual profile/alert	
Name	Quinoline Derivatives
Type of profile	Structural alert
Description/applicability domain	Nitroquinoline Derivatives



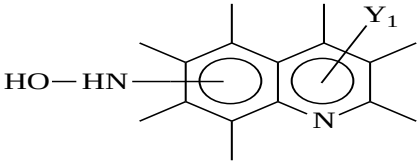
Aminoquinoline Derivatives



(Y_1 can be Cl or Br;
Y_2 can be -Cl, -Br, -COOH
in either ring)

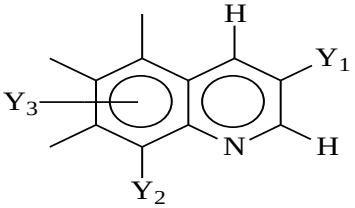
(-NH_2 can be attached to phenyl ring only: one substituent only;
Y_1 is –CH_3 or –C_2H_5 (one substituent only) or –H (all):
can be attached to *any* ring;

N-Hydroxylaminoquinoline Derivatives



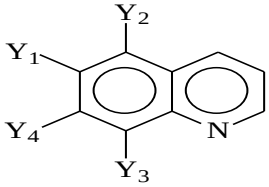
(-NHOH can be attached to any ring: one substituent only;
Y1 is -CH3 or –C2H5 (one substituent only) or –H (all):
can be attached to any ring);

Halogenated Quinolinecarboxylic Acids

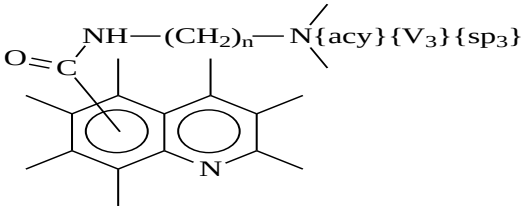
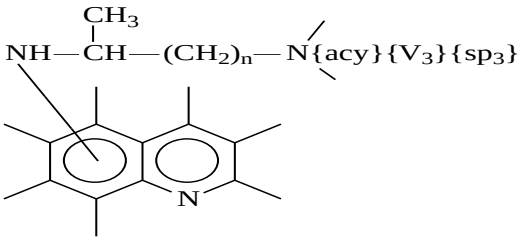
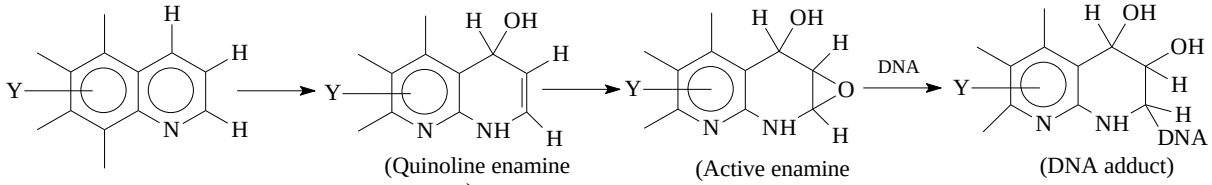


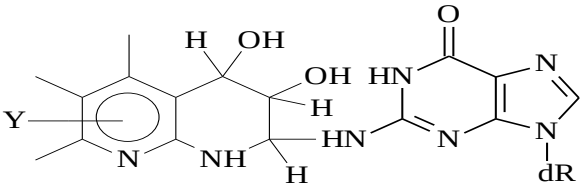
(Y1 is Cl, Br or –COOH;
Y2 is –COOH or –CH3 or -C2H5;
Y3 is –Cl or –Br (number of halogens 1 or 2);
No more than totally four substituents)

Other Quinoline Derivatives

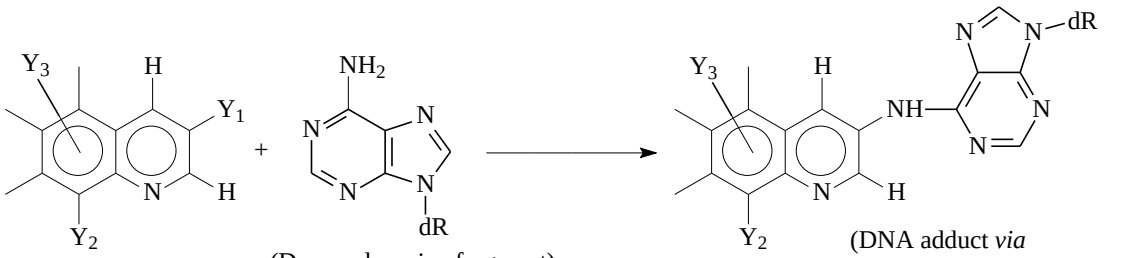


(Y1 – Y4 are –H (all); Y1 is –H or –F or –Cl; Y2 is –H or –OH or -F or –Cl;

	Y3 is –H or –OH or –F or –Cl; Y4 is –H or –F or –Cl) No more than one substituent (-F or -Cl or –OH) DNA Intercalating Agents  (Carboxamide side chain attached to phenyl ring; n = 1 – 3; No more than totally two substituents)  (Aminoalkylamine side chain can be attached to any of the rings; n = 1 – 3; No more than totally two substituents) (see also DNA Intercalators with Carboxamide and Aminoalkylamine Side Chain)
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: SN2 attack on activated carbon atom Mechanistic Domain: SN2 Mechanistic Alert: Direct acting epoxides formed after metabolic activation Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after nitro group reduction and nitrenium ion formation Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after metabolic N-hydroxylation and nitrenium ion formation Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after metabolic nitrenium ion formation
Mechanistic scheme 1 – with external metabolic activation: CYP 450 mediated microsomal/S9 bioactivation (epoxidation) of quinoline and some of its halogenated derivatives:  (Y is F, Cl, Br) (Quinoline enamine structure) (Active enamine epoxide) (DNA adduct) Note: DNA adduct could be, e.g., adduct with guanosine:	



Mechanistic scheme 2 - Direct attack on DNA bases. It is mainly associated with quinoline derivatives of type C (halogenated quinolinecarboxylic acids):



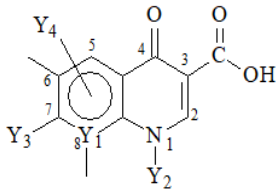
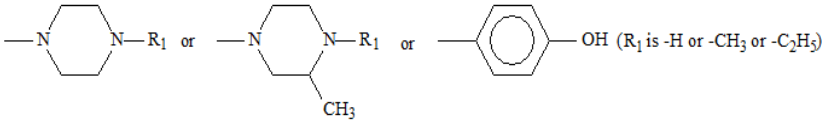
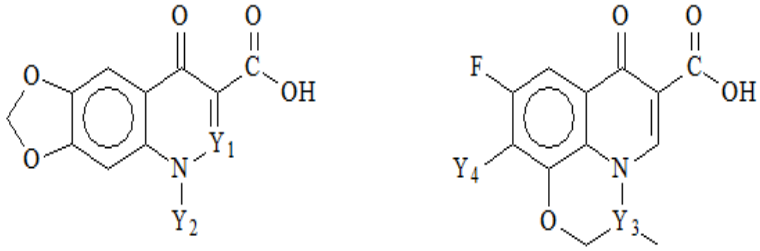
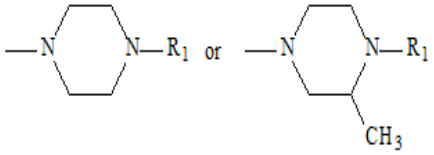
(Deoxyadenosine fragment)

(DNA adduct *via* deoxyguanosine fragment)

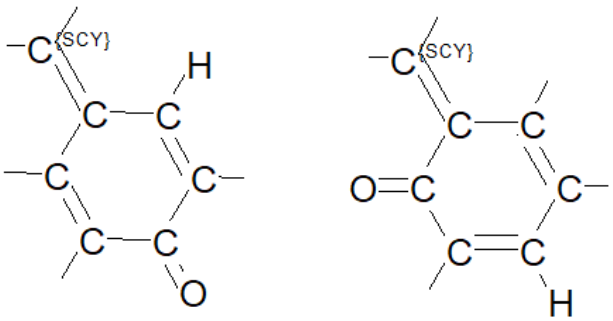
(Y_1 is Cl, Br or -COOH;
Y_2 is -COOH or $-CH_3$ or $-C_2H_5$;
Y_3 is -Cl or -Br (number of halogens 1 or 2);
No more than totally four substituents)

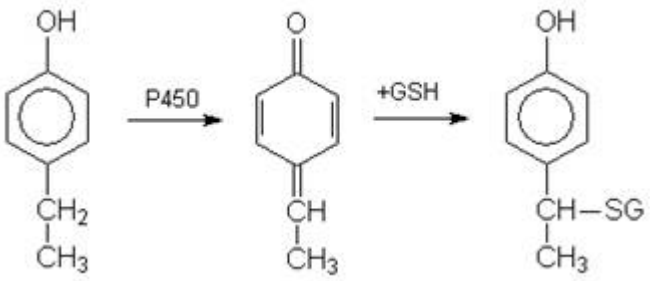
Set of chemicals used for profile development	Quinoline Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Nagao, M., Mutat. Res. **42** (1977), 335 – 342. 2. Willems, M. I., Mutat. Res. **278** (1992), 227 – 236. 3. Miyata, Y., Muitat. Res. **414** (1998), 165 - 169. 4. Suzuki, T., J. Health Sci **53**(3) (2007), 325 – 328. 5. Reigh, G., Carcinog. **17**(9) (1996), 1989 – 1996. 6. *Quinoline* (*CASRN 91-22-5*) Integrated Risk Information System, US-EPA; https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/1004_summary.pdf. Last visited: June, 2021. 7. Arima, Y., Ch. Nishigori, T. Takeuchi, Sh. Oka, K. Morimoto, *4-Nitroquinoline 1-Oxide Forms 8-Hydroxydeoxyguanosine in Human Fibroblasts through Reactive Oxygen Species*, Toxicol. Sci **91**(2) (2006), 382 – 392. 8. *4-Hydroxylaminoquinoline-1-Oxide*, Toxicology Data Network, US National Library of Medicine; Okabayashi, T., Mutagenic Activity of 4-Hydroxylaminoquinoline 1-Oxide, Chem. Pharm. Bull. (Tokyo), 10 (1962), 1127-1128. 9. Ferguson, L. R., W. A. Denny, Genotoxicity of Non-Covalent Interactions: DNA Intercalators (Review), Mutat. Res. **623** (2007), 14 – 23. 10. Snyder, R. D., Possible Structural and Functional Determinants Contributing to the Clastogenicity of Pharmaceuticals, Environ. Molec. Mutag. **51** (2010), 800 – 814. 11. Snyder, R. D., D. Ewing, L. B. Hendry, DNA Intercalative Potential of Marketed Drugs Testing Positive in In Vitro Cytogenetics Assays, Mutat. Res. 609 (2006), 47 – 59. 12. Shubber, E. K., D. J. Kram, J. R. Williams, *Comparison of the Ames*

	Assay and the Induction of Sister Chromatid Exchanges: Results with Ten Pharmaceuticals and Five Selected Agents, Cell Biol. Toxicol. **2**(3) (1986), 379 – 399.

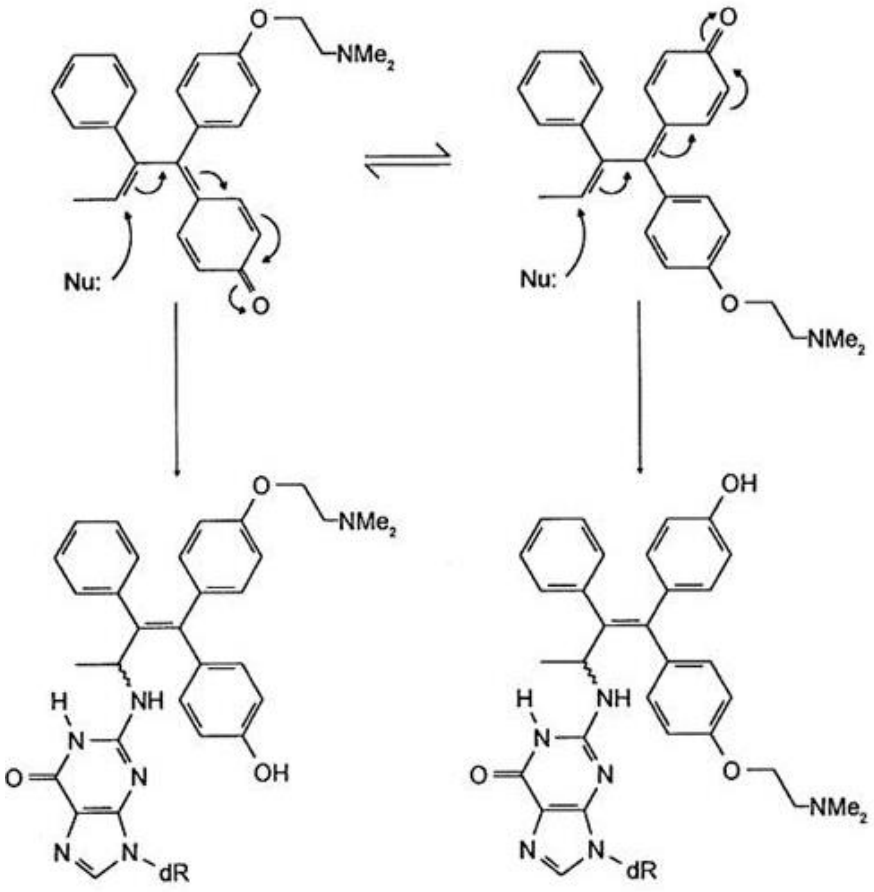
Individual profile/alert	
Name	Quinolone Derivatives
Type of profile	Structural alert
Description/ applicability domain	 (Structure type 1: Fused-ring bicyclic systems) Y_1 can be C or N{V3}; Y_2 can be  or -CH_3 or -CH_2CH_3; Y_3 can be  (R_1 is -H or -CH_3 or -C_2H_5) Y_4 can be -F (positions 6 and 8) or combinations of -F (position 6) and -H (position 8) ***Notes***: 1. Positions 2 and 5 remain non-substituted; 2. If Y_1 is N{V3}, Y_3 can be *also* -CH_3 or -C_2H_5, and if Y_3 is -CH_3 or -C_2H_5 *only*, Y_4 can be -H  (Structures types 2 and 3: Tricyclic fused-ring systems) Y_1 can be C or N{V3}; Y_2 can be  or -CH_3 or -CH_2CH_3; Y_3 can be CH or N{sp3}{V3} Y_4 can be  (R_1 is -H or -CH_3 or -C_2H_5)
Mechanism	Non-covalent interactions DNA intercalation

The mechanism of genotoxicity of quinolone antibiotics involves interaction with the bacterial topoisomerase IV and DNA gyrase enzyme proteins, thereby *indirectly* causing DNA degradation and mutation. These chemicals induce the gyrase enzyme to cleave the DNA with protein covalently bound at the site-specific double-strand scission. The chemicals are highly specific for the bacterial gyrase enzyme, and their bacterial mutagenicity cannot be extended and generalized to mammalian cells. Thus the term "genotoxic" means an increase of the occurrence of DNA lesions by various complex mechanisms, not involving *direct* DNA reactivity [4].	
Set of chemicals used for profile development	Quinolone Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kirkland, D., *Mutat. Res.* **2005**, 584(1 -2), 1 – 256. 2. Albertini, S., *Mutagen*. **1995**, 10(4), 343 – 351. 3. Mamber, S.W., *Antimicrob. Agents Chemother*. **1993**, 37(2), 213 – 217. 4. Gocke, E., Mutat. Res. **1991**, 248(1), 135 – 143. 5. Vashist, J., *Ind. J. Biochem. & Biophys*. **2009**, 147 – 153. 6. Heddle, J., *Antimicrob. Agents and Chemother*. **2002**, 46(6), 1805 – 1815. 7. Peterson, L. R., *Clin. Infect. Diseases*, **2001**, 33(Suppl. 3), S180 – S186.

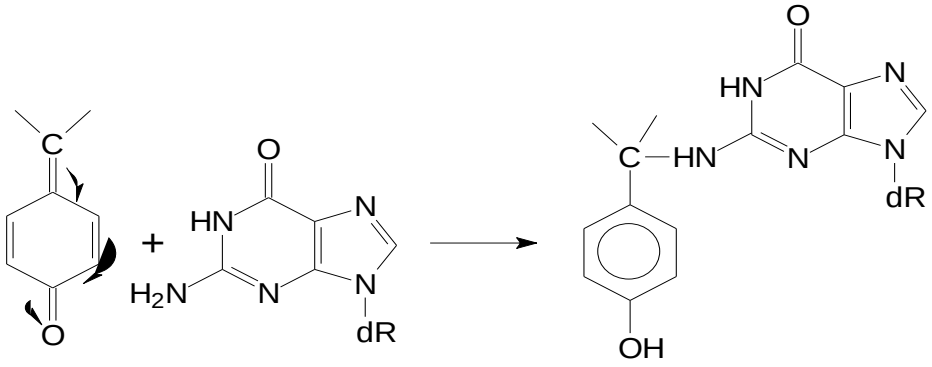
Individual profile/alert	
Name	Quinone methides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS formation after GSH depletion & AN2 Michael addition Quinone type compounds
Results have demonstrated that a series of simple, sterically-unhindered alkylphenols are metabolized to reactive quinone methide intermediates by mammalian liver enzymes. This oxidation mechanism is regarded as common for an increasing number of *p*-alkylphenols and appears to play a significant role in their reported cytotoxic effects, mostly, by glutathione depletion. The following scheme of the formation of glutathione conjugates from 4-ethylphenol *via* quinone methide intermediate was suggested by these authors [3]:	



Tamoxifen is a liver carcinogen in rats and has been shown to increase the risk of specific cancer in women. One of the proposed pathways for the metabolic activation of tamoxifen involves oxidation to 4-hydroxytamoxifen, which may be further oxidized to an electrophilic quinone methide intermediate. It was shown, that the quinone methide intermediate derived from 4-hydroxytamoxifen reacted with DNA to form covalent adducts. The major products, which resulted from 1,8-addition of the exocyclic nitrogen of deoxyguanosine in DNA to the conjugated system of the 4-hydroxytamoxifen quinone methide, were characterized as (*E*)- and (*Z*)-a-(deoxyguanosin-*N*2-yl)-4-hydroxytamoxifen, according to the following general scheme [4]:

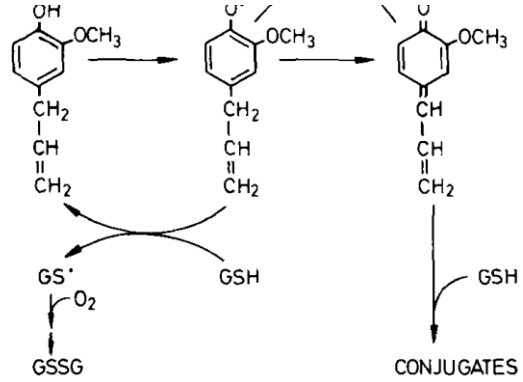


Therefore, based on the above data, the following general scheme of DNA reactivity, and the resulting mutagenicity effects of quinone methide structural fragments can be assumed:

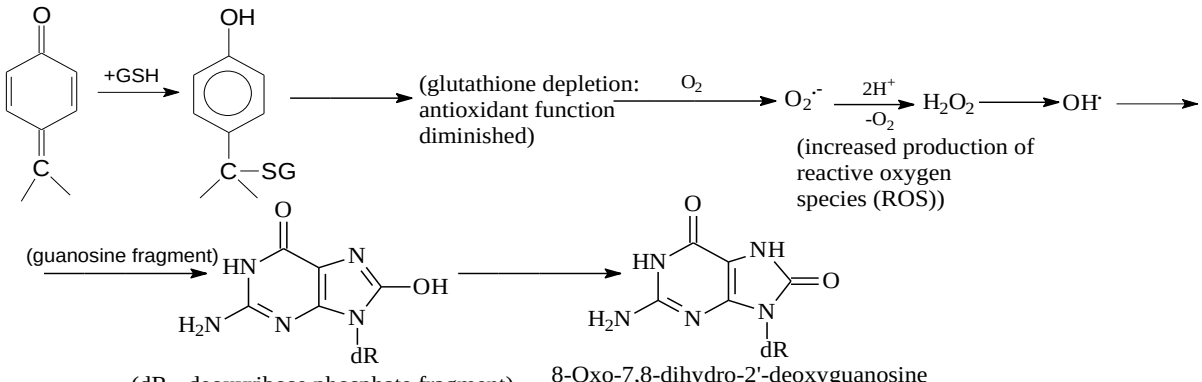


where dR represents desoxyribose fragment.

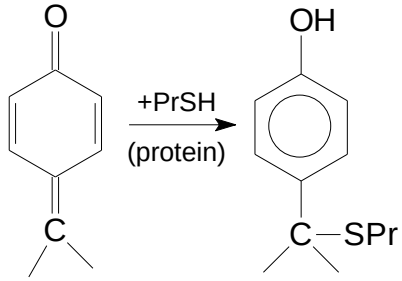
On the other hand, the compound eugenol (1-allyl-3-methoxy-4-hydroxybenzene) extracted from glove oil and marjoram, is widely used as a food flavouring substance and is present in spices such as basil, cinnamon and nutmeg. The genotoxicity of eugenol in V79 cells was evaluated with respect to chromosomal aberration effects. Eugenol was found to induce chromosomal aberration to a significant degree, and S9 liver fraction increased this effect in a dose-dependent manner. The results demonstrated that, the genotoxicity of eugenol was also associated with its topoisomerase II inhibiting activity [5]. Eugenol is known to form the intermediary quinone methide metabolite *by* the following scheme [6]:



Quinone methide is highly-reactive, rapidly forming DNA adducts, and was indicated to also contribute to the induction of chromosome aberrations in V79 cells. Since V79 cells are devoid of CYP- 450 activity, the genotoxicity results could be due to the formation of reactive oxygen species (RSO), resulting from glutathione depletion. This was confirmed by the fact, that 8-hydroxy-20-deoxyguanosine DNA adduct can be produced by eugenol [5]. Therefore, another mechanism of DNA attack may be involved in the overall genotoxicity of quinone methide fragments as follows:

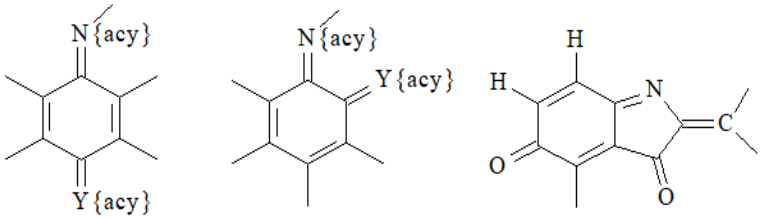


Formation of topoisomerase II inhibition complex, contributing to the chromosomal aberration *via* attack of quinone methide metabolite on the thiol functional groups of cysteine fragments in a protein (enzyme) in a similar mode as that of glutathione conjugation showed above cannot be excluded [7]:



Consequently, it can be assumed that quinone methide intermediates formed during the metabolism of various chemicals can cause both the mutagenicity and chromosome aberration effects.

Set of chemicals used for profile development	Quinone Methides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Sweeny, Mutat. Res. **82**(2), 1981, 275 – 283. 2. Rietjens, Mutat. Res. **574** (1 – 2), 2005, 124 – 138. 3. Thompson, Chem. Res. Toxicol. **8**, 1995, 55 -60. 4. Marquest, Carcinogenesis **18**(10), 1997, 1949 – 1954. 5. Maralhasi, Mutagenesis **21**(3). 2006, 199–204. 6. Thompson, J. Biol. Chem. **264**(2), 1969, 1016 – 1021. 7. Bolton, Chem. Biol. Interact. **107**(3), 1997, 185 – 200.

Individual profile/alert	
Name	Quinoneimine, Thione and Phenoxazinium Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is O or N{V3}); {acy}: acyclic atom (No more than one *additional* substituent on the six-membered ring; (in case of $–CH_3$ and/or $–C_2H_5$ the number of *additional* substituents should be no more than two); No halogens (F, Cl, Br, I) or –OC{sp3} substituent(s) attached; General “mask”: $-SO_3H$)

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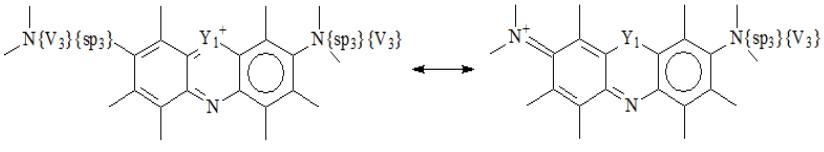
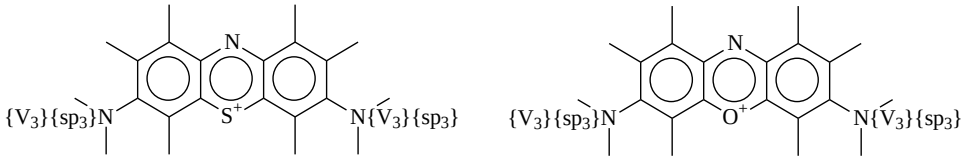
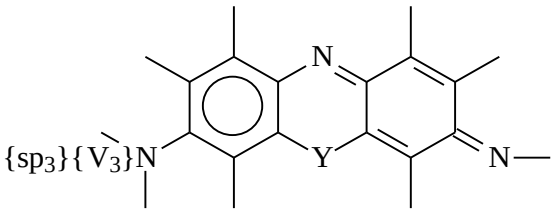
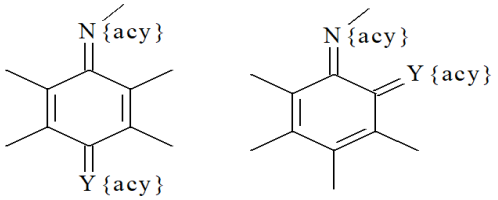
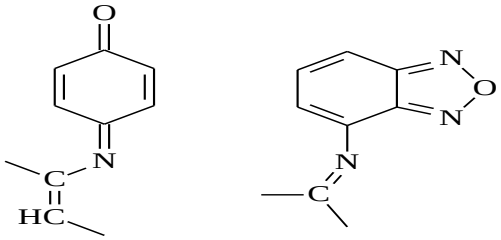


(Thionine and phenoxazinium derivatives)
(Y_1 is S or O)
(No more than one additional substituent attached;
General “mask”: -SO_3H)




(Thionine and Phenoxazine Derivatives)
(Y is S or O)
(No more than one additional substituent attached;
General “mask”: -SO3H)


Quinoneimine Derivatives)
(Y is O or N{V3});
{acy}: acyclic atom
(No more than one additional substituent on the six-membered ring;
(in case of –CH3 and/or –C2H5 the number of additional substituents should be no more than two);
No halogens (F, Cl, Br, I) or –OC{sp3} substituent(s) attached;
General “mask”: -SO3H)


</td>
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<tr>
<td>Mechanism</td>
<td>Radical ROS formation after GSH depletion (indirect), A_N2 Michael-type addition, quinoid structures & Non-covalent interactions DNA intercalation</td>
</tr>
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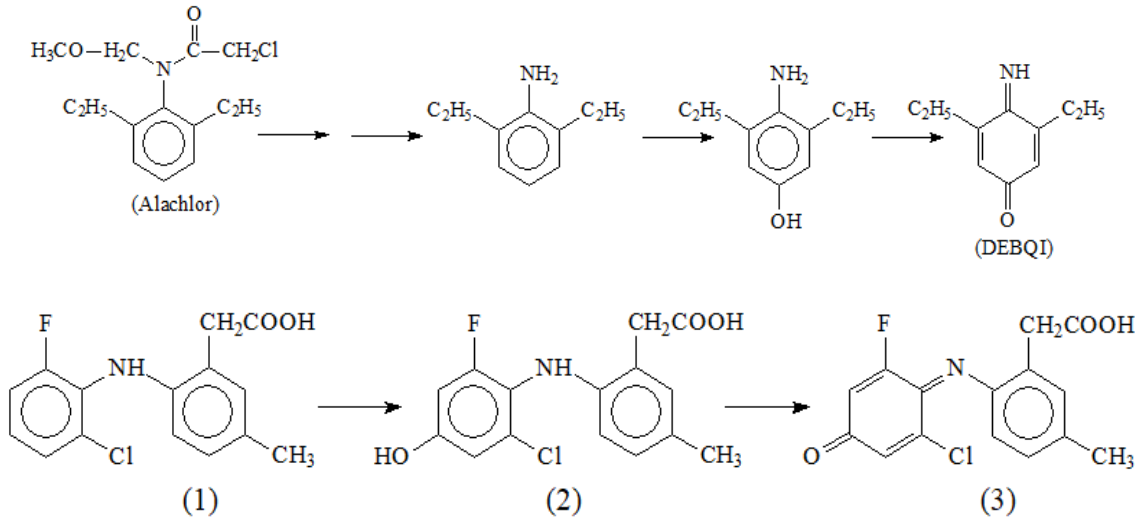
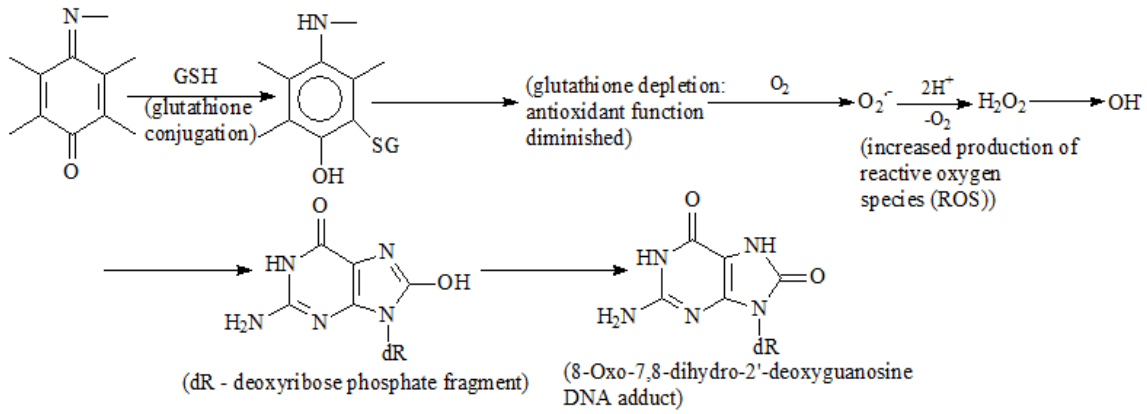
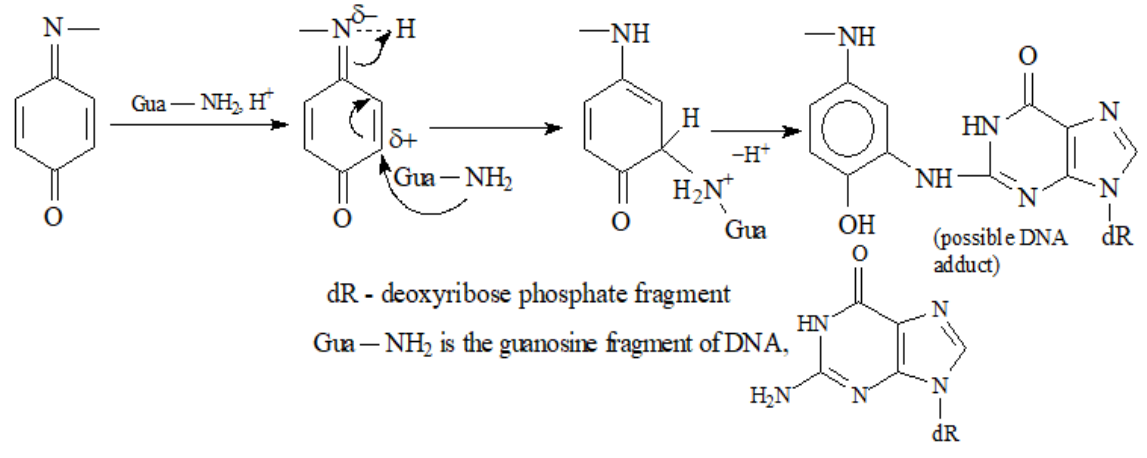


I. **<u>Generation of reactive oxygen species (ROS).</u>** It may be caused by an interaction with protein (enzyme) thiols or glutathione in the microsomal metabolic activation system. This mechanistic scheme seems to be plausible, since it is based on the interaction of "soft" nucleophile with "soft" electrophile as an *initial* molecular event, followed by generation of DNA-damaging ROS:

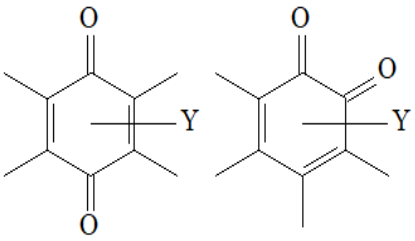
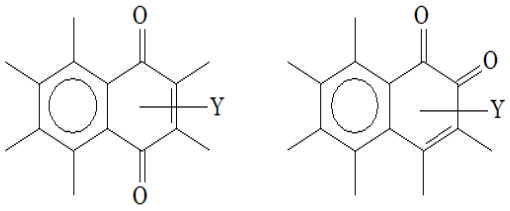
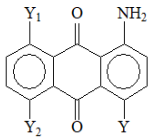
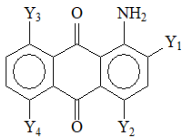
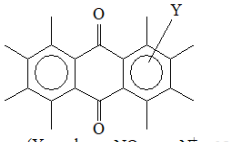
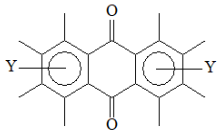


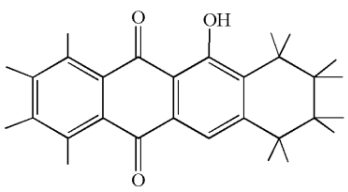
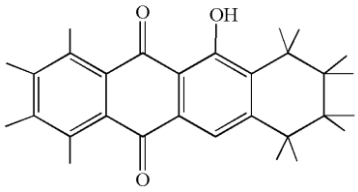
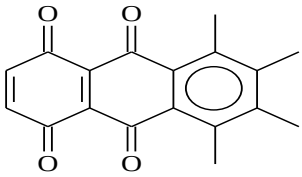
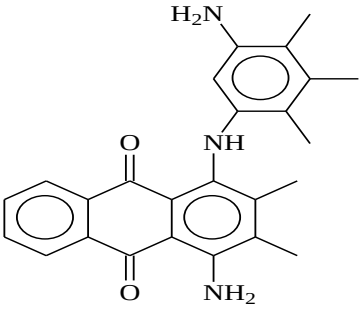
II. **<u>Michael-type addition mechanism</u>.** Such a scheme is regarded as less plausible, since it is based on the direct interaction of "soft" electrophile (quinoneimine derivative) with "hard" nucleophile (DNA base):

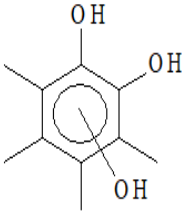
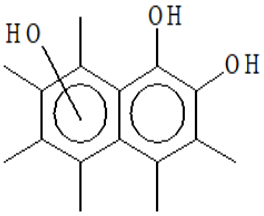
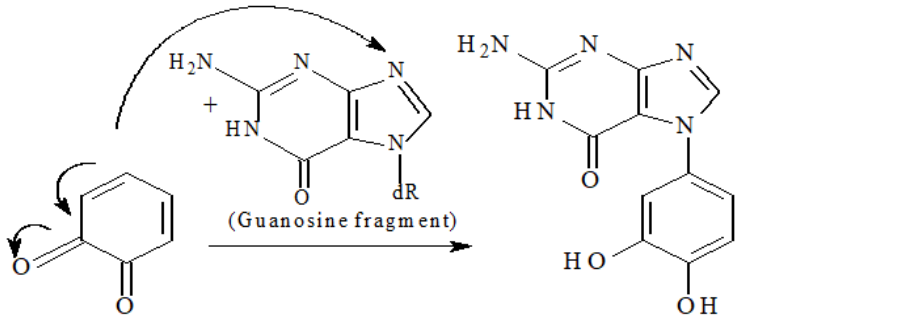


III. **DNA intercalation between DNA base pairs:** This mode of action could be associated with non-covalent interactions, due to the polycyclic planar structure of thionine and phenoxazinium derivatives, and their positively-charged resonance structures.	
Set of chemicals used for profile development	Quinoneimine, Thionine and Phenoxazinium Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Skipper, P. L., Carcinog. **31**(1) (2010), 50 – 58. 2. Rogers, L. K., Chem. Res. Toxicol. **10**(4), 1997, 470 – 476. 3. Cabbot, A. M., Chem. Res. Toxicol. **18**(11) (2005), 1721 – 1728. 4. Hill, A. B., Mutat. Res. **395** (1997), 159 – 171. 5. Stiborova, M., Mutat. Res. **500** (1 - 2) (2002), 49 – 66. 6. Bernadou, J., Proc. Natl. Acad. Sci. USA **81** (1984), 1297 – 1301. 7. Lemke, T. L., Lippincott Williams & Wilkins, 2002; http://www.amazon.com/Foyes-Principles-Medicinal-Chemistry Williams/dp/0683307371#reader_0683307371 Last visited: June, 2021. 8. Thompson, D. C., Mutat. Res. **279** (1992), 83 – 39. 9. Ying Li, Drug Metab. Dispos. **36** (2008), 469 – 473. 10. *Joicela, Lumiracoxib, Assessment Report EMA/CHMP/444155/2011*, Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency; http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2011/11/WC500118339.pdf Last visited: June, 2021. 11. Hesbert, A., Toxicol. Lett. **21**(1) (1984), 119 – 125 12. CCRIS: Indigo, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=482-89-3. Last visited: June, 2021. 13. Huang, M., Drug Metab. Dispos. **36** (2008), 2171 – 2184. 14. *1,4-Benzoquinone Dioxime*, IARC Monographs, Vol. 71, 1999; http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-64.pdf Last visited: June, 2021. 15. Westmoreland, C., Environ. Molec. Mutag. **19** (1992), 71 – 76. 16. Niufar, N. N., Rev. Soc. Quimica de Mexico **46**(4) (2002), 307 – 312. 17. Thionine, CCRIS, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=581-64-6. Last visited: June, 2021. 18. Methylene Blue, CCRIS, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=61-73-4. Last visited: June, 2021. 19. Basic Blue 3, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=33203-82-6. Last visited: June, 2021. 20. Hossain, M., Mol. BioSyst **5** (2009), 1311 – 1322. 21. Hecht, Chr., *J. Phys. Chem. B* 108(29), (**2004)**, 10241 – 10244.

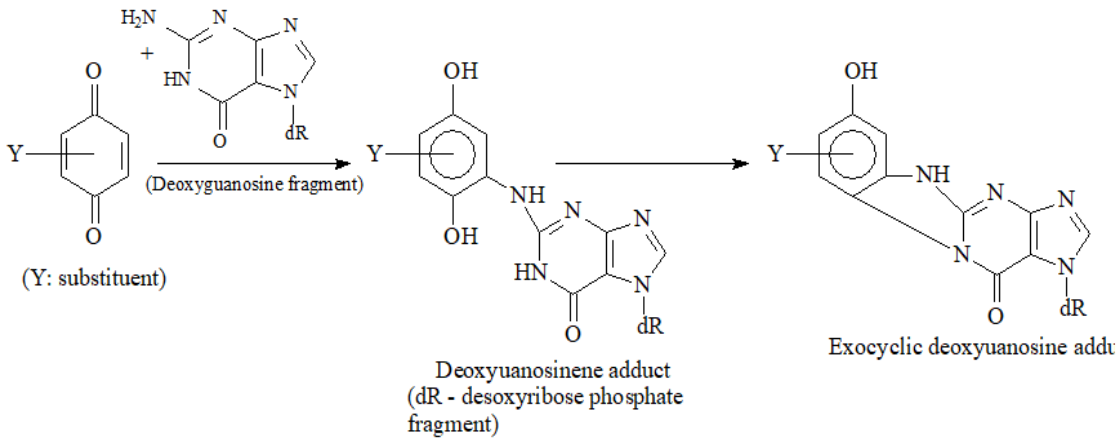
Individual profile/alert	
Name	Quinones and Trihydroxybenzenes

Type of profile	Structural alert
Description/applicability domain	Simple Quinones:  (Y can be Cl, Br (more than one); -CN, $-NO_2$, -C=O, -CHOH or H or C{ar}or N{acy}{V3}or $-CH(CH_3)_2$ or $-C(CH_3)_3$ or combinations with -H), $-CH_2-NH-$ no other substituents; for catechol quinones Y = -OH should be added Naphthoquinones:  1,4-Naphthoquinones 1,2-Naphthoquinones Y can be any combination of substituents such as –H, $-CH_3$, -OH, $-OCH_3$, $-NH_2$, $-NHCH_3$, –Cl, -Br, -CN, $-CX_3$ (X = Cl, Br), $-C(O)CH_3$, $-C(O)OCH_3$; Y can be attached to one or to both rings; No more than totally two fused rings in the molecular structure Anthraquinone Derivatives:  (Y can be -OH or -NH; Y_1, Y_2 can be -OH, $-NH_2$ or -H)  (Y_1 can be -Cl, -Br, -COOH, -OH, $-OCH_2$ or $-NH_2$); Y_2 can be Cl or Br or -H; Y_3, Y_4 can be -OH, $-NH_2$ or -H)  (Y can be $—NO_2$ $—N^{+}\equiv N$ —N=NH N{V_3}—N{V_3}; could be anywhere)  (Y is -OH or $-CH_2OH$ or -H or -O-C{sp3} or $-C(C\{sp3\})_3$ or C{scy}{sp3} or $-NHCH_3$ or $-NHCH_2OH$ or $-NHC_2H_5$ or $-NHCH_2CH_2OH$ or $-NH-C(O)-C_6H_5$ or combinations)

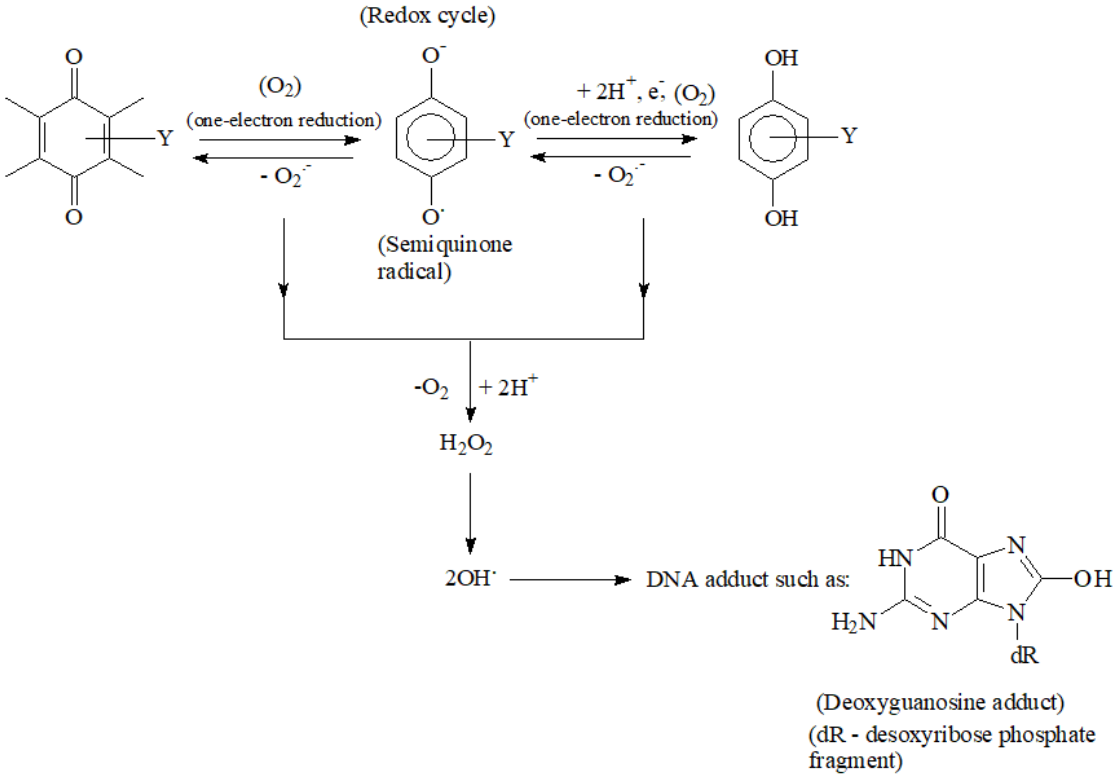
<table>
<tr><td></td><td> 




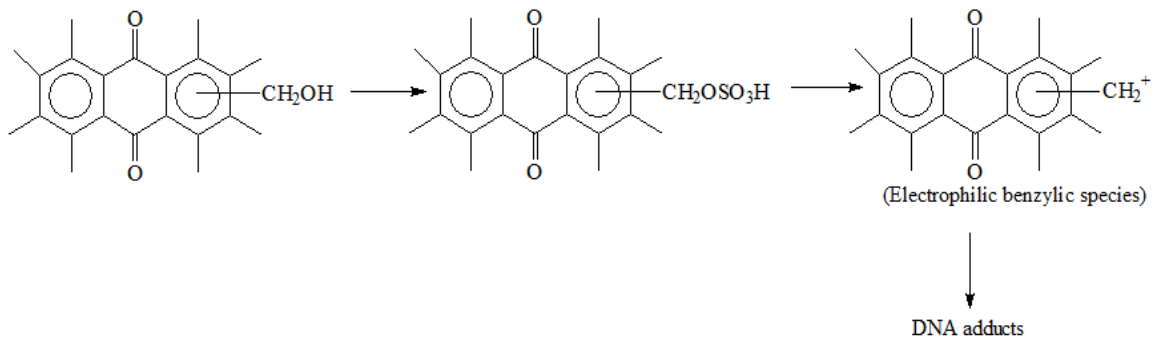
Trihydroxybenzenes:


(Other possible substituents: -H, -CH3,-OCH3, -NH2;
No substituents other than these)</td></tr>
<tr><td>Mechanism</td><td>AN2 Michael-type addition, quinoid structures , Radical ROS generation (indirect) & Non-covalent interactions DNA intercalation SN1 Nucleophilic attack after carbenium ion formation</td></tr>
<tr><td colspan="2">1. Electrophilic mechanism for simple quinones and naphthoquinones:
 

Guanine adduct
(depurination)
dR - desoxyribose phosphate fragment</td></tr>
</table>



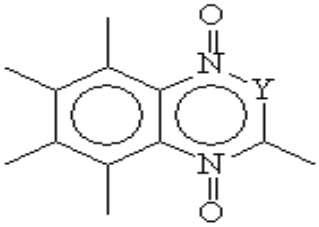
2. Radical mechanism for simple quinones, naphthoquinones, anthraquinone derivatives and trihydroxybenzenes

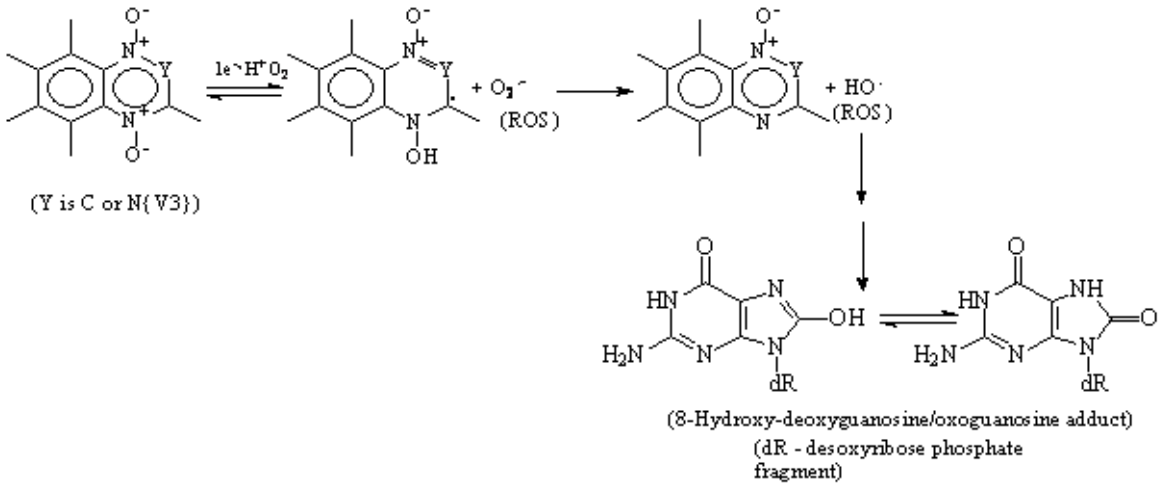




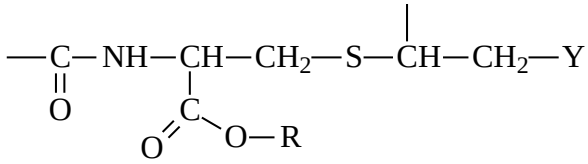
Set of chemicals used for profile development	Quinones and Trihydroxybenzenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Hakura, A., Mutat. Res. **347** (1995), 37 – 43). 2. Nagabhushan, M., *Environ. Mutagen.* 7(6) (1985**)**, 881 – 888. 3. Chanda, S., Drug Metab. Dispos. **36** (2008), 670 -675. 4. Reilly, Chr., Chem. Res. Toxicol. **16** (2003), 336 – 349. 5. Watanabe, K., *Mutat. Res.* 412(1) (1998**)**, 17 - 31). 6. Gocke, E., Mutat. Res. **90**(2) (1981), 91 – 109. 7. Ben-Gurion, R., Mutat. Res. **68**(3) (1979), 201 – 205. 8. Takemura, Y., Bull. Environ. Contam. Toxicol. **84**(3) (2010), 347 - 350. 9. Opinion on 1,2,4-Trihydroxybenzene, COLIPA No. A33, Scientific Committee on Consumer Safety SCCS 11 December **2012**; http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_113.pdf Last visited: June, 2021. 10. Lin, J.K., Mutat. Res. **269**(2) (1992), 217 – 224. 11. Tourino, S., EJEAFChe 7(8) (2008), 3348 – 3352. 12. Hakura, A., Chem. Res. Toxicol. **7** (1994), 559 – 567. 13. DaCosta, Mutat. Res. **650** (2008), 140 – 149. 14. Cavalieri, E., Carcinog. **23**(6) (2002), 1071 – 1077. 15. Hakura, A., Chem. Res. Toxicol. **7** (1994), 559 – 567. 16. Tikkanen, L., Mutat. Res. **124** (1983), 25 – 34. 17. *Opinion Proposing Harmonized Classification and Labelling at Community Level of Acequinocyl*, ECHA/RAC/CLH-O-0000001401-89-01/F, Committee for Risk Assessment RAC, Adopted 28 October 2010. 18. Brown, J. P., Mutat. Res. **66** (1979), 9 – 24. 19. Bosch, R., Mutat. Res. **188** (1987), 161 – 168. 20. Poginsky, B., Carcinogenesis **12**(7) (1991), 1265 – 1271. 21. Westendorf, J., Cell Biol. Toxicol. **4**(2) (1988), 225 – 229. 22. Marzin, D., Eur. J. Cancer Clin. Oncol. 19(5) (1983), 641 – 647. 23. CCRIS: Daunomycin CASRN 20830-81-3, Toxicology Data Network, U.S. National Library of Medicine**;** https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=20830-81-3. Last visited: June, 2021. 24. Benedict, W.F., Cancer Res. **37**(7) Pt 1 (1977) 2209 – 2213.

	25. Bachur, N. R., Br. J. Pharmac. **43** (1971), 828 – 833. 26. CCRIS: Doxorubicin CASRN 23214-92-8, Toxicology Data Network, U.S. National Library of Medicine**;** https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=23214-92-8. Last visited: June, 2021. 27. Bhuyan, B.K., Cancer Res. **43**(11) (**1**983**)**, 5293 - 5297. 28. Kalgutkar, A. S., Current Drug Metabol. **6** (2005), 161 – 225. 29. Gaskell, M., Carcinogenesis **26**(3) (2005), 673 – 680. 30. Park, J. Z., Carcinogenesis **25**(9) (2004), 1727 – 1733. 31. Li, K. M., Carcinogeneis **25**(2) (2004), 289 – 297. 32. Singh, M. W., A. Karmakar, N. Barooah, J. B. Baruah, *Variation in Product in reactions of Naphthoquinone with Primary Amines*, Beil. J. Org. Chem. **3**(10) (2007), 1 – 6. 33. Gaskell, M., Chem. Res. Toxicol. **15** (2002), 1088 – 1095. 34. Xie, Zh., DNA Repair **4** (2005), 1399 – 1409. 35. Yu, D., Chem. Res. Toxicol. **15** (2002), 832 – 842. 36. Kovacic, P., Current Med. Chem. **8** (2001), 773 – 796. 37. Gouda, M. A., Turk. J. Chem. **34** (2010), 651 – 709. 38. Poginsky, B., Carcinogenesis **12**(7) (1991), 1265 – 1271. 39. Double, J. C., J. Pharm. Pharmac. **28** (1976), 166 – 169. 40. Brock, K. H., Mutagen. **6**(1) (1991), 35 – 46.

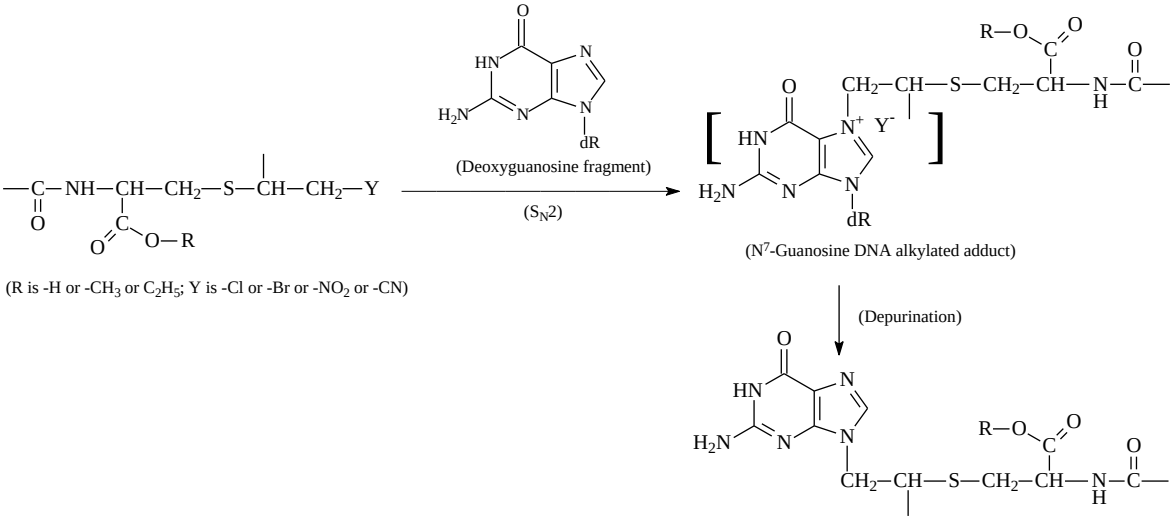
Individual profile/alert	
Name	Quinoxaline-Type 1,4-Dioxides
Type of profile	Structural alert
Description/applicability domain	 (Y is C or N{V3})
Mechanism	Radical ROS generation
The following scheme for generation of ROS and formation of DNA adducts can be assumed [5]:	



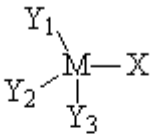
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Yoshimura, Mutat. Res.90(1) (1981**)**, 49 – 55. 2. Nunoshiba, Mutat. Res. **217**(3) (1989**)**, 203 - 209. 3. Beutin, Antimicrob. Agents Chemother.20(3) (1981**),** 336 - 343. 4. Voogd, Mutat. Res. 78 (1980) 233 – 242. 5. Ganley, Bioorg. & Med. Chem. **9** (2001), 2395 – 2401. 6. Liu, Toxicol. Lett. **195** (2010), 51 - 59.

Individual profile/alert	
Name	S-Activated Cysteine Derivatives
Type of profile	Structural alert
Description/applicability domain	 (R is -H or –CH3 or C2H5; Y is –Cl or –Br or –NO2 or -CN)
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: Alkylation, nucleophilic substitution on activated sp3-carbon atom
Newly synthesized S-haloethyl conjugates of cysteine and glutathione, as well as selected methyl ester and N-acetyl derivatives were tested for their ability to act as direct mutagens in Salmonella typhimurium TA98 and TAl00. In the strain TA100, where mutation of a specific guanine by base-pair substitution produces reversion, all compounds were found to be mutagenic, but the levels of mutagenicity did not correlate with the levels of DNA alkylation. The ratio of mutations to the amount of adducts varied at least 14-fold among the various N7-guanyl adducts examined. It is known that many simple electrophilic species react predominantly at the N7-position of guanine, because this position is highly electronegative and nucleophilic, and is also exposed to the major DNA groove. S-(2-Chloroethyl)glutathione was found to be the most potent mutagen, although it produced only intermediate levels of alkylation. This has indicated that: (i) N7-alkylguanine residues can be quite	

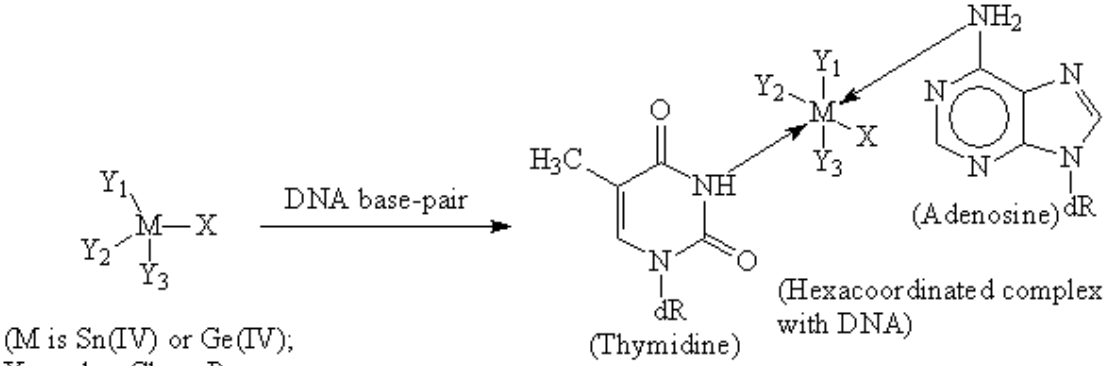
mutagenic and (ii) small differences in the structures of a single adduct can significantly alter mutagenicity [1].
Based on the above discussions, bearing in mind also the process of depurination, following the initial formation of DNA guanosine adducts [1, 2], the following simplified mechanistic schemes can be expertly proposed:



Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Humphreys, W. G., D. H. Kim, J. L. Cmarik, Ts. Shimada, F. P. Guengerich, Comparison of the DNA-Alkylating Properties and Mutagenic Responses of a Series of S-(2-Haloethyl)-Substituted Cysteine and Glutathione Derivatives, Biochem. 29 (1990), 10342 – 10350. 2. Thap, Pr., E. K. Kim, M. R. Nepal, KiSun Jeong, M. J. Kang, K. Noh, S. Lee, H. G. Jeong, J. Ho Lee, T. Ch. Jeong, E. S. Lee, Identification of a N7-guanine adduct of 1-bromopropane in calf thymus DNA by mass spectrometry, Mol. Cell Toxicol. 12 (2016), 7 – 14.

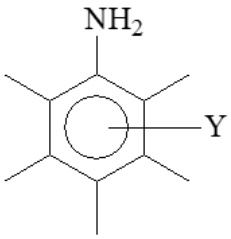
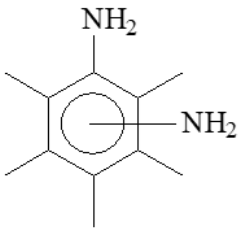
Individual profile/alert	
Name	Short-Chain Alkytin and Alkylgermanium Halides
Type of profile	Structural alert
Description/applicability domain	 (M is Sn(IV) or Ge(IV); X can be -Cl or -Br; Y_1, Y_2 can be -Cl or -Br or -$(CH_2)_nH$ (n = 1 - 4) Y_3 can be -$(CH_2)_nH$ (n = 1 - 4))

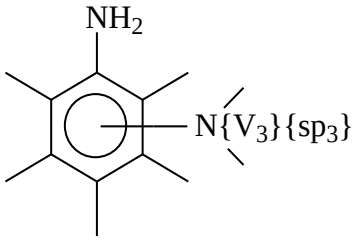
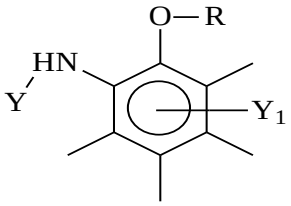
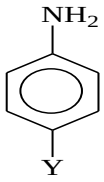
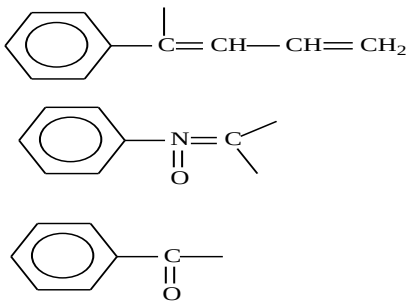
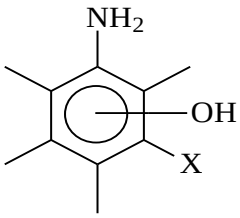
Mechanism	S_N2 Coordination with nucleoside bases

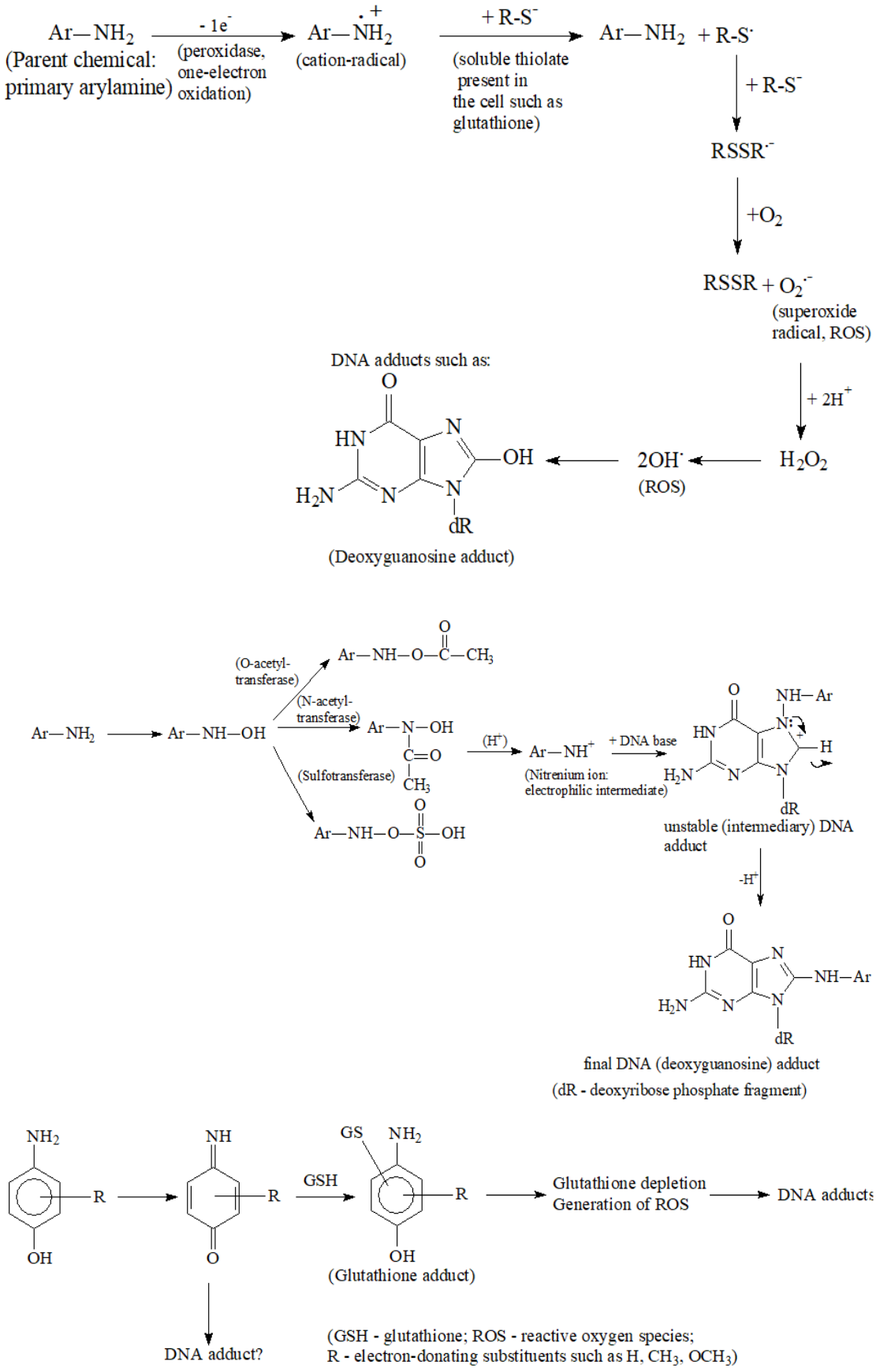


(M is Sn(IV) or Ge(IV);
X can be -Cl or -Br;
Y_1, Y_2 can be -Cl or -Br or $-(CH_2)_nH$ (n = 1 - 4)
Y_3 can be $-(CH_2)_nH$ (n = 1 - 4))

Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Hamasaki, TMutat. Res., 300(3-4) (1993**)**, 265 - 271. 2. Li, Toxicol. Appl. Pharmacol. **64** (1982), 482 – 485. 3. Shoukry, The Scientific World Journal, (**2013**), 1 – 7. 4. Rastogi, J. Appl. Chem. (**2014**), 1 – 5.

Individual profile/alert	
Name	Single-Ring Substituted Primary Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	 (Y can be N{V3}, C{sp3}, O-C{sp3}; No more than four substituents; Single-ring aromatic system; Total "masks": $-SO_3H$ and aniline $C_6H_5NH_2$  (No more than two $-NH_2$ groups)

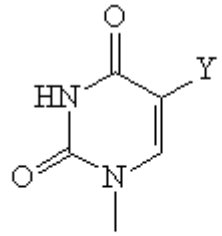
	 (No more than totally three amino groups)  (R is H or -CH_2-; Y_1 is X (Cl or Br) or C{any}; No more than totally three substituents) Y is H or CH=O  Y can be as follows:   X is Cl or Br; No more than totally three sustitients including the existing - NH_2)
Mechanism	S_N1 Nucleophilic attack after nitrenium ion formation & Radical ROS generation (indirect) Radical ROS generation by quinoid structure formation and glutathione depletion for p-disubstituted aminophenols and phenylenediamines

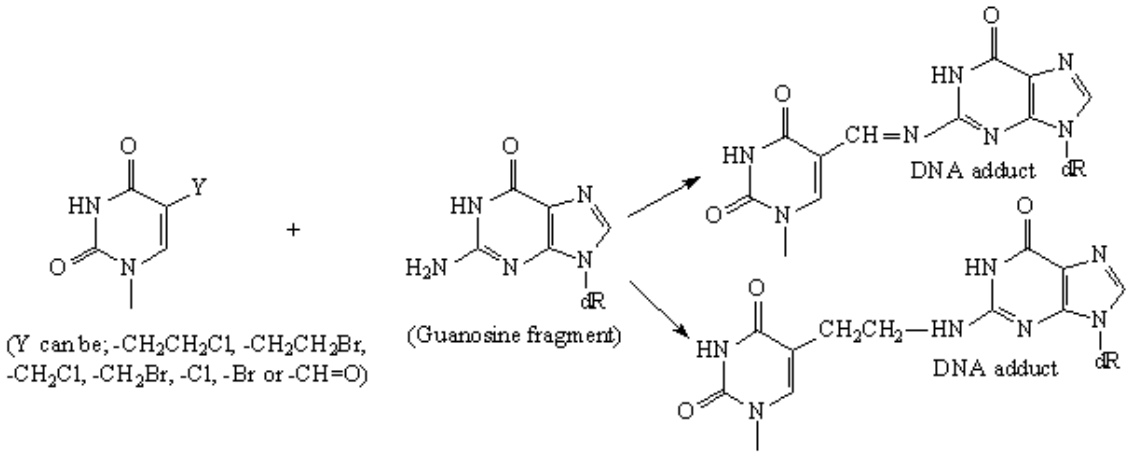


Set of chemicals used	Single-Ring Substituted Primary Aromatic Amines

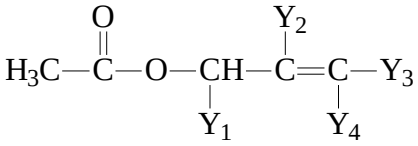
for profile development	
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Ames, Br. N., H. O. Kammen, E. Yamasaki, *Hair Dyes Are Mutagenic: Identification of a Variety of Mutagenic Ingredients*, Proc. Nat. Acad. Sci USA **72**(6) (1975), 2423 – 2427. 2. Garner, R. C., C. A. Nutman, *Testing of Some Azo Dyes and Their Reduction Products for Mutagenicity Using Salmonella Typhimurium TA 1538*, Mutat. Res. **44** (1977), 9 – 19. 3. Zimmer, D., J. Mazurek, G. Petzold, B. K. Bhuyan, *Bacterial Mutagenicity and Mammalian Cell Damage by Several Substituted Anilines*, Mutat. Res. **77** (1980), 317 – 326. 4. Thompson, Chr. Z., L. E. Hill, J. K. Epp, G. S. Probst, *The Induction of Bacterial Mutation and Hepatocyte Unscheduled DNA Synthesis by Monosubstituted Anilines*, Environ. Mutag. **5** (1983), 803 – 811. 5. Ashby, J., R. W. Tennant, *Definitive Relationships Among Chemical Structure, Carcinogenicity and Mutagenicity for 301 Chemicals Tested by the US NTP*, Mutat. Res. **257** (1991), 229 – 306. 6. Chung, K. T., L. Kirkovsky, A. Kirkovsky, W. P. Purcell, *Review of Mutagenicity of Monocyclic Aromatic Amines: Structure-Activity Relationships*, Mutat. Res. **387** (1997), 1 – 16. 7. Kranendonk, M., J. N. M. Commandeur, A. Laires, J. Rueff, N. P. E. Vermeulen, *Characterization of Enzyme Activities and Cofactors Involved in Bioactivation and Bioinactivation of Chemical Carcinogens in the Tester Strains Escherichia coli K12 MX100 and Salmonella typhimurium LT2 TA100*, Mutag. **12**(4) (1997), 245 – 254. 8. Lang, B., M. M. Iba, *Peroxidative Activation of 3,3'-Dichlorobenzidine to Mutagenic Products in the Salmonella typhimurium Test*, Mutat. Res. **191** (1987), 139 – 143. 9. Subrahmany, V. V., P. J. O·Brien, *Peroxidase Catalysed Oxygen Activation by Arylamine Carcinogens and Phenol*, Chem.-Biol. Interactions **56** (1985), 185 – 199. 10. Makena, P. S., K. T. Chung. *Evidence that 4-Aminobiphenyl, Benzidine and Benzidine Congeners Produce Genotoxicity Through Reactive Oxygen Species*, Environ. Molec. Mutagenesis **48** (2007), 404 – 413. 11. Kalgutkar, A. S., I. Gardner, R. S. Obach, Chr. I. Shaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O,Donnell, J. Boer, Sh. P. Harriman, *A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups*, Curr. Drug Metabol. **6**(3), 2005, 161 – 225. 12. Shamovsky, I., L. Ripa, L. Borjesson, Chr. Mee, B. Norden, P. Hansen, C. Hasselgren, M. O,Donovan, P. Sjo, *Explanation for Main Features of Structure-Genotoxicity Relationships of Aromatic Amines by Theoretical Studies of Their Activation Pathways in CYP1A2*, JACS **133** (2011), 16168 – 16185. 13. Humphreys, W. G., F. F. Kadlubar, F. Peter Guengerich, *Mechanism of C8 Alkylation of Guanine Residues by Activated Arylamines: Evidence of Initial Adduct Formation at the N7 Position*, Proc. Natl. Acad. Sci USA, **89** (1992), 8278 – 8282. 14. Skipper, P. L., M. Y. Kim, H. L. P. Sun, G. N. Wogan, St. R. Tannenbaum, *Monocyclic Aromatic Amines as Potential Human Carcinogens: Old is New Again*, Carcinog. **31**(10) (2010), 50 – 58.

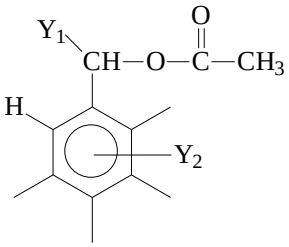
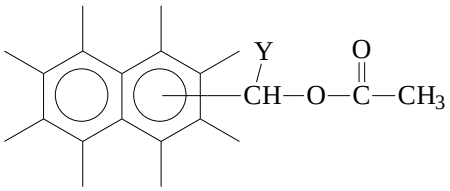
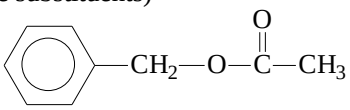
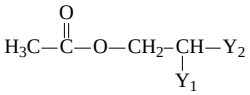
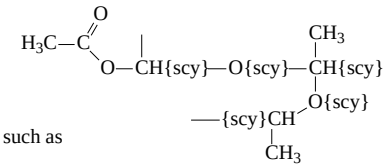
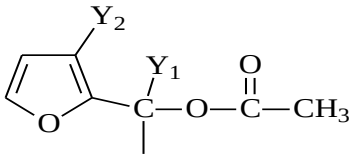
15. Nitrenium Ion; https://www.wikidoc.org/index.php/Nitrenium_ion. Last visited: June, 2021.
16. Guengerich, F. P., A. Parikh, E. F. Johnson, T. H. Richardson, C. von Wachenfeldt, J. Cosme, Fr. Jung, C. P. Strassburg, M. P. Mannis, R. H. Tukey, M. Prichard, S. Fournel-Gigleux, Br. Burchell, *Heterologous Expression of Human Drug-Metabolizing Enzymes*, Drug Metabol. Dispos. **25**(11) (1997), 1234 – 1241.
17. Glatt, H., W. Meini, *Use of Genetically Manipulated Salmonella typhimurium Strains to Evaluate the Role of Sulfotransferases and Acetyltransferases in Nitrofen Mutagenicity*, Carcinogenesis **25**(5) (2004), 779 – 786.
18. Westwood, I. M., S. J. Holton, F. Rodriges-Lima, J. M. Dupret, S. Bhakta, M. E. M. Noble, E. Sim, *Expression, Purification, Characterization and Structure of Pseudomonas aeruginosa Arylamine N-Acetyltransferase*, Biochem. J. **385** (2005), 605 – 612.
19. Beland, FR., W. B. Melchior Jr., L. L. G. Mourato, M. A. Santos, M. M. Marques, *Arylamine-DNA Adduct Conformation in Relation to Mutagenesis*, Mutat. Res. **376** (1997),13 – 19.
20. NTP Results Report: Results, Status and Publication Information of All NTP Chemicals Produced from Chemtrack System (08/10/00). http://www.predictive-toxicology.org/data/ntp/original_ntp_data.txt; https://ntpsearch.niehs.nih.gov. Last visited: June, 2021.
21. *3,4-Dichloroaniline*, The MAK Collection for Occupational Health and Safety, 19 June 2013; http://onlinelibrary.wiley.com/doi/10.1002/3527600418.mb9576e4013/pdf.

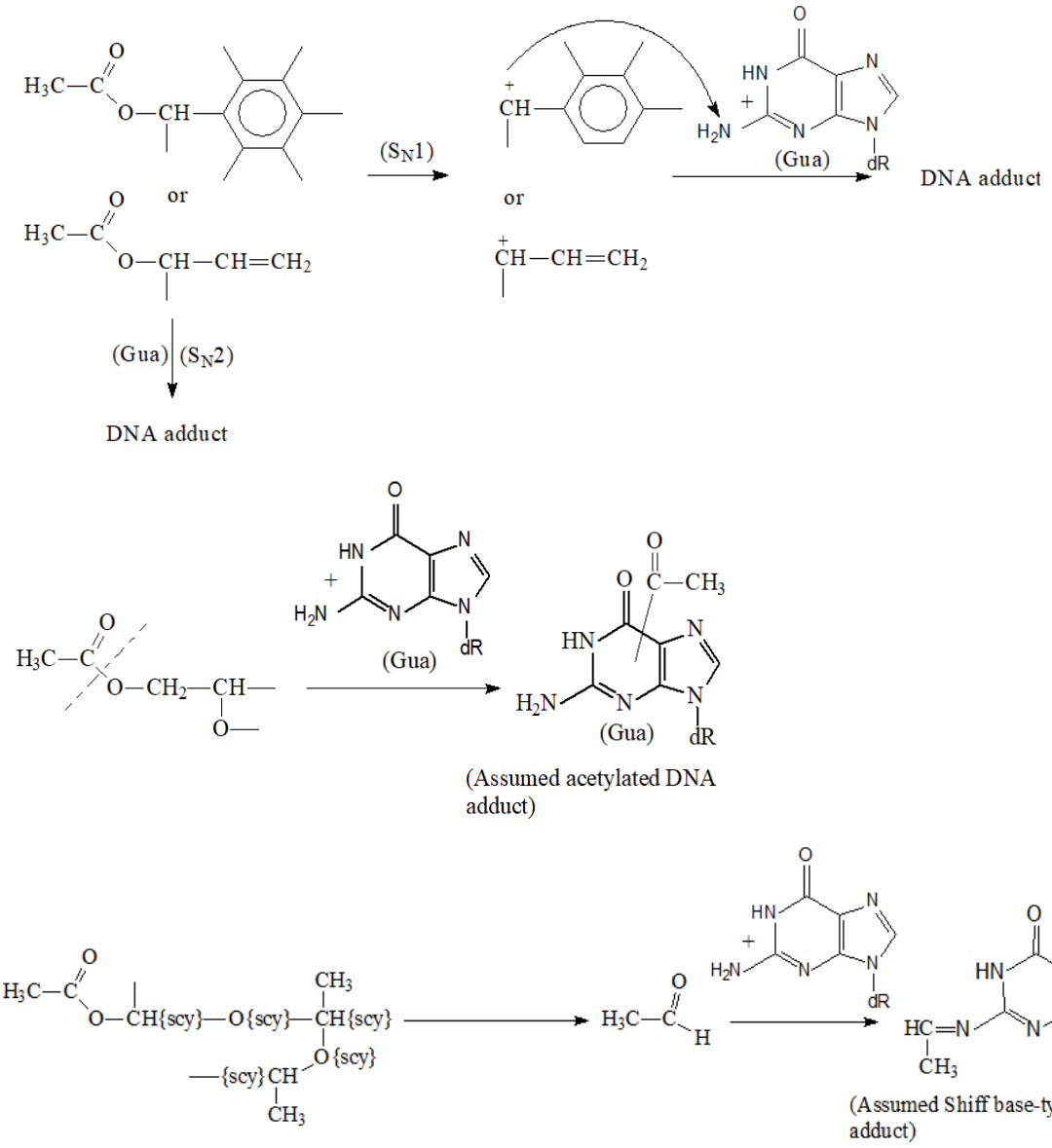
Individual profile/alert	
Name	Specific 5-Substituted Uracil Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y can be; -CH_2CH_2Cl, -CH_2CH_2Br, -CH_2Cl, -CH_2Br, -Cl, -Br or -CH=O)
Mechanism	A_N2 Shiff base formation, S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom and Non-covalent interactions DNA intercalation
Formation of covalent adducts, DNA or DNA/protein cross-linking – schemes of formation of some possible DNA adducts are given below:	



Set of chemicals used for profile development	Specific 5-Substituted Uracil Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Suter, Mutat. Res. **568**(2) (**2004**), 195 - 209. 2. Szinai, Eur. J. Drug Metabol. Pharmacokinet. **16**(2) (1991), 129 – 136. 3. Privat, Mutat. Res. **354** (1996), 151 – 156.

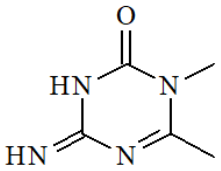
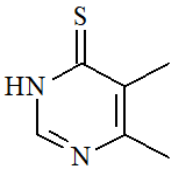
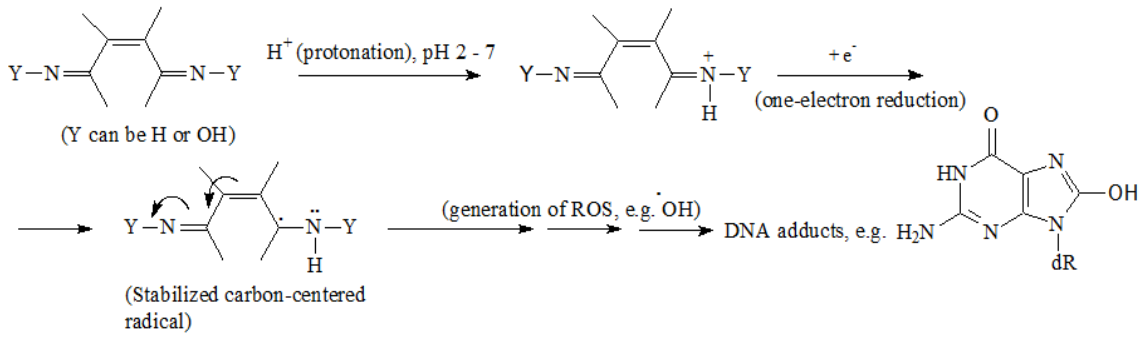
Individual profile/alert	
Name	Specific Acetate Esters
Type of profile	Structural alert
Description/applicability domain	Allyl acetate derivatives  (Y_1: -H or C{ar}; Y_2, Y_3: -H or electron-withdrawing substituents such as -O-, -NO_2, -CN, -C(O)-, -CHO capable of conjugation); Y_4: -H or -C: number of C-atoms in Y_4 0 - 2) Benzyl acetate derivatives

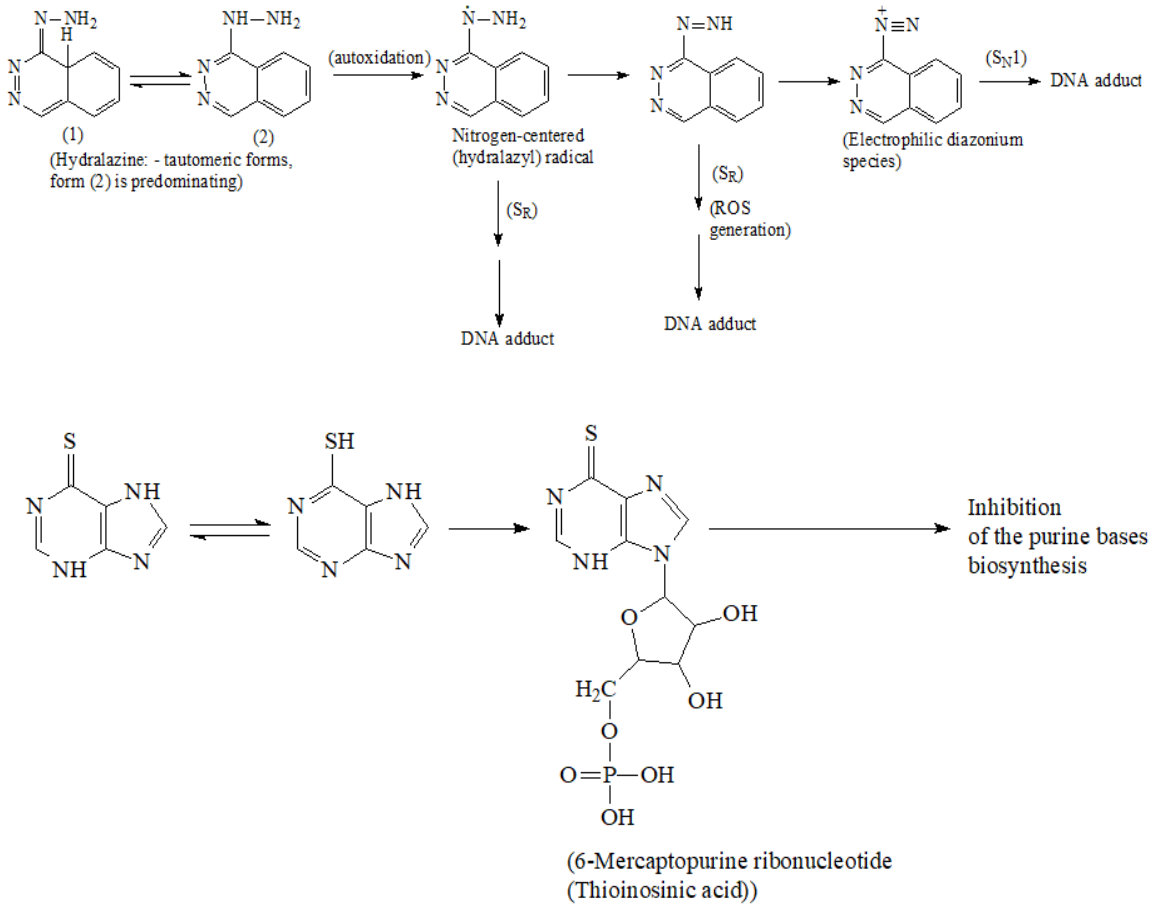
	 (Single-ring, Y_1: -H or C=C; Y_2: electron-withdrawing substituents such as -O-, -NO_2, -CN, -C=O, -CHO, -OC=O); no more than three substituents)  (Fused-ring polycyclic derivative; Y can be -H or -CH_3) "Masks": -SO_3H (general);  Other specific acetate esters  (Y_1 and Y_2 can be OH and -CH_2OH or H and -O-CH_3 respectively; or -H and electron-withdrawing substituents such as -NO_2, -CN, -C=O, -CHO, -OC=O)  Important notes for clarification regarding structural alert (IV): 1. Only one Y1 (or Y2) can be H; Y1=Y2=H is “forbidden”; 2. Y1 and Y2 should be always different types of substituents, i.e., only one –OH, -CH2OH or –OCH3 attached to the same carbon atom. 3. Y1 and Y2 can be only combinations of one H and one electron-withdrawing substituent such as –NO2, -CN, -C=O, -CH=O, -OC=O.  (Y_1 is -H or C{ar}; Y_2 is -H or EWG such as -O-, NO_2, -CN, -C=O, -CH=O, -OC=O)
Mechanism	S_N1 Nucleophilic attack after carbenium ion formation, S_N2 Acylation, S_N2 at sp3 carbon atom & A_N2 Shiff base formation after aldehyde release



Set of chemicals used for profile development	Specific Acetate Esters
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Zeiger, E., Mutat. Res. **290** (1993), 53 – 61. 2. Rogan, E. G., Chem. Biol. Interact. **58** (1986), 253 – 273. 3. Auerbach, S. S., Toxicol. **253**(1 – 3) (2008), 79 – 88 4. Johanson, G., Crit. Rev. in Toxicol. **30**(3) (2000), 307 – 345 5. Tenant, R.W., Mutat. Res. **257** (1991), 209 – 227. 6. Glatt, H., Mutag. **27**(1) (2012), 41 – 48. 7. *NTP Technical Report on the Comparative Toxicity Studies of Allyl Acetate (CAS No. 591-87-7), Allyl Alcohol (CAS No. 107-18-6) and Acrolein (CAS No. 107-02-8) Administered by Gavage to F344/N rats and B6C3F1 Mice*, Tox. Rep. Ser. 48 (2006) 1 – 73, A1-H10 (Abstract);

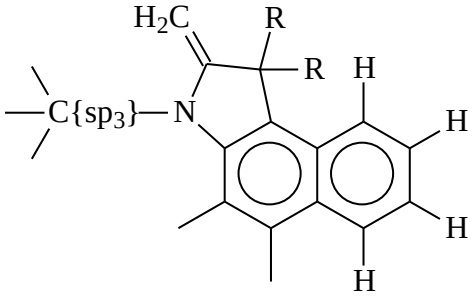
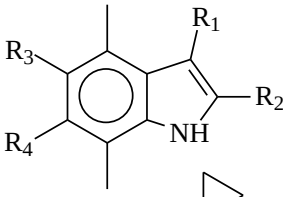
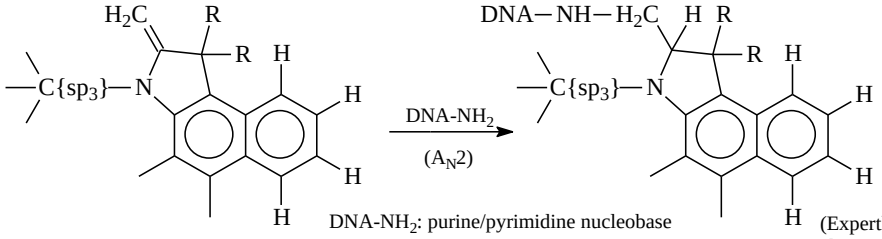
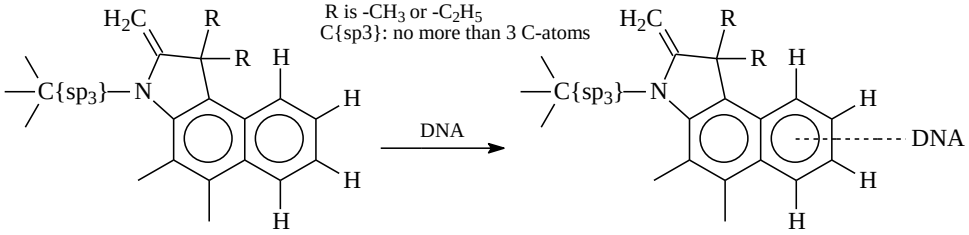
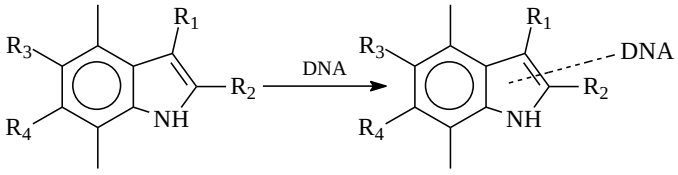
	https://www.ncbi.nlm.nih.gov/pubmed/17160105, last visited 06.2021. 8. *Acetin, Chemical Carcinogenesis Research Information System (CCRIS)*; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=26446-35-5. Last visited: June, 2021.

Individual profile/alert	
Name	Specific Imine and Thione Derivatives
Type of profile	Structural alert
Description/applicability domain	(1) —C{scy}=C{scy}—C{scy}=N{acy}{V3}— (2) —C{scy}=N{scy}{V3}—C{scy}=S (3) —N{scy}{V3}=N{scy}{V3}—C{scy}=N{acy}{V3}— {scy} - cyclic atom; {acy}: acyclic atom; V - valency (4)  (5) 
Mechanism	S_R ROS formation , S_N1 Nucleophilic substitution on diazonium ion & Non-specified Incorporation into DNA/RNA, due to structural analogy with nucleoside bases
	

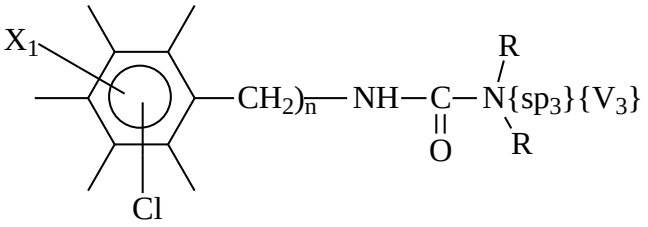
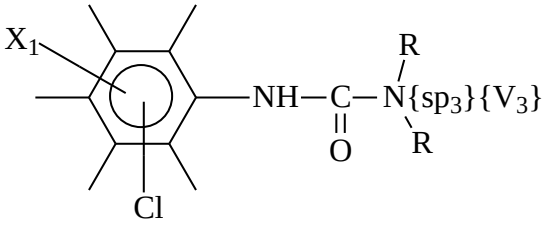


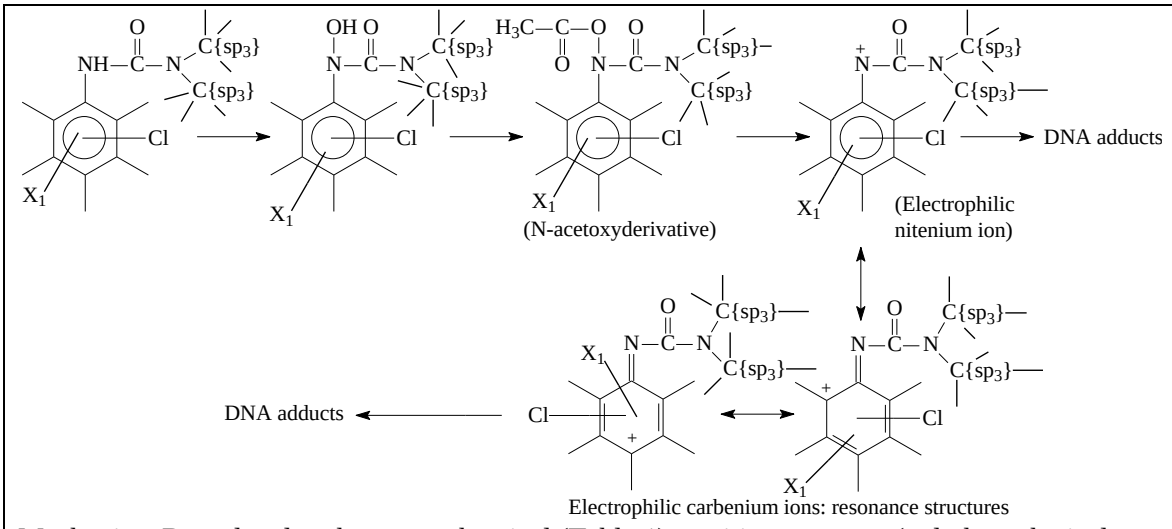
Set of chemicals used for profile development	Specific Imine and Thione Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *1,4-Benzoquinone Dioxime*, IARC Monographs, Vol. 71, 1999; ISBN-13 (PDF): 978-92-832-1571-4. 2. Westmoreland, C., Environ. Molec. Mutag. **19** (1992), 71 – 76. 3. Niufar, N. N., Rev. Soc. Quimica de Mexico **46**(4) (2002), 307 – 312. 4. Sinha, B., Biochem. Pharmacol. **32**(22) (1983), 3279 – 3284. 5. Yamamoto, K., Biochem. Pharmacol. **41** (6/7) (1991), 905 – 914. 6. Chlopkiewicz, B., Toxicol. Lett. **110** (1999), 203 – 207. 7. Benedict, W. F., Canc. Res. **37** (1977), 2209 – 2213. 8. Seino, Y., Canc. Res. **38** (1978), 2148 – 2156. 9. Pommer, Y., Cold Spring Harbor Press, Ed. By M. L. DePamphilis, 1 – 28; http://discover.nci.nih.gov/pommier/ReplicationInhibitorsText.pdf. Last visited 09.2019. Last visited: June, 2021. 10. Christman, J. K., Oncogene **21** (2002), 5483 – 5495. 11. Kelecsenyi, Z., Mutag. **15**(1) (2000), 25 – 31.

Individual profile/alert	
Name	Substituted Benzoindoline and Indole Derivatives

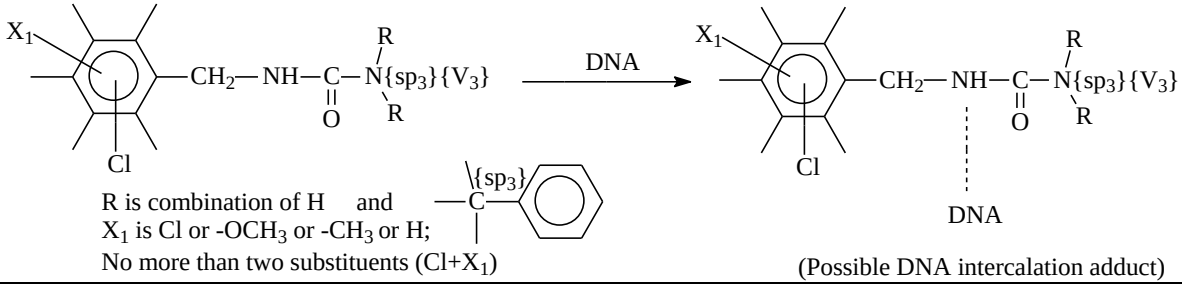
Type of profile	Structural alert
Description/applicability domain	 (R is CH_3 or C_2H_5) C{sp_3}: no more than three C-atoms)  R_1, R_2 are $-CH_3$ (both) or R_1 is  (Y is -CN or $-NO_2$); R_2 is -OH); R_3 and R_4 are H OR R_1, R_2 are $-CH_3$ (both); one of R_3, R_4 is $-CH_3$, the other is H; or both R_3 and R_4 are H
Mechanism	AN2 Nucleophilic addition to C=C-bond Non-covalent interactions DNA intercalation
	 DNA-NH_2: purine/pyrimidine nucleobase with exocyclic -NH_2 groups) (Expertly assumed DNA adduct, possibly eliciting mutagenicity) R is $-CH_3$ or $-C_2H_5$ C{sp3}: no more than 3 C-atoms  (Expertly assumed DNA intercalative adduct)  (Expertly assumed DNA intercalative adduct)
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in

	this profiler according to the references listed below.
References	1. Weems, J. M., N. S. Cutler, Ch. Moore, W. K. Nichols, D. Martin, E. Makin, J. G. Lamb, G. S. Yost, 3-Methylindole is Mutagenic and a Possible Pulmonary Carcinogen, Toxicol. Sci 112(1) (2009), 59 – 67. 2. Curvall, M. I. Florin, T. Jannson, Mutagenicity of some indoles and related compounds in the Ames test, Toxicol. 23 (1982) 1 – 10.

Individual profile/alert	
Name	Substituted Chlorophenylalkylurea Derivatives
Type of profile	Structural alert
Description/applicability domain	 (n = 0 - 1); R is CH_3 (both) or combination of H and C{sp3}; X_1 is Cl or -OCH_3 or -CH_3 or H; No more than two substituents (Cl+X_1)
Mechanism	SN1 Nucleophilic attack after nitrenium and/or carbenium ion formation Non-covalent interactions DNA intercalation
Mechanism A - related to halophenyl urea derivatives listed in Table 2: Ames-positive with metabolic activation:  R is CH_3 (both) X_1 is Cl or -OCH_3 or -CH_3 or H; No more than two substituents (Cl+X_1)	

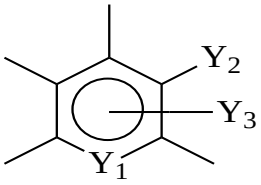


Mechanism B – related to the target chemical (Table 1), positive as parent (only hypothetical scheme):

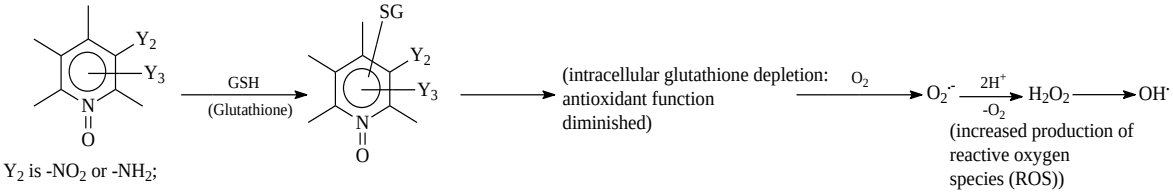


Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Seiler, J. P., Herbcidal Phenylalkylureas as Possible Mutagens. I. Mutagenicity Tests with Some Urea Herbicides, Mutat. Res. 58 (1978), 353 – 359. 2. Seiler, J. P., Herbicidal Phenylalkylureas as Possible Mutagens. II. Chemical Basis of Mutagenic Activity, Pest. Biochem. Physiol. 12 (1979), 183 – 190. 3. NITE – Chemical Management Field – GHS Classification Result, Japan, Cumyluron; https://www.nite.go.jp/chem/english/ghs/15-meti-0005e.html. Last visited: June, 2021.

Individual profile/alert	
Name	Substituted Nitropyridines, Aminopyridines and N-Oxides
Type of profile	Structural alert

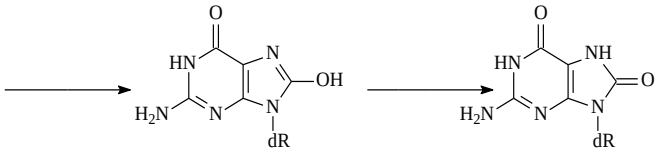
Description/applicability domain	 Y_1 is N{ar} or N{ar}=O; Y_2 is $-NO_2$ or $-NH_2$; Y_3 is $-CH_3$ or CH=O or $-CH_2-CH=O$
Mechanism	Radical Radical mechanism via ROS formation AN2 Schiff base formation

The following simplified mechanistic schemes associated with the observed bacterial mutagenicity effects can be expertly proposed:



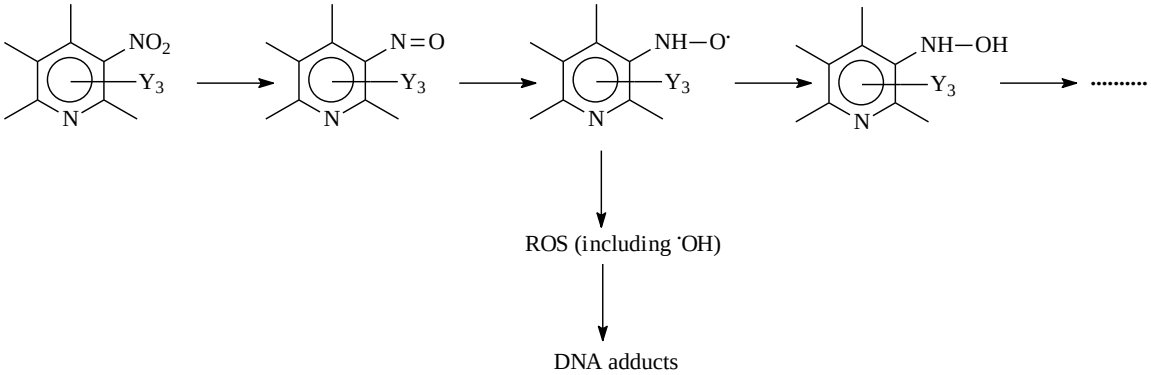
Y_2 is $-NO_2$ or $-NH_2$;

Y_3 is $-CH_3$ or CH=O or $-CH_2-CH=O$

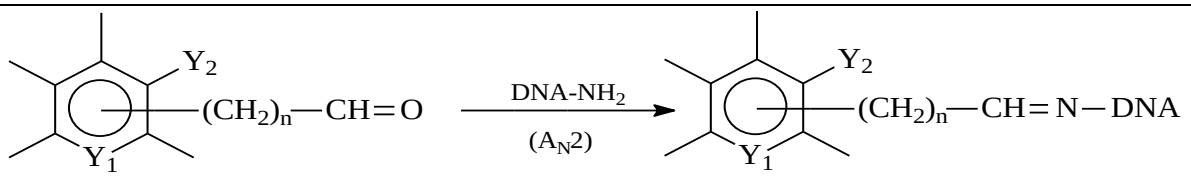


(dR - deoxyribose phosphate fragment)

(8-Oxo-7,8-dihydro-2'-deoxyguanosine DNA adduct)



If Y3 is –CH=O or –CH2CH=O, a third mechanistic scheme associated with Schiff base formation is regarded as possible:



(n = 0- 1)

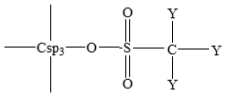
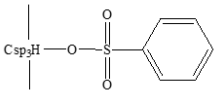
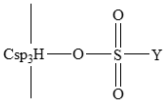
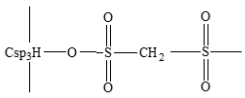
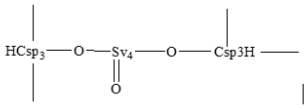
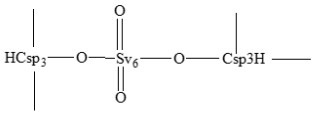
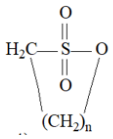
(DNA adduct)

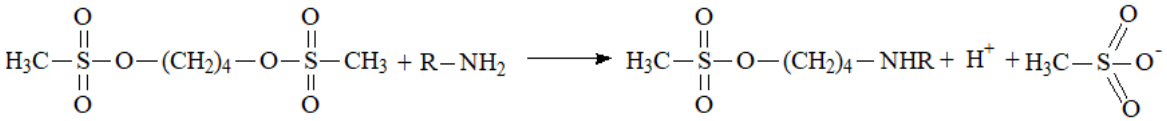
Y_1 is N{ar} or N{ar}=O;

Y_2 is $-NO_2$ or $-NH_2$

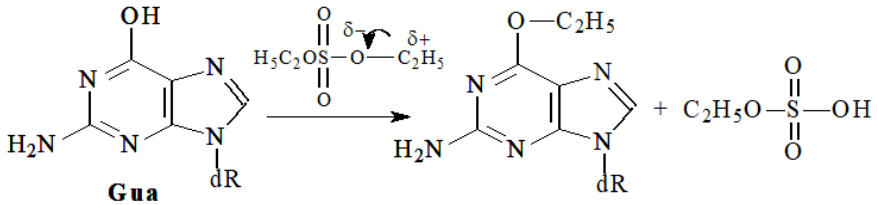
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gorrod, J. W., L. A. Damani; Some factors involved in the N-oxidation of 3-substituted pyridines by microsomal preparations in vitro, Xenobiotica 9(4) (1979), 209 – 218. 2. 1,3-Dinitrobenzene, Biological Test Results; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=99-65-0#section=Biological-Test-Results. Last visited: June, 2021. 3. 3-Nitroaniline, Biological Test Results; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=99-09-2#section=Biological-Test-Results. Last visited: June, 2021. 4. Plošnik A, Vračko M, Sollner Dolenc M. Mutagenic and carcinogenic structural alerts and their mechanisms of action, Arh. Hig. Rada Toksikol 67 (2016), 169 - 182 5. Arima, Y., Ch. Nishigori, T. Takeuchi, Sh. Oka, K. Morimoto, A. Utani, Y. Miyachi, 4-Nitroquinoline 1-Oxide Forms 8-Hydroxydeoxyguanosine in Human Fibroblasts through Reactive Oxygen Species, Toxicol. Sci 91(2) (2006), 382 – 392. 6. Kovacic, P., J. D. Jacintho, Mechanisms of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer, Current Med. Chem. 8 (2001), 773 – 796.

Individual profile/alert	
Name	Sulfonates and Sulfates
Type of profile	Structural alert

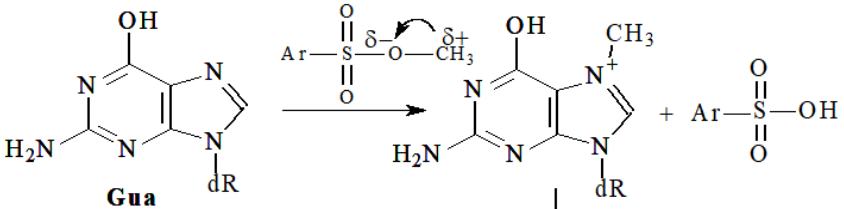
Description/applicability domain	 Structure (I) - Y= H, F;  Structure (II) – single aromatic ring only  Structure (III) – Y= -CH_3, -CH_2CH_3;  Structure (IV)  Structure (V)  Structure (VI)  Structure (VII) – n=2-4
Mechanism	S_N2 at sp3-carbon atom (alkylation)



(R-NH_2: biological macromolecule (e.g., adenine or guanine fragment in DNA)



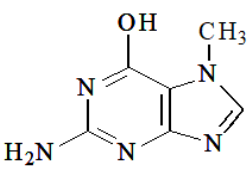
Gua

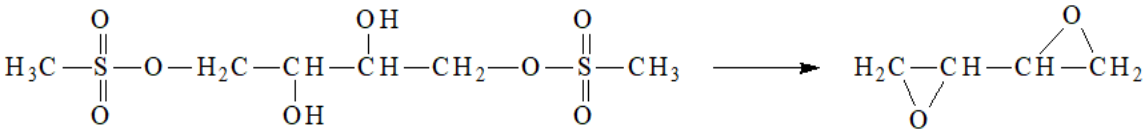


Gua

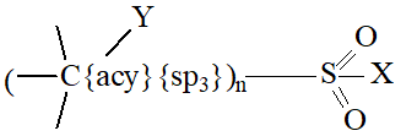
(dR - deoxyribose phosphate fragment)

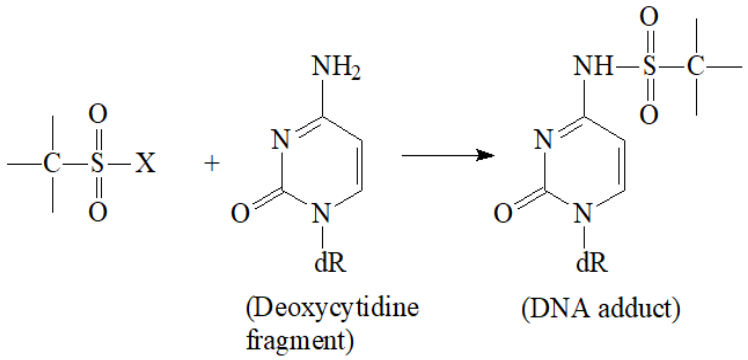
("depurination")



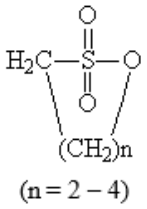
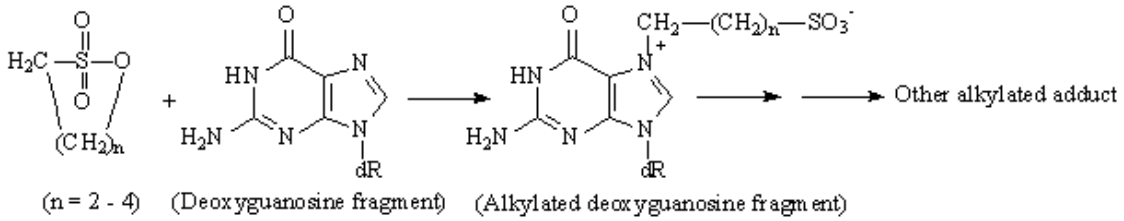


Set of chemicals used for profile development	Sulfonates and Sulfates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Colvin, M., *Alkylating Agents and Platinum Antitumor Compounds* (In Ch. 51, Section 12: Chemotherapeutic Agents, Holland-Frei Cancer Medicine, 6th Ed., Kufe DW, Pollock RE, Weichselbaum RR, et al. (Editors), Hamilton (ON): BC Decker; 2003; http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=cmed6.figgrp.12445 Last visited: June, 2021. 2. Kovacic, P., Current Med. Chem. **8**, (2001), 773 – 796. 3. Couch, D. B., Mutat. Res. **57**(2) (1978), 217 - 224. 4. Sanderson, B. J. S., Mutat. Res. **355** (1996), 41 – 57. 5. Kazius, J., J. Med. Chem. **48** (2005), 312 – 320. 6. Hopppe, H., Canc. Res. **38** (1978), 1595 – 1600. 7. McCann, J., Proc. Nat. Acad. Sci. USA **72**(12) (1975), 5135 – 5139. 8. Abu-Shakra, A., Mutat. Res. **470**(1) (2000), 11 – 18. 9. Zeiger, E., Environ. Mol. Mutagen. **13**(4) (1989), 343 – 346. 10. Hartley, J. A., Brit. J. of Cancer **79**(2) (1999), 264 – 266.

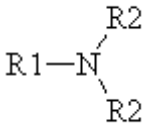
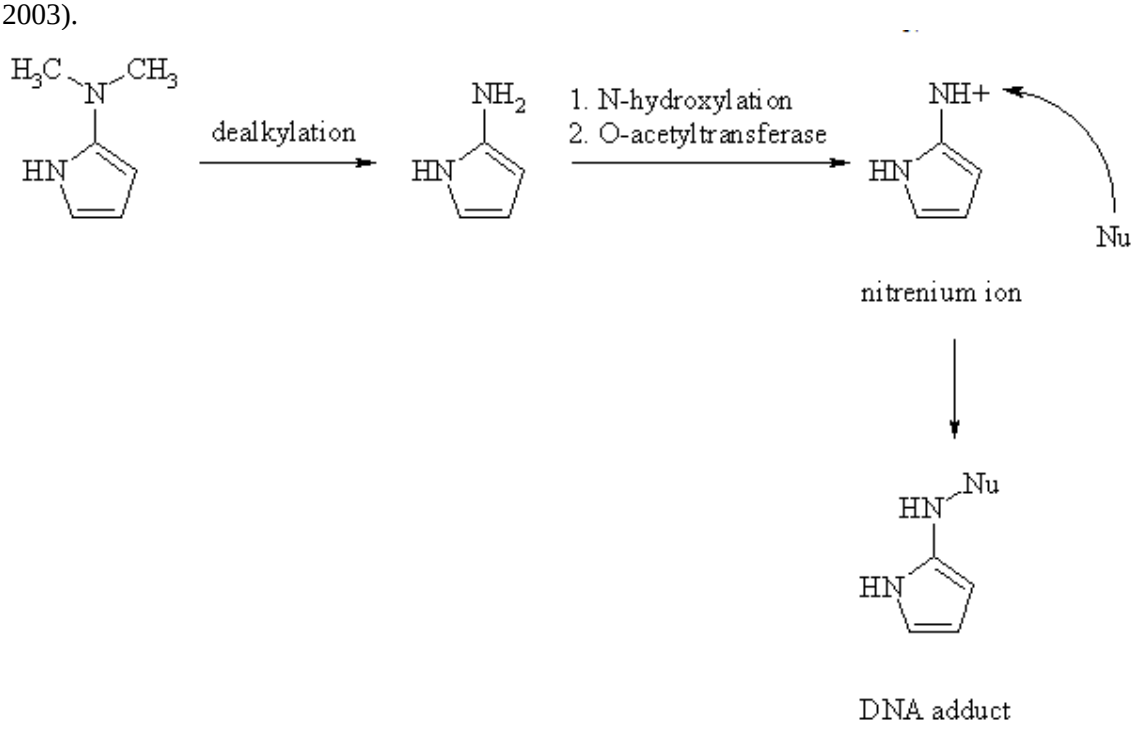
Individual profile/alert	
Name	Sulfonyl Halides
Type of profile	Structural alert
Description/applicability domain	 (X is F, Cl or Br; n = 1 - 3; can be also isopropyl radical; Y is X attached as substituent to the alkyl chain; or -H; or their combinations) **(Small-molecule alkanesulfonyl halides)**
Mechanism	**S_N2 attack on sulfur atom**



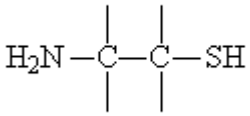
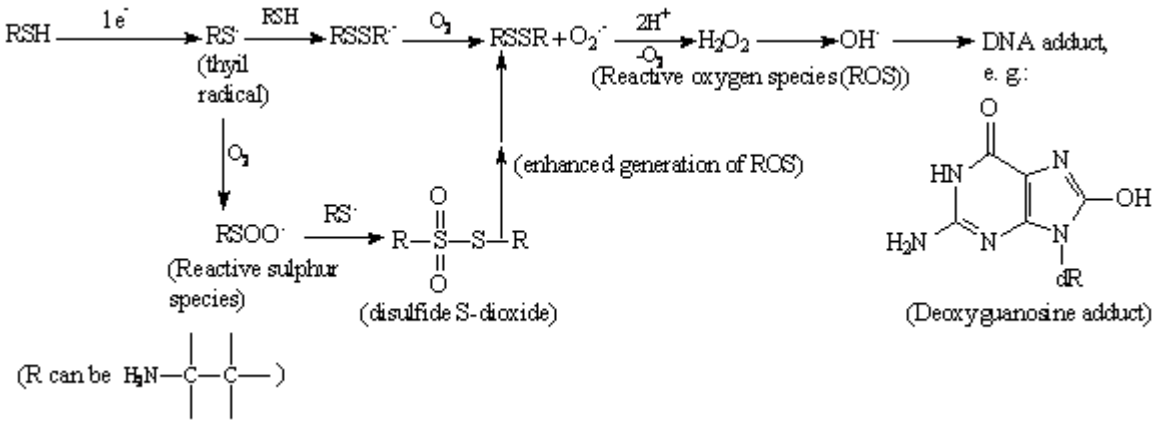
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Sawatari, K., Ind. Health **39** (2001), 341 – 345. 2. Supek, Fr., Invest New Drugs **26** (2008), 97 – 110). 3. *4-Methylbenzenesulfonyl Chloride CAS No. 98-59-9*, SIDS Final Assessment Report for SIAM 17, Arona, Italy, 11 – 14 November 2003, OECD SIDS. 4. Tsuchiya, Y., Water Sci & Technol. **25**(2) (1992), 123 – 130 (Abstract).

Individual profile/alert	
Name	Sultones
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**Ring opening S_N2 (alkylation)**
DNA-alkylating capability and the *in vitro* genotoxicity of sultones can be expertly suggested: 	
Set of chemicals used for profile development	Sultones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in

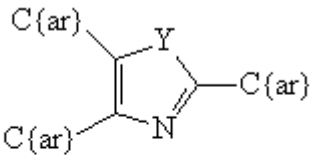
	this profiler according to the references listed below.
References	1. *1,3-Propane Sultone, Exposure Data*, IARC Monographs Vol. 71, 1095 – 1102; ISBN-13 (PDF): 978-92-832-1571-4. 2. *1,4-Butane Sultone* [MAK Value Documentation, 1992], The MAK Collection for Occupational Health and Safety; DOI: 10.1002/3527600418.mb163383isme0004. 3. Kubinski, J. Bacteriol. **136**(3) (1978), 854 – 866). 4. Golker, Chem.-Biol. Interact. **14** (1976), 195 – 202. 5. Hemminki, Carcinog. **4**(7) (1983), 901 – 904).

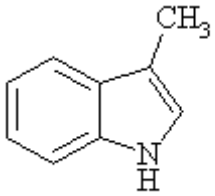
Individual profile/alert	
Name	Tertiary aromatic amine
Type of profile	Structural alert
Description/applicability domain	 R1 = any five membered heterocyclic ring system (the heterocyclic ring can contain any combination of carbon, nitrogen, oxygen or sulphur in which R is connected via a carbon atom) R2 = any combination of methyl, ethyl
Mechanism	SN2 reaction Nitrenium ion formation
Protected secondary and tertiary aromatic amines (methyl and ethyl) undergo metabolism to a reactive nitrenium ion. This ion can bind to DNA via an SN1 mechanism (Kalgutkar et al 2005, Jones et al 2003).  	
Set of chemicals used for profile development	Not appliciable – all chemicals are private and can`t be disclosed.
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.

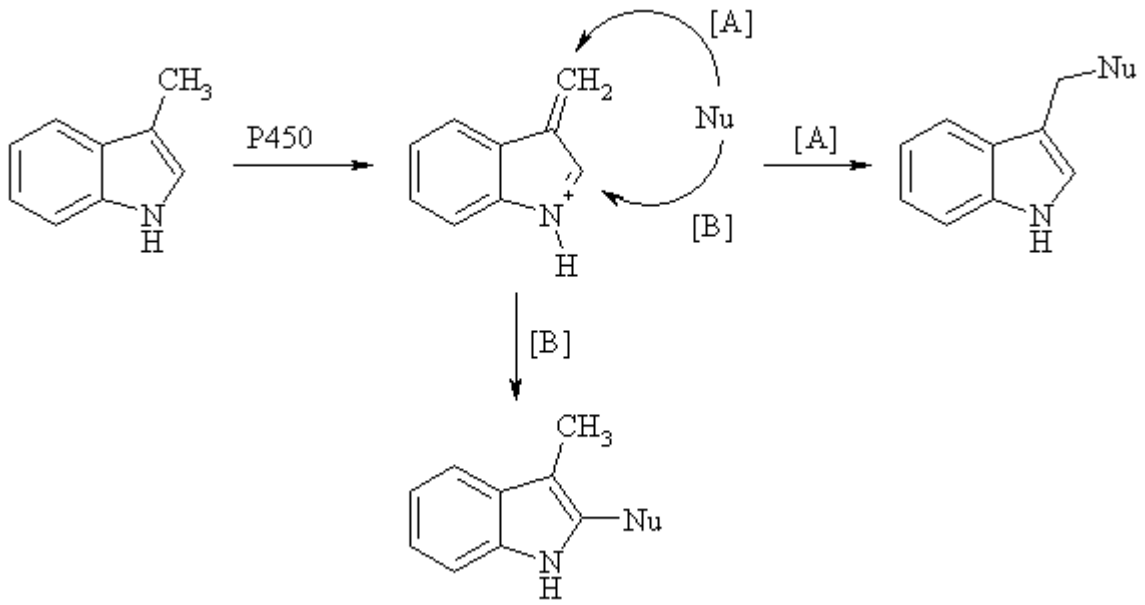
References	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225

Individual profile/alert	
Name	Thiols
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS generation (indirect)
	
Set of chemicals used for profile development	Thiols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Stark, A. A., Carcinog. 9(5) (1988), 771 – 777. 2. Sen, Ch. K., Am. J. Clin. Nutr. **72** (2000, 653S - 669S. 3. Jacob, C., Biochem. Soc. Transact **32** (2004), 1015 – 1017; http://www.biochemsoctrans.org/bst/032/bst0321015.htm. 4. Giles, G. I., Free Radic. Biol. Med. **31**(10), (2001), 1279 – 1983. 5. Kiley, P. J., PloS Biol. **2**(11) (2004), e400; https://doi.org/10.1371/journal.pbio.0020400, last visited 06.2021. 6. Giles, G. I., Biochem. J. 364 (2002), 579 – 585.

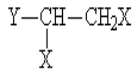
Individual profile/alert	
Name	Triarylimidazole and Structurally Related DNA Intercalators
Type of profile	Structural alert

Description/applicability domain	 (Y can be N(V3)(sp3), -S-(V2), -O-) (C(ar): carbon atom as part of arene ring)
Mechanism	Non-covalent interactions DNA intercalation
The chemical mechanisms accompanied by the formation of a covalent adducts are expected to be characteristic for *Salmonella typhimurium* strains, related to base pair substitutions (strains TA100, TA102 and TA1535). However, DNA intercalations operate with the strains associated with induction of frameshift mutations (TA97, TA98, TA1537 and TA1538). Substituted triphenylimidazoles were suggested to belong to the class of DNA intercalating agents [1], probably due to the multi-cyclic planar molecular system and conjugation effects.	
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Enoch, Mutat. Res. **743** (2012), 10 – 19. 2. Mercangoz, A., B. A. Tuylu, *Detection of Mutagenic Effects of 2*,4,5-Trisubstituted *Phenyl Imidazole and Its Derivatives in Ames/Salmonella/Test System*, Turk. J. Biol. **24** (2000), 57 – 64 (Abstract); http://journals.tubitak.gov.tr/biology/issues/biy-00-24-1/biy-24-1-5-96048.pdf. Last visited: June, 2021.

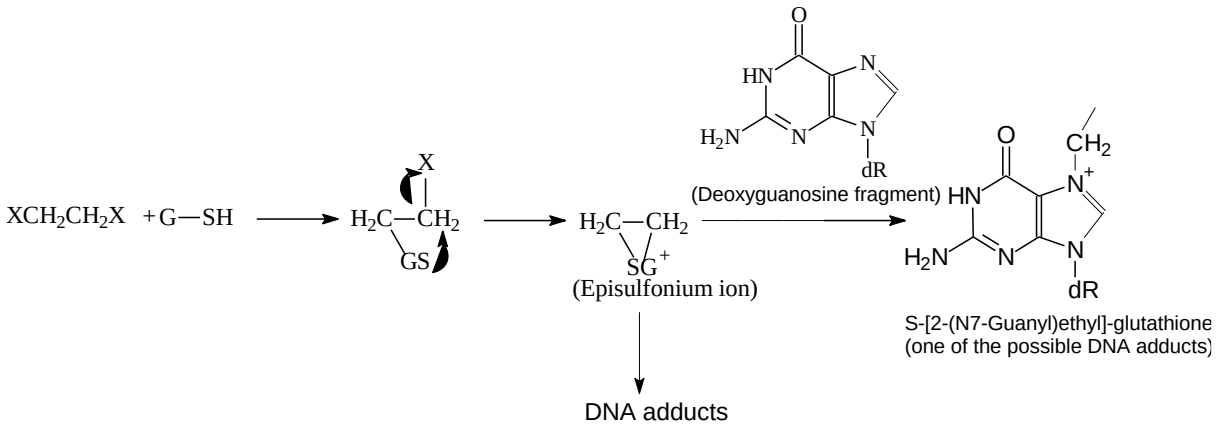
Individual profile/alert	
Name	Tri-Methylindole derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Michael addition with biological nucleophiles



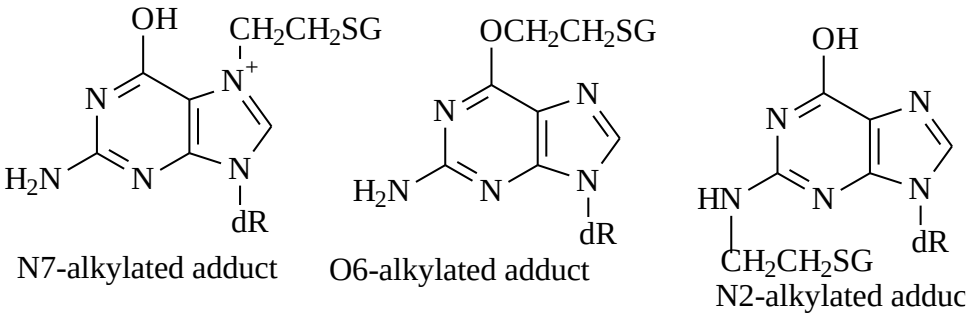
Set of chemicals used for profile development	No chemicals in the local training set
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225 Regal KA et al (2001) Chemical Research in Toxicology, 14, p1014-1024

Individual profile/alert	
Name	Vicinal Dihaloakanes
Type of profile	Structural alert
Description/applicability domain	 (Y is –H, -$(CH_2)_nH$ (n = 1, 2), -$O(CH_2)_nH$ (n = 0 -2), -CH_2-O-, C{acy}{sp2}; No other halogens bound to Y)
Mechanism	Internal S_N2 reaction with aziridinium and/or cyclic sulfonium ion formation and DNA alkylation
1,2-dichloroethane is reasonably anticipated to be a human carcinogen, based on sufficient evidence of carcinogenicity in experimental animals. *In vivo* and *in vitro* studies in rodents have revealed that the primary metabolic pathway for 1,2-dichloroethane probably involves conjugation with glutathione, and the compound shows bacterial mutagenicity. This is S_N2 (bimolecular nucleophilic attack) of glutathione GSH on the electron-deficient carbon of 1,2-dichloroethane (also for 1,2-dibromoethane, 1,2-dichloropropane, etc.) and S-(2-chloroethyl)-glutathione adduct is formed. One of the further possible metabolic pathways is the loss of chloride ion with the formation of *episulfonium ion*, which is highly reactive. This ion is believed to be the reactive *electrophilic* intermediate that results in covalent reaction with biopolymers such as DNA, and is believed to determine the	

mutagenic potential of this class of organic halides [1 – 4, 6]:



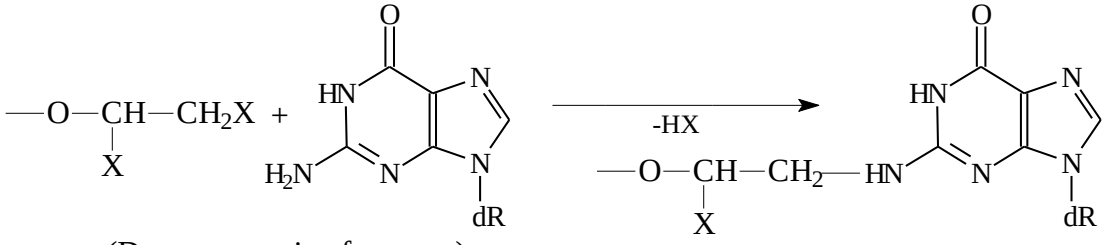
The major product of this reaction is S-[2-(N7-guanyl)ethyl]glutathione, but N2- and O6-guanyl adducts are also formed, and all three adducts are potentially mutagenic [3]:



(dR - deoxyribose phosphate fragment)

Similar mechanism of *in vitro* metabolic activation by forming episulfonium cation as reactive intermediate has also been suggested for structurally similar short-chain compounds such as 1,2-dibromo-3-chloropropane [5].

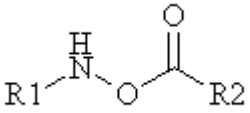
Beside 1,2-dichloroethane, 1,2-dibromoethane belonging to this class of compounds was also found to possess bacterial mutagenicity [7]. Short-chain vicinal dihalolakanes with halogen attached to terminal carbon atom are assumed to act by direct alkylation mechanism, too. Other short-chain vicinal haloalkane derivatives with electron-withdrawing heteroatoms adjacent to the –CHX fragment such as 1-methoxy-1,2-dichloroethane, 2,3-dibromo-propanol, etc., are believed to cause also direct mutagenicity by alkylation mechanism:



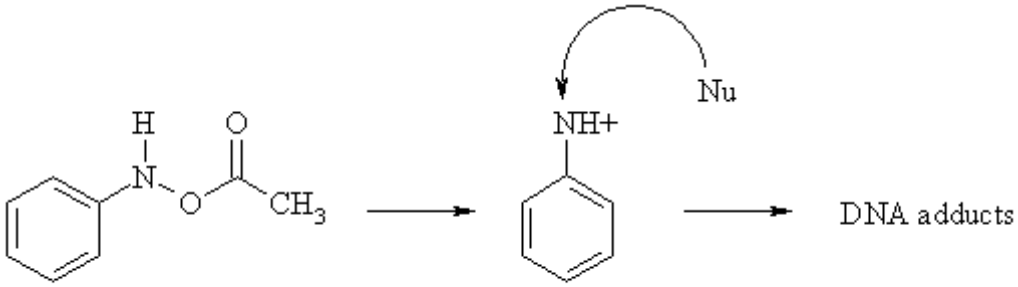
(Deoxyguanosine fragment)

(One possible DNA alkylated adduct)

Set of chemicals used for profile development	Vicinal Dihaloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Anders, Drug Metabol. Rev. **36** (3 – 4) (2004), 583 – 594. 2. *Public Health Goal for 1,2-Dichloropropane in Drinking Water*, Office of Environmental Health Hazard Assessment, California EPA, February 1999; http://www.oehha.ca.gov/water/phg/pdf/12dcp_f.pdf. 3. Guengerich, Environ. Health Persp. **76** (1987), 15 – 18. 4. Liu, J. Biol. Chem. **277** (40) (2002), 37920 - 37928. 5. 5. Miller, J. Toxicol. Environ. Health: Current Issues **19**(4) (1986), 503 – 518. 6. Rannug, Chem.-Biol. Interact. **20** (1978), 1 – 16. 7. Strubel, K., Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128.

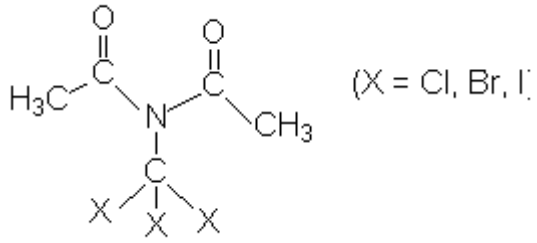
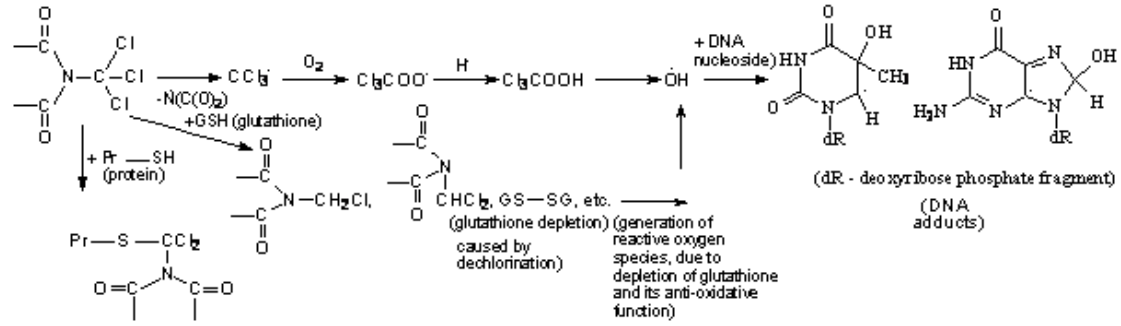
Individual profile/alert	
Name	Aromatic ester hydroxylamine
Type of profile	Structural alert
Description/applicability domain	 R1 = aromatic carbon atom R2 = any carbon atom
Mechanism	SN1 reaction Nitrenium ion formation

Desterification to produce a reactive nitrenium ion capable of reacting with DNA via an SN1 mechanism is the most likely mechanism (Jones et al 2003).

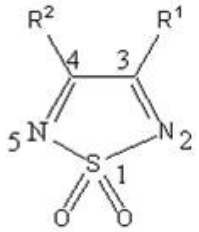


Nu = biological nucleophile

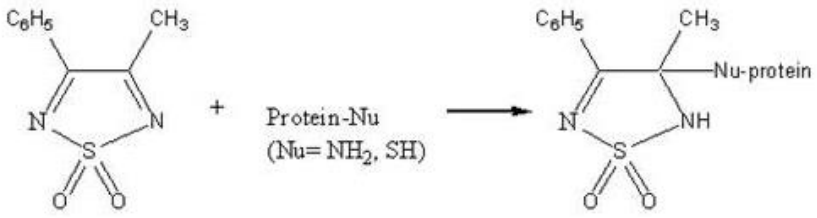
Set of chemicals used for profile development	Not appliciable
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Jones CR et al (2003) Chemical Research in Toxicology, 16, p1251-1263

Individual profile/alert	
Name	N-Trihalomethyldiacylimides
Type of profile	Structural alert
Description/applicability domain	 (X = Cl, Br, I)
Mechanism	Acylation Direct acylation involving a leaving group
	
Set of chemicals used for profile development	Not Appliciable
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Schneider, M., G. B. Quistad, J. E. Casida, *Glutathione Activation of Chloropicrin in the Salmonella Mutagenicity Test*, Mutat. Res. **439**(2), 1999, 233 – 238. 2. Sparks, S. E., G. B. Quistad, J. E. Casida, *Chloropicrin: Reactions with Biological Thiols and Metabolism in Mice*, Chem. Res. Toxicol. **10**(9), 1997, 1001 – 1007. 3. IPCS Inchem Home, FAO Meeting Report No. PL/1965/10/2, *Evaluation of the Hazards to Consumers Resulting from the Use of Fumigants in the Protection of Food*, WHO/Food Add/28.65, Food and Agriculture Organization of the United Nations, World Health Organization, 1965 4. Toxicological Review of Carbon Tetrachloride (CAS No. 56-23-5), In Support of Summary Information on the Integrated Risk Information System (IRIS), March 2010, US-EPA, Washington DC; 5. Kovacic, P., J. D. Jacintho, *Mechanisms of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer*, Current Medic. Chem. **8**,

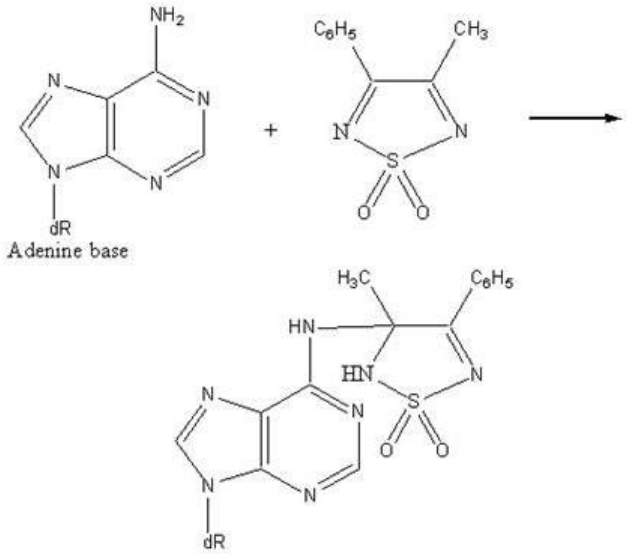
2001, pp. 773 – 796.
6. Witherell, H. L., R. A. Hiatt, M. Replogle, J. Parsonnet, *Helicobacter pylori Infection and Urinary Excretion of 8-Hydroxy-2-Deoxyguanosine, an Oxidative DNA Adduct*, Canc. Epidemiol. Biomarkers & Prevention **7** (1998), 91 – 96.
7. Wiseman, H., B. Halliwell, *Damage to DNA by Reactive Oxygen and Nitrogen Species: Role in Inflammatory Disease and Progression to Cancer*, Biochem. J. 313 (1996), 17 – 29.

Individual profile/alert	
Name	Thiadiazoledioxide Derivatives
Type of profile	Structural alert
Description/applicability domain	 Where R^1 and R^2 are Hydrogen, Alkyl or Aryl
Mechanism	1,2,5-Thiadiazole 1,1-dioxide derivatives

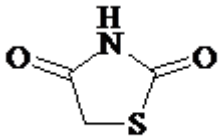
It was found that 3-methyl-4-phenyl-1,2,5-thiadiazole 1,1-dioxide was a moderate skin sensitizer [5]. The mechanism of interaction with skin proteins is presented below:

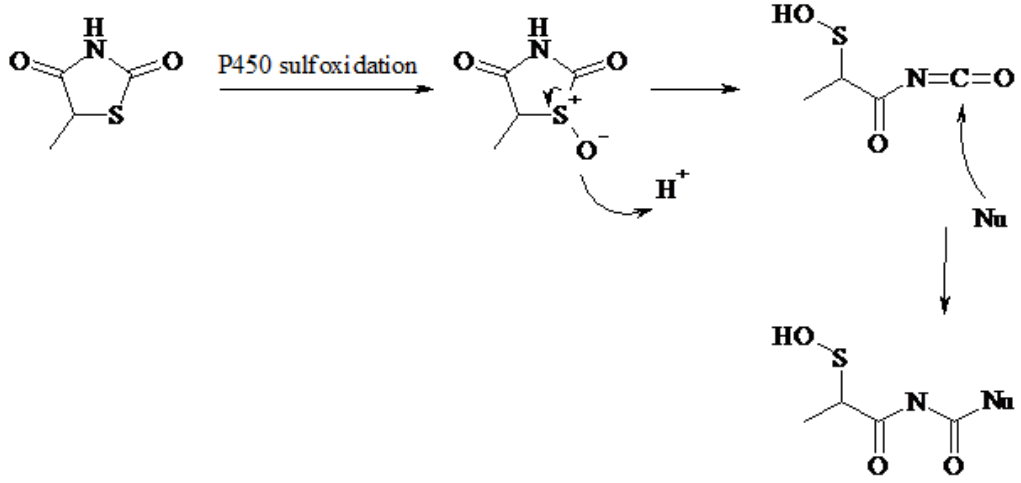


It may be assumed that thiadiazole dioxide derivatives can bind to the amino groups in DNA bases regardless of their lower nucleophility.



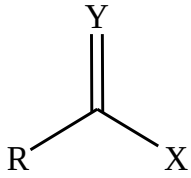
Set of chemicals used for profile development	Not Appliciable
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. J. A. Caram, can. J. Chem. 1996, 74(8), 1564-1571. 2. V. J. Aran, Adv. Heterocycl. Chem. 1988, Vol.44, 81-197 3. R. Y. Wen, J. Org. Chem. 1975, Vol.40(19), 2743-2746. 4. J. A. J. Phys. Org. Chem. 2003, 16(4), 220-225. 5. G. Patlewicz, Chem. Res. Toxicol. 2008, 21(2), 521-541.

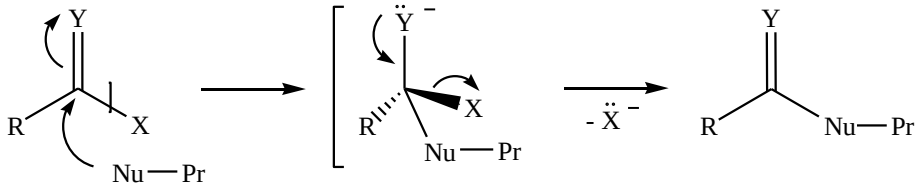
Individual profile/alert	
Name	Thiazolidinediones
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Acylation P450 Mediated Activation to Isocyanates or Isothiocyanates
The most likely mechanism for DNA binding that has been suggested involves a P450 mediated sulfoxidation. This reactive intermediate species then undergoes ring scission to produce an isocycanate. This isocyanate undergoes an acylation mechanism with a biological nucleophile such as DNA (Bedir et al 2008, Kalgutkar et al 2005)	



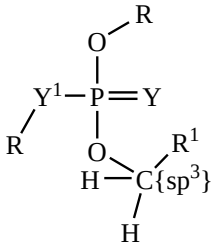
Set of chemicals used for profile development	Not Appliciable
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Bedir A et al (2008) Environmental and Molecular Mutagenesis, 49, p185-191. Kalgutkar AS et al (2005) Current Drug Metabolism, 6, p161-225.

Protein binding alerts:

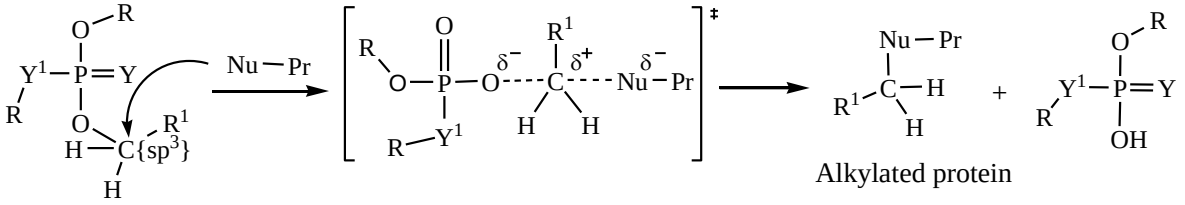
Individual profile/alert	
Name	(Thio)Acyl and (thio)carbamoyl halides, cyanides, azides, etc.
Type of profile	Structural alert
Description/applicability domain	 **Y** = O, S; **X** = F, Cl, Br, I, C≡N, N_3; **R** = C{sp^3}, N{v3}; O-C{sp3}; **Classification:** a) (Thio)Acyl halides: Y = O, S; X = F, Cl, Br, I; R = C{sp^3} b) (Thio)Carbamoyl halides: Y = O, S; X = F, Cl, Br, I; R = N{v3} c) (Thio)Acyl cyanides: Y = O, S; X = C≡N; R = C{sp^3} d) (Thio)Carbamoyl cyanides: Y = O, S; X = C≡N; R = N{v3}

	e) Thio)Acyl azides: Y = O, S; X = N_3; R = C{sp^3} f) (Thio)Carbamoyl azides: Y = O, S; X = N_3; R = N{v3} g) Alkyl halocarbonates: Y = O; X = F, Cl, Br, I; R = O-C{sp3}
Mechanism	Acylation, Direct acylation involving a leaving group
The acylation mechanistic domain involves the attack of a (thio)carbonyl (or (thio)carbonyl-type) compound by a biological nucleophile such as cysteine or lysine. In an acylation reaction, the (thio)carbonyl group is attached to an electronegative 'leaving group' (for example halogen, C≡N, N3, etc.) which is expelled during the course of the reaction [2]. A common mechanism via acylation reaction for compounds with active fragments as reported above is presented in Figure 1 below:  Nu = -S^-, -NH_2	
Set of chemicals used for profile development	(Thio)Acyl and (thio)carbamoyl halides, cyanides, azides, etc.
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis*, **1987**, 2(5), 357-365. 2. Enoch, S.J., Ellison, C.M., Schultz, T.W., Cronin, M.T., A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit., Rev. Toxicol.*, **2011**, 41(9), 783-802. 3. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213.

Individual profile/alert	
Name	(Thio)Phosphates
Type of profile	Structural alert

Description/applicability domain	 **Y** = O, S; **Y¹** = O, S; **R** = any C; R^1 = H, CH_3 **Classification:** 1. Phosphates: **Y** = O 2. Thiophosphates: **Y** = S
Mechanism	S_N2, Nucleophilic substitution at sp^3 carbon atom

The alkyl (thio)phosphates is shown to be capable of binding covalently to proteins via an SN2 reaction at an sp3 hybridized carbon atom [2]. The mechanism of SN2 alkylation reaction is shown in Figure 1[3]:

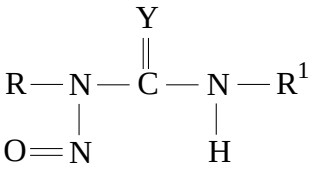


Y = O, S; Y^1 = O, S;
R = any C; R^1 = H, CH_3
Nu = -SH, $-NH_2$

Fig. 1. Nucleophilic substitution at sp3 carbon atom for (thio)phosphonates with Cys and Lys protein nucleophiles

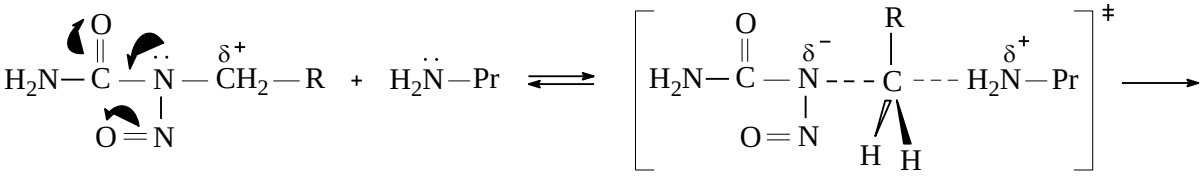
Set of chemicals used for profile development	(Thio)Phosphates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Timoroğlu, İ., Yüzbaşıoğlu, D., Ünal, F., Yılmaz, S., Aksoy, H., Çelik, M., Assessment of the genotoxic effects of organophosphorus insecticides phorate and trichlorfon in human lymphocytes. *Environ. Toxicol.*, **2014**, 29(5), 577-587. 2. Enoch, S.J., Ellison, C.M., Schultz, T.W., Cronin, M.T., A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit., Rev. Toxicol.*, **2011**, 41(9), 783-802. 3. Bedford, C.T., Robinson, J., The alkylating properties of organophosphates. *Xenobiotica*, **1972**, 2(4), 307-337. 4. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances

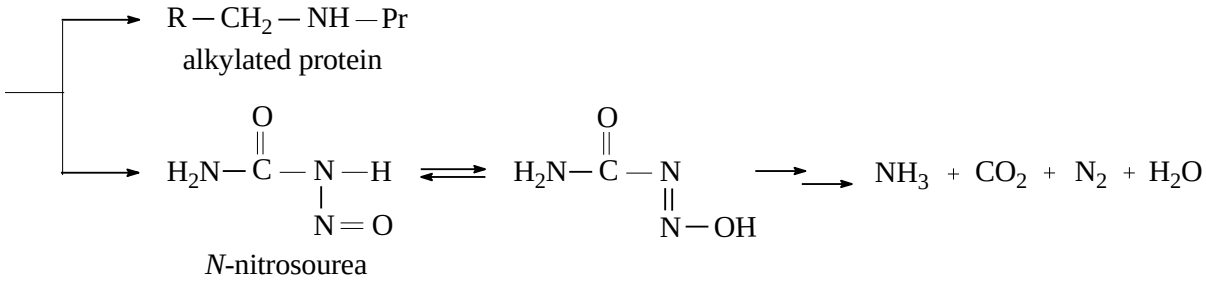
tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213.
5. Trimethyl phosphate, CAS No 512-56-1, NTP, Genetic Toxicology - Mammalian Cell Cytogenetics, Study ID 445223_CA. https://manticore.niehs.nih.gov/cebssearch/genetox/002-02970-0002-0000-2/ Last visited: July, 2021.
6. Woo, Y-T., Arcos, J.C., Argus, M.F., Phosphorous containing alkylating agents. Carcinogenicity and structure-activity relationships. Other biological properties. Activating metabolism. Environmental significance. United States Environmental Protection Agency, Chemical Hazard Identification Branch, **1982**, pp 484-485.

Individual profile/alert	
Name	Alkylated nitrosoureas and nitrosoguanidines
Type of profile	Structural alert
Description/applicability domain	 where: R = $(Csp^3)_n$ – acy at n ≥ 1, preferably linear or branched C_1–C_5 alkyl groups; R may also include an acyl group $C(=O)Csp^3$ (acy) and a nitro group; R^1 = H atom; $(Csp^3)_n$ – acy at n ≥ 1; $C(=O)Csp^3$ (acy), Csp^2 (aryl), nitro group, etc.; Y = O and NH.
Mechanism	S_N2, Protein alkylation via direct attack at the *N*-alkyl group S_N1 and S_N2, DNA and protein alkylation via the formation of alkyldiazonium ion

Protein alkylation via direct attack at the *N*-alkyl group

The alkylation of proteins by the nitrosoureas and nitrosoguanidines takes place as a nucleophilic substitution reaction at the active electrophilic center of *N*-alkyl group, involving the electron-withdrawing effect of the neighboring groups [8]. In this case, the alkyl group CH_2R becomes bound to the protein through the amino or sulfhydryl group (Scheme 1).



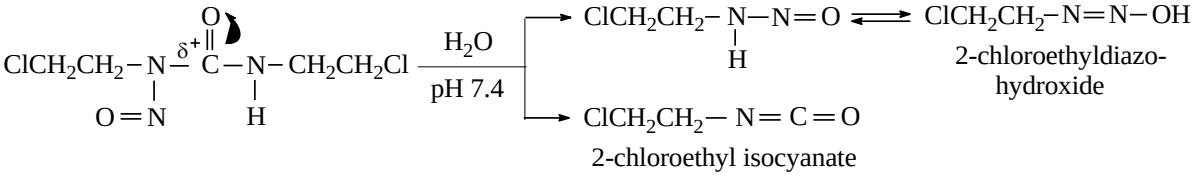


DNA and protein alkylation via the formation of alkyldiazonium ion

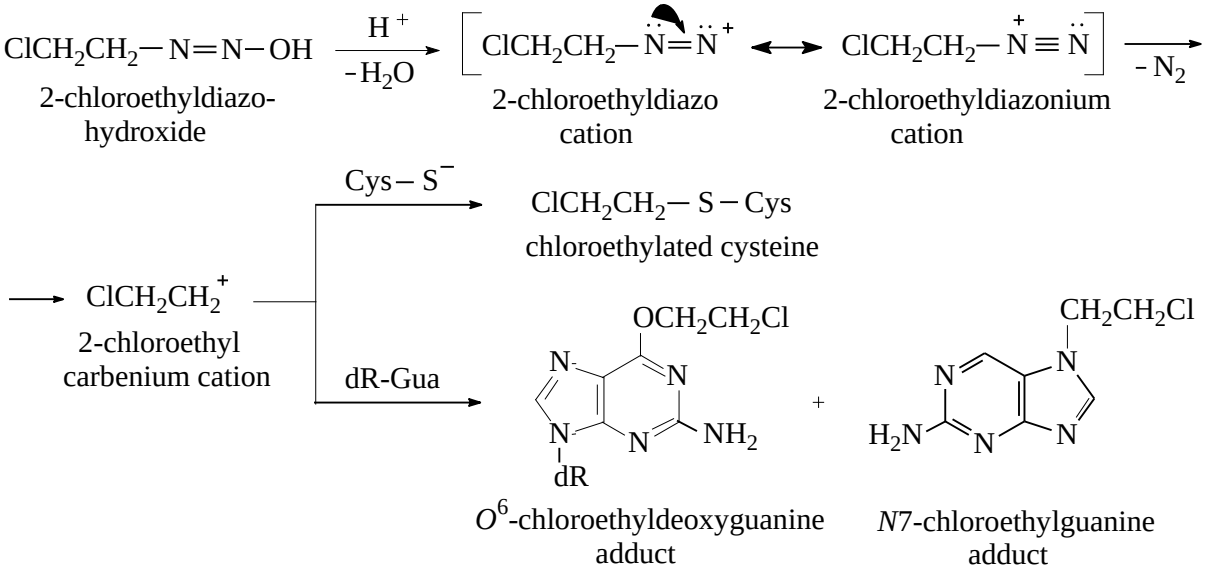
It is well known that alkylnitrosoureas may undergo non-enzymatic decomposition (i.e. hydrolysis) under physiological conditions yielding alkyldiazohydroxide and subsequently alkyldiazonium ion [6,10]. For example, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) decomposes spontaneously in aqueous media at pH 7.4, yielding 2-chloroethyldiazohydroxide and 2-chloroethyl isocyanate (Scheme 2a). 2-Chloroethyldiazohydroxide gives rise to the reactive 2-chloroethyl carbenium ion that can alkylate proteins and DNA bases mainly in the O^6- and $N7$-positions of deoxyguanine (Scheme 2b). Modification of proteins (mainly lysine amino groups) is thought to be due to the carbamoylating activity of 2-chloroethyl isocyanate (Scheme 2c) [10]. Moreover, it is tentatively suggested that the cytocidal effect of BCNU, generally attributed to its alkylating activity, may be potentiated by one of its metabolites, 2-chloroethyl isocyanate, through an inhibition of the repair of damaged DNA [11].

Scheme 2

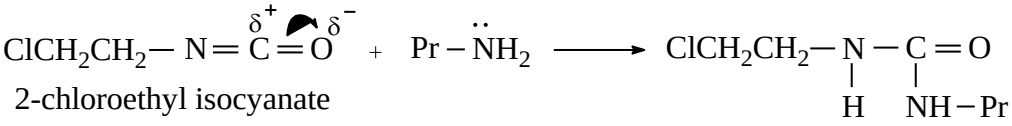
a)



b)



c)



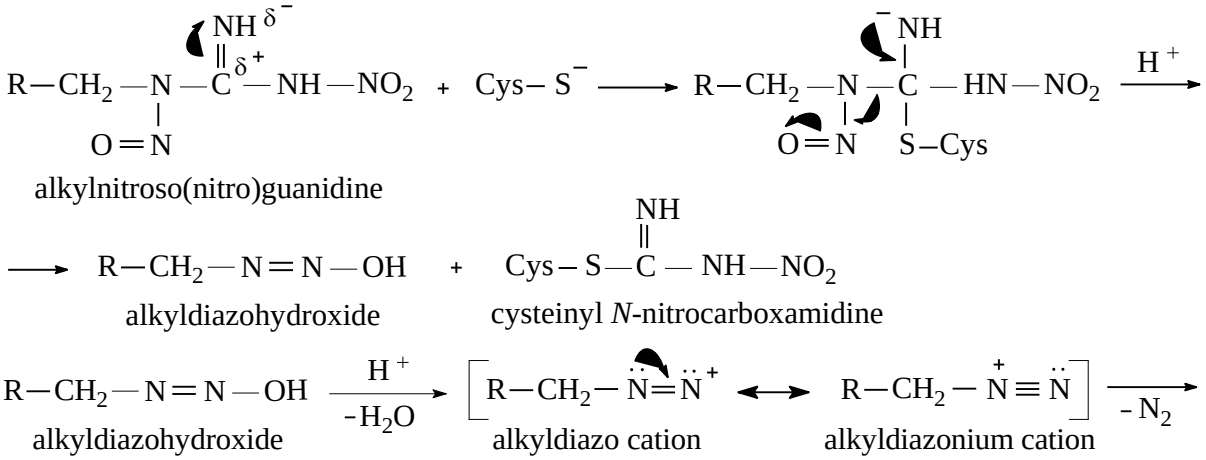
2-chloroethyl isocyanate

protein carbamoylation

Generally, the less reactive agents may bind predominantly with the stronger nucleophiles such as the 7-position of guanine. Highly reactive reagents, such as diazonium ions, are less selective and alkylate the less nucleophilic sites, such as the O^6-position of guanine. For example, *N*-propyl-*N*-nitrosourea and *N*-butyl-*N*-nitrosourea react to form predominantly straight-chain adducts at the 7-position of guanine and branched adducts at the O^6-position. This results indicate that the more nucleophilic sites on the DNA (i.e., *N*7-position of guanine) react with primary diazonium ions via an S_N2 mechanism while the less nucleophilic sites (i.e., O^6-position of guanine) react via an S_N1 mechanism with the secondary carbenium ions produced from decomposition and subsequent rearrangement of the primary diazonium ion [7,12]. It is also believed that alkylation at oxygen residues of DNA bases results in mutagenic events, while *N*-alkylation leads to cytotoxic lesions [13].

Many authors were found that the mutagenicity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and its ethyl-, propyl- and butyl-analogs is highly dependent on where their reactions with thiols take place [9,12,14,15]. Alkylated nitroso(nitro)guanidines are known to react preferentially with thiols (GSH, Cys-SH) thereby forming the alkyldiazohydroxyde and subsequently, the highly reactive and mutagenic alkyldiazonium ion. The predominant reaction of thiol-enhanced decomposition of alkylated nitroso(nitro)guanidines is related to the nucleophilic attack of the cysteine thiolate ion on the iminocarbon of guanidine moiety. The subsequent release of a short-lived intermediate alkyldiazonium ion can alkylate mainly O^6- and *N*7-positions of guanine (Scheme 3).

Scheme 3



alkylnitroso(nitro)guanidine

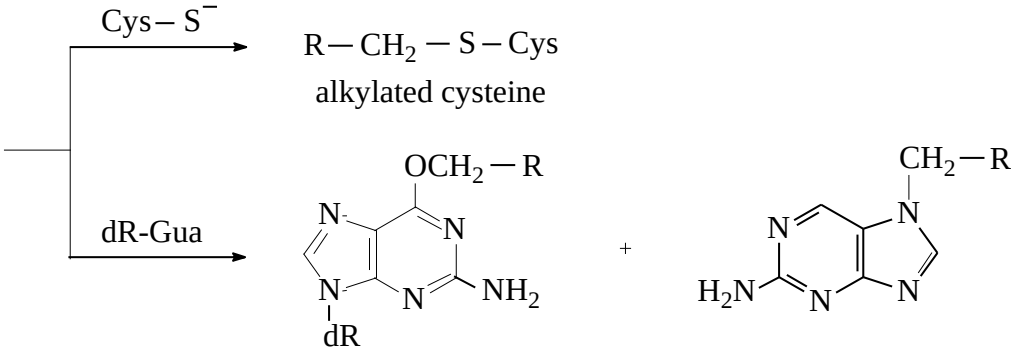
alkyldiazohydroxide

cysteinyl *N*-nitrocarboxamidine

alkyldiazohydroxide

alkyldiazo cation

alkyldiazonium cation

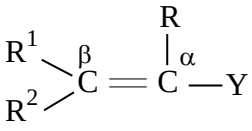


O^6-alkyldeoxyguanine adduct $N7$-alkylguanine adduct

Set of chemicals used for profile development	Alkylated nitrosoureas and nitrosoguanidines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Cai, T., Janczuk, A.J., Nitric oxide donors: chemical activities and biological applications. Chem. Rev., 2002, 102(4), 1091-1134. 2. Wilhelm, D., Bender, K., Knebel A., Angel, P., The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. Mol. Cell Biol., 1997, 17(8), 4792-4800. 3. Ishidate, M. Jr., Odashima, S., Chromosome tests with 134 compounds on Chinese hamster cells in vitro - a screening for chemical carcinogens. Mutat. Res., 1977, 48(3-4), 337-353. 4. Ishidate, M. Jr., Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutat. Res., 1988, 195(2), 151-213. 5. Thust, R., Mendel, J., Schwarz, H., Warzok, R., Nitrosated urea pesticide metabolites and other nitrosamides. Activity in clastogenicity and SCE assays, and aberration kinetics in Chinese hamster V79-E cells. Mutat. Res., 1980, 79(3), 239-248. 6. Buckley, N., A regioselective mechanism for mutagenesis and oncogenesis caused by alkylnitrosourea sequence-specific DNA alkylation. J. Am. Chem. Soc., 1987, 109(25), 7918-7920. 7. Spratt, T.E., Zydowsky, T.M., Floss, H.G., Stereochemistry

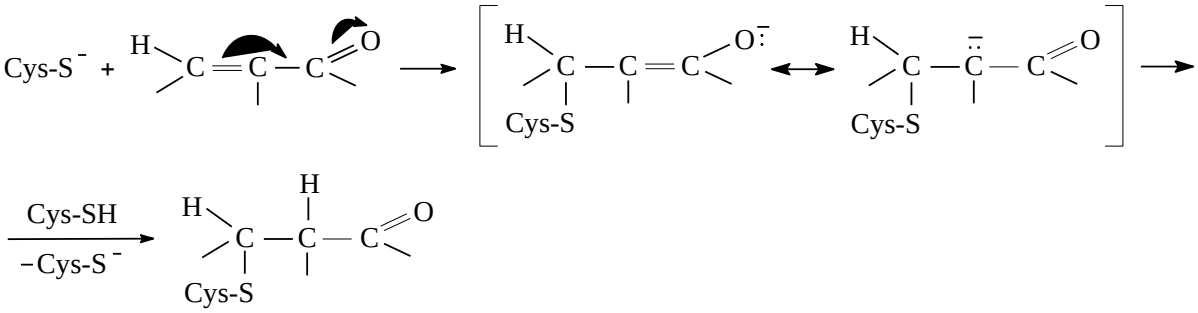
	of the in vitro and in vivo methylation of DNA by (R)- and (S)-N-[2H1,3H]methyl-N-nitrosourea and (R)- and (S)-N-nitroso-N-[2H1,3H]methyl-N-methylamine. Chem. Res. Toxicol., 1997, 10(12), 1412-1419. 8. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. Chem. Res. Toxicol., 2007, 20(1), 44-60. 9. Cain, J.D., A Theoretical Study of the Mechanism of the Alkylation of Guanine by N-Nitrso Compounds. PhD Thesis, University of North Carolina, Chapel Hill, USA, 1992. 10. Wiencke, J.K., Wiemels, J., Genotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Mutat. Res., 1995, 339(2), 91-119. 11. Kann, H.E. Jr, Kohn, K.W., Lyles, J.M., Inhibition of DNA repair by the 1,3-bis(2-chloroethyl)-l-nitrosourea breakdown product 2-chloroethyl isocyanate. Cancer Res., 1974, 34(2), 398-402. 12. Lawley, P.D., Thatcher, C.J., Methylation of deoxyribonucleic acid in cultered mammalian cells by N-methyl-N′-nitro-N-nitrosoguanidine. The influence of cellular thiol concentrations on the extent of methylation and the 6-oxygen atom of guanine as a site of methylation. Biochem. J., 1970, 116(4), 693-707. 13. Jansen, J.G., Mohn, G.R., Vrieling, H., van Teijlingen, C.M., Lohman, P.H., van Zeeland, A.A., Molecular analysis of hprt gene mutations in skin fibroblasts of rats exposed in vivo to N-methyl-N-nitrosourea or N-methyl-N-nitrosourea. Cancer Res., 1994, 54(9), 2478-2485. 14. Romert, L., Jenssen, D., Mechanism of N-acetylcysteine (NAC) and other thiols as both positive and negative modifiers of MNNG-induced mutagenicity in V79 Chinese hamster cells. Carcinogenesis, 1987, 8(10), 1531-1535. 15. Romert, L., Swedmark, S., Jenssen, D., Thiol-enhanced decomposition of MNNG, ENNG and nitrosocimetidine: relationship to mutagenicity in V79 Chinese hamster cells. Carcinogenesis, 1991, 12(5), 847-853.

Individual profile/alert	
Name	alpha,beta-Unsaturated Carbonyls and Related Compounds
Type of profile	Structural alert

Description/applicability domain	 where: Y can be: –CHO, –C≡N, –COR′ with R′ = Csp^3 (scy), Csp^2, –N<, –OC; R can be H atom, CH_3 group, Csp^2(scy), Csp^2(aryl); R should not include Csp^3(acy) containing more than two carbon atoms and OCsp^3 groups. $R^1 \neq R^2$ and can be H, (Csp^3)n at n = 1or 2, Csp^2(aryl, vinyl), etc.
Mechanism	A_N2, Michael addition to activated double bonds

α,β-unsaturated carbonyls can undergo nucleophilic addition of different thiol-containing compounds to the electrophilic β-carbon. Thiols are the softest of the biological nucleophiles and the most likely target for soft electrophiles like Michael acceptors. Michael addition reaction of thiols proceeds via the attack of the corresponding tiolate anion to the β-carbon of the unsaturated carbonyl compound, which leads to the formation of a stable enolate ion [Schultz et al., 2005]. In the presence of excess thiol as a proton source the corresponding thioether adduct is formed [Miyata et al., 1991]. The mechanism for α,β-unsaturated carbonyl compounds is shown in Scheme 1.

Scheme 1

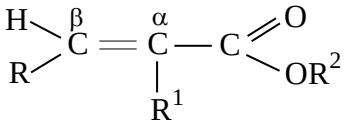


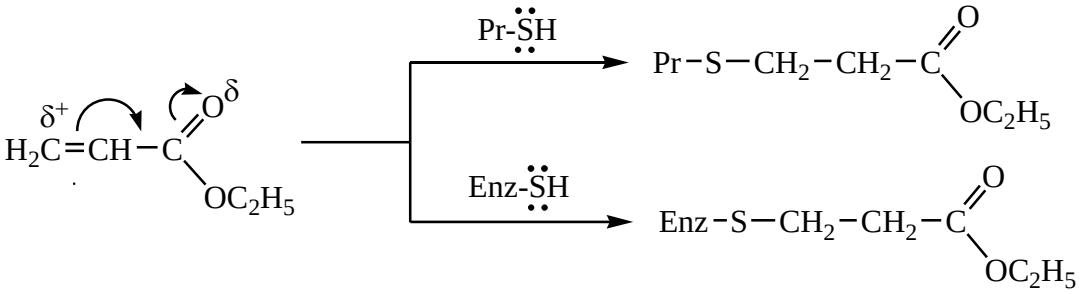
The reactivity of polarized alkenes depends on the nature of the substituents R, R1, and R2. It is apparently that the electron-withdrawing groups on Cβ will activate the double bond although to a lesser extent than on Cα. Conversely, electron-donating groups such as methyl, ethyl, propyl, etc. will deactivate the double bond, more so when they are on Cα than when they are on Cβ. Moreover, the decrease in the reactivity of α,β-unsaturated carbonyls was found to be influenced by the combination of both steric and electronic factors [Schultz et al., 2005].

Set of chemicals used for profile development	alpha,beta-Unsaturated Carbonyls and Related Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.

References	1. T.W. Schultz, J.W. Yarbrough, R.S. Hunter, A.O. Aptula, Verification of the structural alerts for Michael acceptors. Chem. Res. Toxicol., 2007, 20(9), 1359–1363. 2. A.O. Aptula, G. Patlewicz, D.W. Roberts, T.W. Schultz, Nonenzymatic glutathione reactivity and in vitro toxicity: A non-animal approach to skin sensitization. Toxicol In Vitro, 2006, 20(2), 239–247. 3. S.J. Enoch, J.C. Madden, M.T.D. Cronin, Identification of mechanisms of toxic action for skin sensitisation using a SMARTS pattern based approach. SAR QSAR Environ. Res., 2008, 19(5-6), 555–578. 4. T.W. Schultz, K. Rogers, A.O. Aptula, (2009). Read-across to rank skin sensitization potential: Subcategories for the Michael acceptor domain. Contact Dermatitis, 2009, 60(1), 21–31. 5. R.M. LoPachin, D.S. Barber, T. Gavin, Genotoxicity and carcinogenicity of acrylamide: a critical review. Toxicol. Sci., 2008, 104(2), 235–249. 6. H. Tsuda, C.S. Shimizu, M.K. Taketomi, M.M. Hasegawa, A. Hamada, K.M. Kawata, N. Inui, Acrylamide: induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations. Mutagenesis, 1993, 8(1), 23–29. 7. B.I. Ghanayem, M.R. Elwell, S.R. Eldridge, Effects of the carcinogen, acrylonitrile, on forestomach cell proliferation and apoptosis in the rat: comparison with methacrylonitrile. Carcinogenesis, 1997, 18(4), 675–680. 8. D.W. Roberts, G. Patlewicz, A.O. Aptula, Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse Local Lymph Node Assay. Chem. Res. Toxicol., 2007, 20(1), 44-60. 9. G.Y. Patlewicz, Z.M. Wright, D.A. Basketter, C.K. Pease, J.P. Lepoittevin, E. Gimenez Arnau, Structure-activity relationships for selected fragrance allergens. Contact Dermatitis, 2002, 47(4), 219-226. 10. O. Miyata, T. Shinada, I. Ninomiya, T. Naito, T. Date, K. Okamura, S. Inagaki, Stereospecific nucleophilic addition reactions to olefins. Addition

	of thiols to α,β-unsaturated carboxylic acid derivatives. J. Org. Chem., 1991, 56(23), 6556-6564. 11. S.R. Ahlfors, O. Sterner, C. Hansson, Reactivity of contact allergenic haptens to amino acid residues in a model carrier peptide, and characterization of formed peptide-hapten adduct. Skin Pharmacol. Appl. Skin Physiol., 2003, 16(1), 59-68. 12. T.W. Schultz, J.W. Yarbough, E.L. Johnson, Structure-activity relationships for reactivity of carbonyl-containing compounds with glutathione. SAR QSAR Environ. Res., 2005, 16(4), 313-322.

Individual profile/alert	
Name	alpha,beta-Unsaturated Carboxylic Acids and Esters
Type of profile	Structural alert
Description/applicability domain	 R = H atom, Csp3 (scy), Csp2 (scy), C(=O)OC R1 = H atom, Csp3, Csp2 (aryl) R2 = H atom, Csp3 (acy, scy), Csp2 (aryl, vinyl). Positive results for the in vitro chromosomal damage were seen mostly when ester hydrocarbon chain length (Csp3 acy) is between 1-10 carbon atoms [1]. Negative results were obtained in in vitro chromosome aberration test in Chinese hamster V79 cells for methacrylates, containing C12-C18 alkyl ester groups which may be branched or linear and may be even- or odd-numbered in chain length [2].
Mechanism	A_N2, Michael addition to *α,β*-unsaturated acids and esters
Michael addition, a nucleophilic addition on C_β atom of the double bond is suggested as the predominant reaction mechanism of *α,β*-unsaturated esters with intracellular nucleophiles, e.g., free sulfhydryl groups found in proteins, reduced glutathione, and in active sites of enzymes [9,15] The formation of corresponding adducts is presented in Scheme 1: **Scheme 1**	



The difference in the reactivity of acrylates and methacrylates is probably determined by various factors. For example, hydrophilicity and lipophilicity (or hydrophobicity) were correlated with toxic potency, though these parameters are inversely related. Lower-molecular-weight substances were more toxic than those with high molecular weight, and straight-chain esters were less injurious compared with the corresponding branched-chain molecules [11]. It was also found that multifunctional acrylates and methacrylates (esters with greater than one functional vinyl group) required lower concentrations than monofunctional compounds to induce maximal cytotoxic, mutagenic, and clastogenic responses [16].

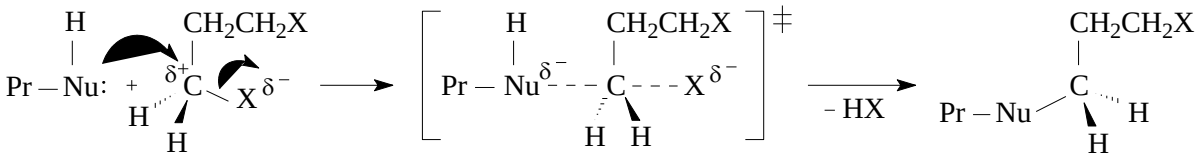
Set of chemicals used for profile development	alpha,beta-Unsaturated Carboxylic Acids and Esters
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. F.R. Johanssen, B. Vogt, M. Waite, R. Deskin, Mutagenicity assessment of acrylate and methacrylate compounds and implications for regulatory toxicology requirements. *Regul. Toxicol. Pharmacol.*, **2008**, 50(3), 322-335. 2. Food Contact Substance Notification No. 732: Environmental Assessment, May 31, **2007**. 3. E.O. Dillingham, W.H. Lawrence, J. Autian, G. Schmalz, *J. Biomed. Mater. Res.*, **1983**, 17(6), 945-957. 4. F.R. Johannsen, B. Vogt, M. Waite, R. Deskin, *Regul. Toxicol. Pharmacol.*, **2008**, 50(3), 322-335. 5. M. Ishidate Jr., M.C. Harnois, T. Sofuni, *Mutat. Res.*, **1988**, 195(2), 151-213. 6. M.M. Moore, K. Harrington-Brock, C.L. Doerr, K.L. Dearfield, *Mutagenesis*, **1989**, 4(5), 394-403. 7. K.L. McCarthy, W.C. Thomas, M.J. Aardema, J.L. Seymour, D.L. Putman, L.L. Yang, R.D. Curren, R. Valencia, *Food Chem. Toxicol.*, **1992**, 30(6), 505-515. 8. Methacrylic Acid, SIDS Initial Assessment Profile, OECD SIDS, **2001**. 9. A.P. Freidig, H.J.M. Verhaar, J.L.M. Hermens, *Environ.*

	Toxicol. Chem., **1999**, 18(6), 1133-1139. 10. D.W. Potter, T.B. Tran, *Toxicol. Lett.*, **1992**, 62(2-3), 275-285. 11. W. Geurtsen, G. Leyhausen, *J. Dent. Res.*, **2001**, 80(12), 2046-2050. 12. F.P. Carney, C.A. Morris, B. Milthorpe, J.L. Flanagan, M.D. Willcox, *Eye Contact Lens*, **2009**, 36(6), 320-328. 13. B.I. Ghanayem, L.T. Burka, H.B. Matthews, *Fundam. Appl. Toxicol.*, 1987, 9(3), 389-397. 14. J.M. Sanders, L.T. Burka, H.B. Matthews, *Drug Metab. Dispos.*, **1988**, 16(3), 429-434. 15. T.J. McCarthy, E.P. Hayes, C.S. Scwartz, G. Witz, *Fundam. Appl. Toxicol.*, **1994**, 22(4), 543-548. 16. K.L. Dearfield, C.S. Millis, K. Harrigton-Brock, C.L. Doerr, M.M. Moore, *Mutagenesis*, **1989**, 4(5), 381-393.

Individual profile/alert	
Name	alpha,omega-Dihaloalkanes
Type of profile	Structural alert
Description/applicability domain	$X^1—(CH_2)_n—X^2$ n = 3 - 6; $X^1 = X^2$ or $X^1 \neq X^2$ and X^1, X^2 = J, Br, Cl
Mechanism	S_N2, Nucleophilic substitution at sp^3 carbon atom

The disubstituted haloalkanes 1,3-dibromopropane and 1-bromo-3-chloropropane have been found to be positive in in vitro chromosomal aberration assays without metabolic activation [1-3]. According to many authors, the clastogenicity of these compounds is strongly dependent upon the carbon chain length (4 > 5 > 3 ~ 6) as well as the type of halogen involved [1-3]. The order of leaving group ability is I > Br > Cl, which is in accordance with the order in nucleophilic participation processes of different halogen atoms [4]. SN2 mechanism between α,ω-activated dihaloalkane and protein nucleophiles is shown in Scheme1.

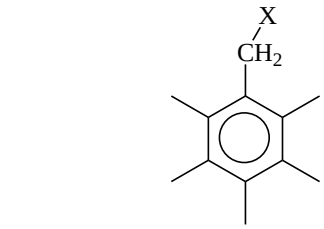
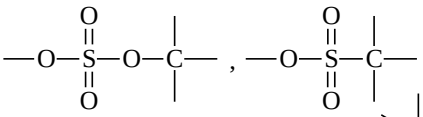
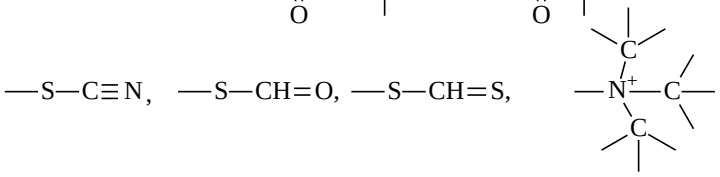
Scheme 1



X = J, Br, Cl ; Nu–H = $-NH_2$, -SH

Set of chemicals used for profile development	alpha,omega-Dihaloalkanes

Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kim, Y.-J., Ryu, J.-C., Evaluation of the genetic toxicity of synthetic chemicals (XIV)-*in vitro* chromosomal aberration assay with 11 chemicals in Chinese hamster lung cells. *Mol. Cell. Toxicol.*, **2006**, 2(2), 89-96. 2. Buijs, W., van der Gen, A., Mohn, G.R., Breimer, D.D., The direct mutagenic activity of alpha,omega-dihalogenoalkanes in Salmonella typhimurium. Strong correlation between chemical properties and mutagenic activity. *Mutat. Res.*, **1984**, 141(1), 11-14. 3. Morita, T., Hayashi, M., Nakajima, M., Tanaka, N., Tweats, D.J., Morikawa, K., Sofuni, T. Practical issues on the application of the GHS classification criteria for germ cell mutagens. *Regul. Toxicol. Pharmacol.*, **2009**, 55(1), 52-68. 4. Solomons T.W.G., Fryhle C.B., Organic Chemistry, 7th ed., John Wiley & Sons, Inc., **2000**, pp. 61–62.

Individual profile/alert	
	alpha-Activated benzyls
Type of profile	Structural alert
Description/applicability domain	 X is -Cl, -Br, -I, F,   *Note:* Where applicable, benzylic carbon is bound to the other functionalities via their O-atom

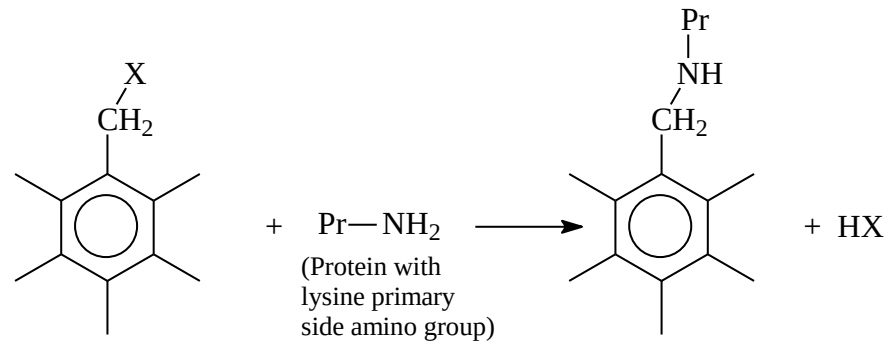
Mechanism	SN2, Nucleophilic subsitution on benzilyc carbon atom

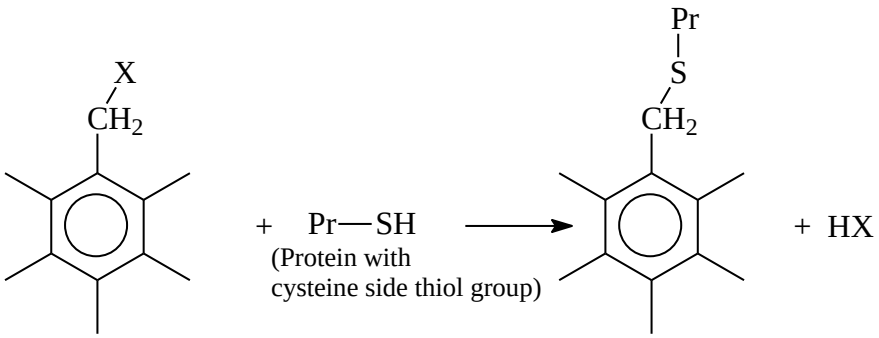
The genotoxic activity of various alkyl nitrites has been documented. Nitrite functionality enhances the reactivity by increasing the electrophilicity of the benzylic carbon atom, and acting as a good leaving group (Chemical 5, Table 1) [6]. In addition, the biological activity of organic nitrites is likely to be initiated by benzylic-type electrophiles capable of modification of cysteine residues in proteins [7].

According to another publication [8], by analogy with Cl and other halogens attached to the benzylic carbon, functionalities such as –OSO2R, –OSO2OR and –SCN (Table 1, Chemicals (4), (6) and (7)) can be regarded as "pseudohalides", since their conjugated acids are strong. Therefore, these functionalities are assumed to be good leaving groups acting by SN2 mechanism. Such compounds exhibit protein binding capabilities, and are strong skin sensitizers [8].

Therefore, despite the lack of reported experimental data on the in vitro CA for such compounds, it could be expertly assumed that chemicals (4) – (7) may also possess reactivity towards histone/non-histone proteins and may form adducts, thereby acting as in vitro genotoxins.

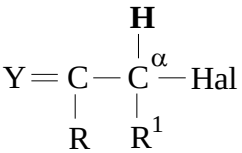
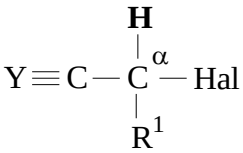
On the basis of the above discussion, and the fact that benzylic carbon may interact as both the "soft" and "hard" electrophile, the following SN2-type reaction mechanistic schemes which may elicit CA by protein binding can be expertly proposed:





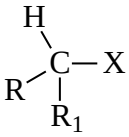
Set of chemicals used for profile development	alpha-Activated benzyls
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which

	causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Benzyltrimethylammonium Chloride, ECHA Registration Dossier; https://www.echa.europa.eu/el/web/guest/registration-dossier/-/registered-dossier/13489/7/7/1. Last visited: July, 2021. 4. Japan Chemical Database; https://dra4.nihs.go.jp/mhlw_data/home/file/file611-19-8.html. Last visited: July, 2021. 5. Application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes, EMA/CHMP/ICH/458894/2015, Committee for Human Medicinal Products; 23 July 2015; https://www.ema.europa.eu/en/documents/scientific-guideline/application-principles-ich-m7-guideline-calculation-compound-specific-acceptable-intakes-step-2b_en.pdf. Last visited: July, 2021. 6. Benigni, R., C. Bossa, Mechanisms of Chemical Carcinogenicity and Mutagenicity: A Review, Chem. Rev. 2011, 111, 2507 – 2536. 7. Dunlap, T., S. Abdul-Hay, R. Esala, P. Chadrasena, et al., Nitrates and NO-NSAIDs in Cancer Chemoprevention & Therapy: In Vitro Evidence Querying the NO Donor Functionality, Nitric Oxide. 2008, 19(2), 115 – 124. 8. Roberts, D. W., A. M. Api, R. J. Safford, J. F. Lalko, Principles for identification of High Potency Category Chemicals for which the Dermal Sensitisation Threshold (DST) approach should not be applied, Regulatory Toxicology and Pharmacology, 2015, 72, 683 – 693.

Individual profile/alert	
Name	alpha-Activated Haloalkanes
Type of profile	Structural alert
Description/applicability domain	Query I and Query II   where **Hal** = Cl, Br, I; **Y** = Csp^2(vinyl,acy), Csp, or Oxygen

<table>
<tr><td></td><td>atom;
R = H, OH, Nsp^3(acy)–Csp^2(aryl); R^1 = H, Csp^3(acy), Csp^3–Hal (Hal = F, Cl, Br, I)

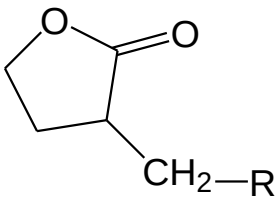
Query III

 
(X is Cl, Br, I)

R$_1$ is H or any C-atom

R is —C≡C, —C≡N,

-C=C, -C=S, -C=O, -NO$_2$
Query IV

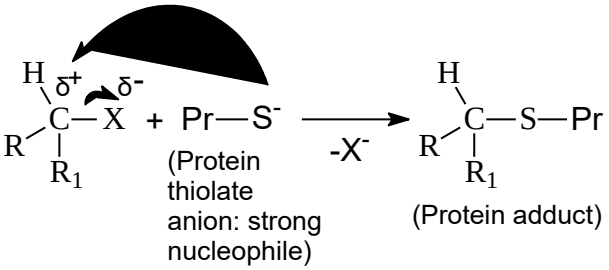
 
R=Cl, Br, I</td></tr>
<tr><td>Mechanism</td><td>S_N2, Alkylation by nucleophilic substitution at sp^3-Carbon atom</td></tr>
<tr><td colspan="2">Alpha-Activated haloalkanes possess electron-withdrawing functional groups or sp2(sp)-carbon atoms directly bound to the alpha-C{sp3}-carbon atom. Chemicals from this sub-class can undergo nucleophilic aliphatic substitution reactions, in which the carbon-halogen bond is subject to heterolytic cleavage. Due to the presence of activated C-X bond with the carbon atom being sufficiently electrophilic center, halogens which are weak bases act as good leaving groups.

Chemicals such as allyl chloride, 1,3-dichloropropene, 3,4-dichloro-1-butene, 2-bromopropanoic acid, alachlor, butachlor, etc. have been tested in in vitro chromosomal aberration assays with and without metabolic activation. Positive results were obtained in the Chinese hamster ovary or lung cells without and, in some cases, with metabolic activation [3 - 8].

The compounds containing allylic or propargylic moiety such as allyl chloride, 1,3-dichloropropene and 3,4-dichloro-1-butene, propargyl bromide, etc. have shown direct mutagenic and clastogenic activities in the absence of external S9 mix. This effect is explained by nucleophilic substitution reactions, leading to the alkylation of DNA and proteins [9].</td></tr>
</table>

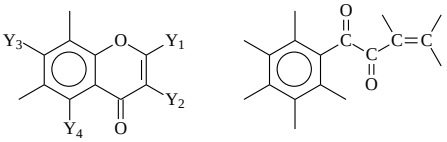
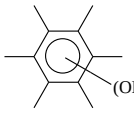
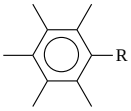
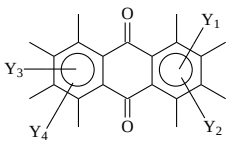
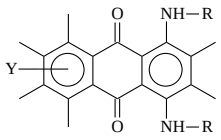
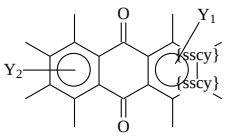
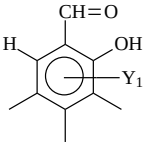
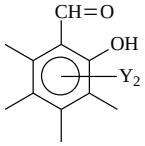
Other compounds with electron-withdrawing carbonyl-containing functionalities, adjacent to the halogen atom such as alachlor and butachlor can also undergo displacement reactions with strong nucleophiles and elicit in vitro genotoxic effects such as CA [10]. It has been shown that such compounds form glutathione conjugates through nucleophilic attack on the alpha-carbon atom [11]. In addition, alachlor S-cysteinyl-protein adducts were examined as potential biomarkers of exposure to alachlor, which is genotoxic and carcinogenic herbicide [12].

The proposed mechanistic scheme of the SN2-type reaction of alpha-activated haloalkane derivatives with the thiol functional groups of the cysteine fragments in histone/non-histone proteins is shown below:



Set of chemicals used for profile development	alpha-Activated Haloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. K.S. Loveday, M.H. Lugo, M.A. Resnick, B.E. Anderson, E. Zeiger, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals. *Environ. Mol. Mutagen*., **1989**, 13(1), 60-94. 2. Office of Environmental Chemicals Safety Environmental Health Bureau, Ministry of Health & Welfare in Japan, Toxicology Testing Reports of Environmental Chemicals, Chemicals Investigation Promoting Committee, Vol. 4, **1996**, p. 529. 3. 3,4-Dichlorobut-1-ene, CAS No. 760-23-6: SIDS Initial Assessment Report, OECD SIDS, **2001**. 4. M.F. Lin, C.L. Wu, T.C. Wang, Pesticide clastogenicity in Chinese hamster ovary cells. *Mutat. Res*., **1987**, 188(3), 241-250. 5. E. Eder, T. Neudecker, D. Lutz, D. Henschler, Mutagenic potential of allyl and allylic compounds. Structure-activity relationship as determined by alkylating and direct *in vitro* mutagenic properties. *Biochem. Pharmacol*., **1980**, 29(7), 993–998.

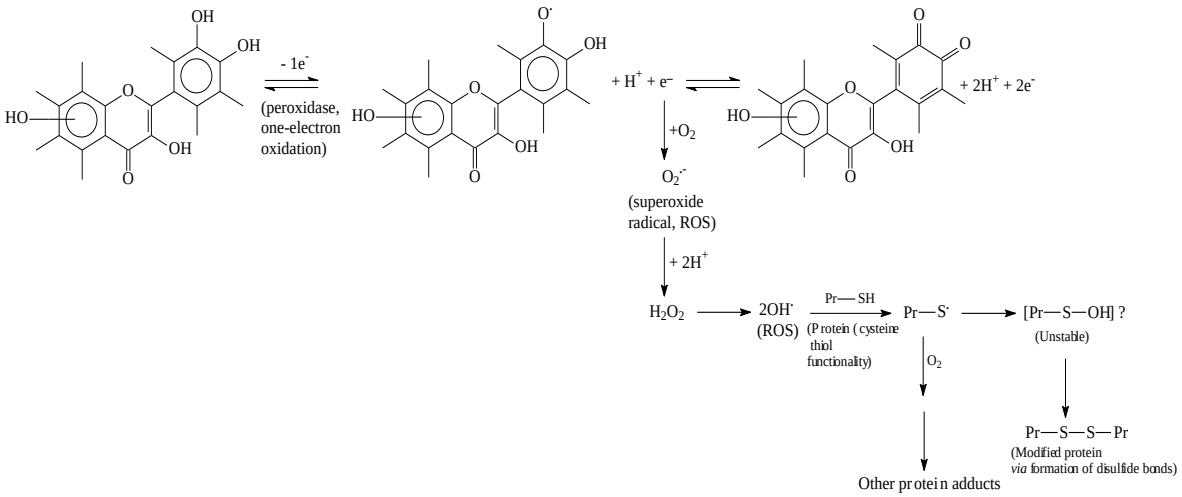
	6. K.A. Lippa, S. Demel, I.H. Lau, A.L. Roberts, Kinetics and mechanism of the nucleophilic displacement reactions of chloroacetanilide herbicides: investigation of *alpha*-substituent effects. *J. Agric. Food Chem.*, **2004**, 52(10), 3010-3021. 7. D.M. Stamper, O.H. Tuovinen, Biodegradation of the herbicides alachlor, metolachlor, and propachlor. *Crit. Rev. Microbiol.*, **1998**, 24(1), 1-22. 8. G.R. Lambert, W.T. Padgett, M.H. George, K.T. Kitchin, S. Nesnow, Quantitative analysis of alachlor protein adducts by das chromatography-mass spectrometry. *Anal. Biochem.*, **1999**, 268(2), 289-296.

Individual profile/alert	
Name	Arenecarbonyl Compounds
Type of profile	Structural alert
Description/applicability domain	A. Flavonoid Compounds:  Y_1 can be H or  (n = 1 - 3) Y_2 is H or -OH or  (R is $-OCH_3$ (1) or -OH (1 - 3)) Y_3 is -OH; Y_4 is H or -OH B. Anthraquinone Derivatives:  (Y_1 is -OH (1 - 3); Y_2 is $-CH_2OH$ (1); or $-CH_3$ (1); or $-CH_2CH_3$ (1); or C{ar} (1) or -CH=O (1) or $-C(O)CH_3$ (1); Y_3 is H (all) or -OH (1 - 2); Y_4 is -C(O)OH (1 - 2) (if Y_3 is -OH)  (R is H (both) or $-NHCH_2$ (both) or combinations); Y is -OH (1 - 2) or H  (Y_1 is -OH (1 - 2); Y_2 is H combined with -OH (1 - 2); or H combined with $-OCH_3$ (1)) C. Salicylaldehydes:  (Y_1 is H (all); or -OH (0 - 2); or Cl (0 - 2); or $-NO_2$ (0 - 2)  (Y_2 is H (all); or $-CH_3$ (0 - 2) (No more than totally 4 substituents)

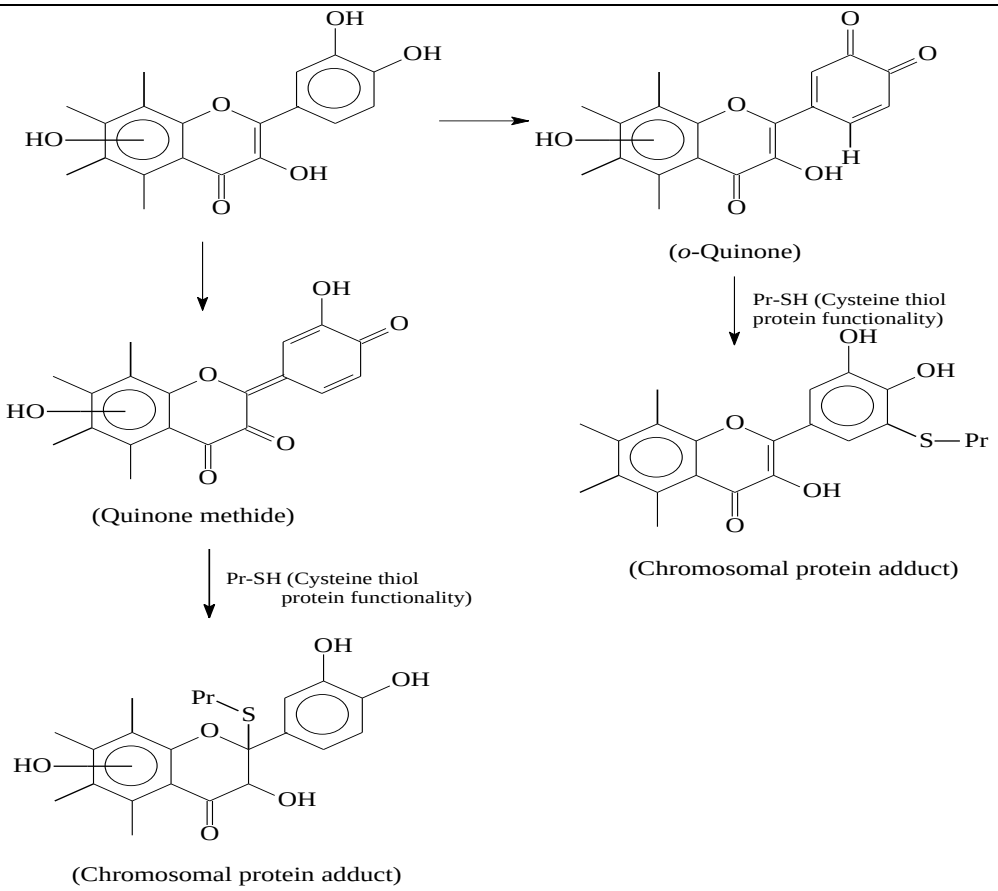
Mechanism	AN2, Schiff base formation AN2, Michael-type addition, quinoid structures Radical, ROS generation Non-covalent interactions, DNA/protein intercalation

A. **Flavonoid compounds**

Oxidative stress and generation of ROS – radical mechanism. Despite their beneficial effects as antioxidants, many flavonoids show genotoxicity in both bacterial and mammalian experimental systems. This is due to their activity as pro-oxidants, generating free radicals with the active participation of endogenous peroxidase enzymes in mammalian eukaryotic cells. Reactive oxygen species (ROS) damage DNA, and cause inhibition of DNA-associated non-histone protein enzymes, such as topoisomerase. This can result in DNA strand breaks, mutations, or chromosomal aberrations (CA) [6]. Experiments in aqueous solutions have indicated that the various thiol compounds (cysteine, cysteamine, glutathione, captopril, N-acetylcysteine, etc.) are efficiently oxidized by hydroxyl radical (HO.) generated as ROS [7]. The following mechanistic scheme associated with radical generation of ROS can be thus inferred (Fig. 1).



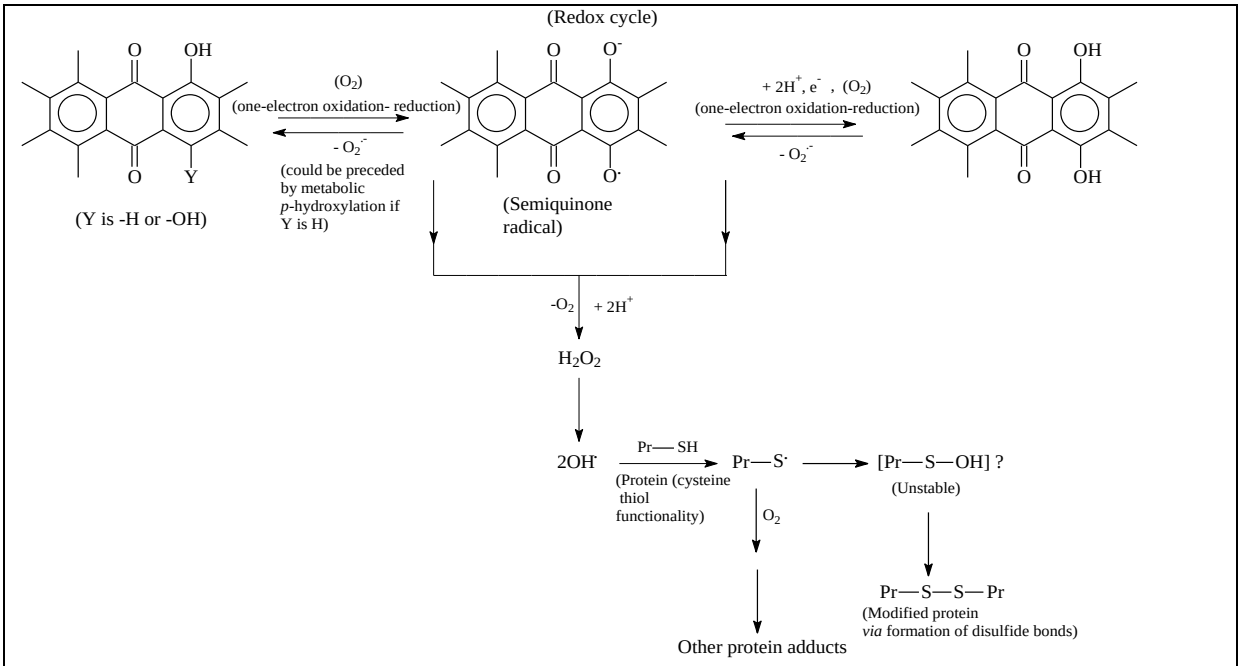
Protein adduct formation – AN2-type Michael addition. The presence of two catechol-type hydroxyl groups in ring B of flavonoids such as quercetin has been recognized as an important structural prerequisite for mammalian cell genotoxicity. Also, other flavonoids listed in Table 1 such as kaempferol, biochanin A, morin and formononetin could form catechol-type products after metabolic activation with S9 mix. This gives rise to further formation of o-quinone and quinone methide reactive electrophilic species which can alkylate biological macromolecules, including DNA and proteins. Moreover, the presence of other electron-donating functionalities with +M-effect such as methoxy groups attached in appropriate locations also acts in such direction after metabolic activation [8]. For instance, quercetin can generate active o-quinone/quinone methide metabolites [9, 10]. Thus the following mechanistic scheme for protein adduct formation can be expertly proposed (Fig, 2):



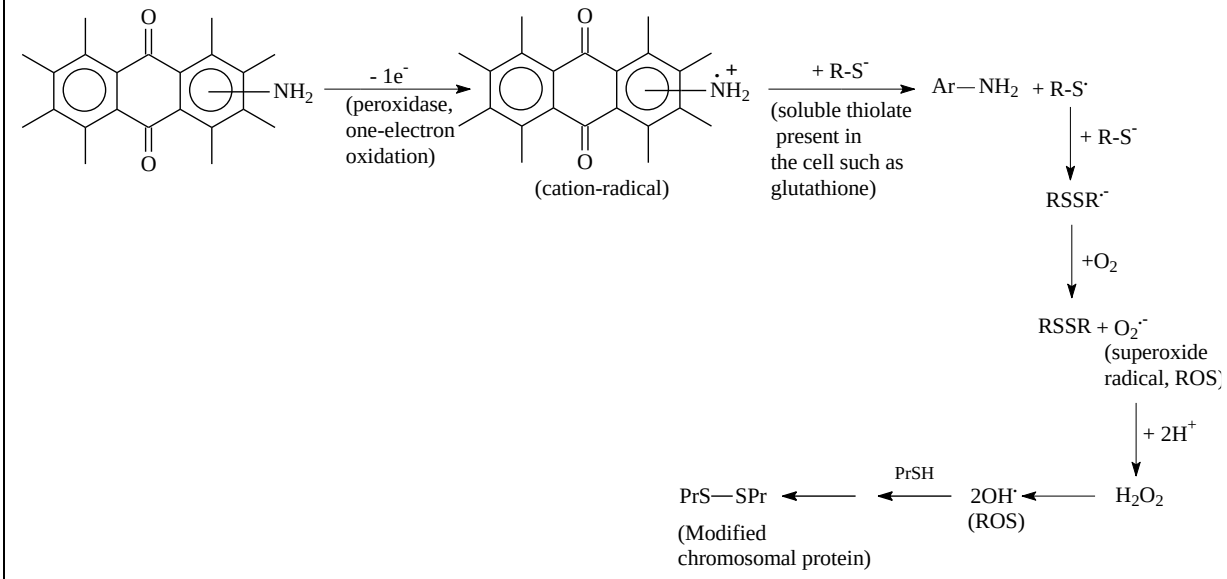
Intercalation – non-covalent interaction with DNA/protein complex. Flavonoids such as Quercetin can also bind to chromosomal DNA by non-covalent interactions such as intercalation [11]. This could be due to the combination of large planar molecules and electron-donating substituents such as –OH and –OCH3 groups with +M-effect, attached to the benzenoid aromatic rings in appropriate positions.

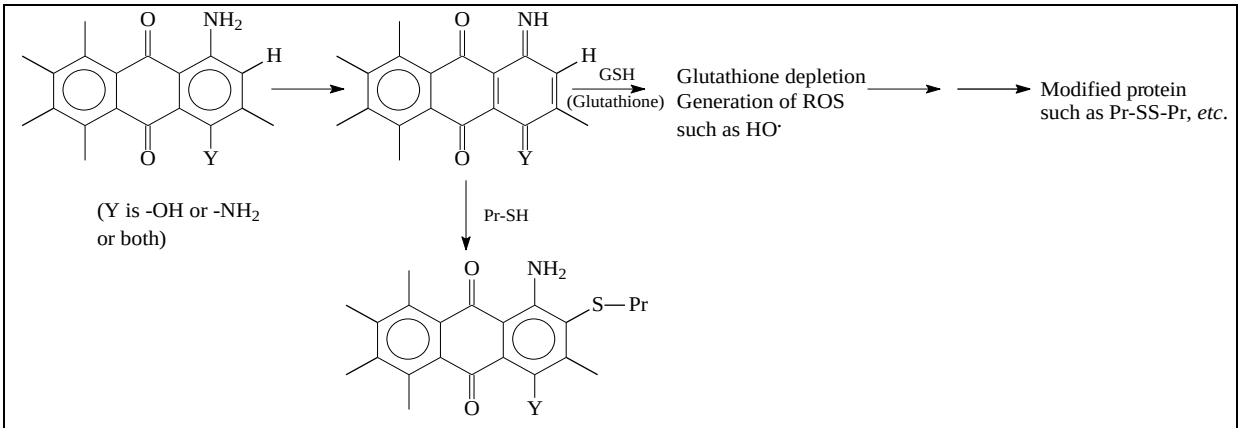
B. Anthraquinone Derivatives

Oxidative stress and generation of ROS – radical mechanism. Peroxidase enzymes might be present in eukaryotic cells, which are associated with endogenous generation of oxygen intermediates. Generally, genotoxicity by oxygen intermediates may be caused by oxidative stress as a result of intracellular species, which can undergo one-electron oxidation-reduction reactions catalyzed by peroxidases to radical species. The latter interact with oxygen to form reactive oxygen species (ROS), which can attack the biological macromolecules such as DNA and proteins causing genotoxicity. Such processes can be mediated by thiols and/or glutathione present in the cells [24 - 26]. It should be noted that generation of ROS may occur after CYP-450-catalyzed metabolic hydroxylation to produce hydroquinone (or catechol)-type metabolites of hydroxyanthraquinones. The process is enhanced by the presence of electron-donating substituents with +M-effect such as –OH and –NH2 functional groups Scheme of such type of generation of ROS and subsequent protein modification is shown below (Fig. 3:



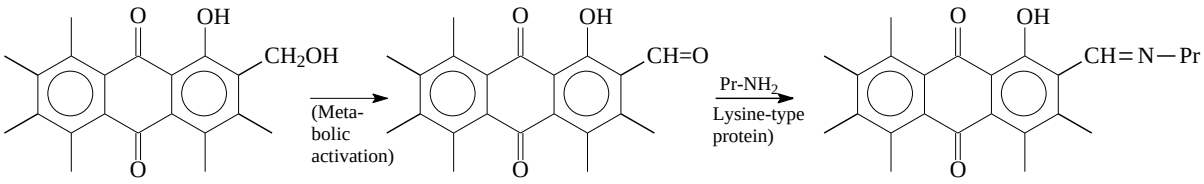
Similar mechanistic schemes associated with generation of ROS and protein modification can be proposed for aminoanthraquinones [24, 25, 27], (Figs. 4a, b):





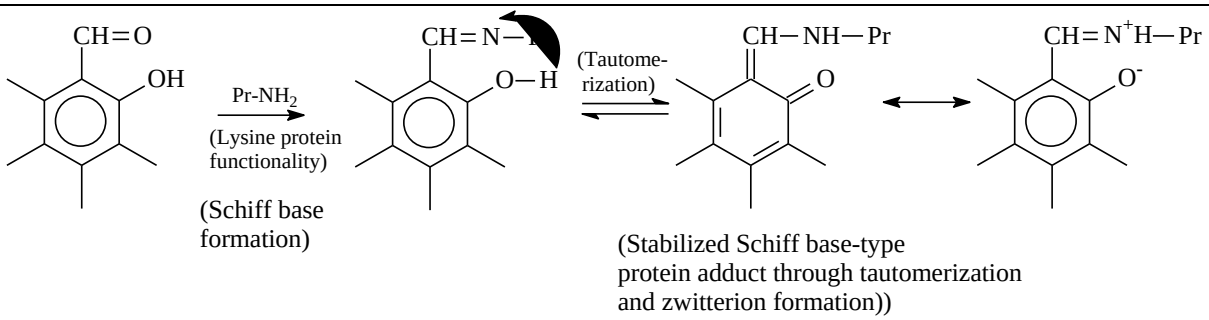
Intercalation – non-covalent interaction with DNA/protein complex. Anthraquinone derivatives can also bind to chromosomal DNA/protein complex by non-covalent interactions such as intercalation. This could be due to the combination of large planar molecules and electron-donating substituents such as –OH and –NH2 groups with +M-effect, attached to the benzenoid aromatic rings in appropriate positions. The additional presence of other polar groups such as –COOH would facilitate intercalation. Thus planar tricyclic and tetracyclic ring systems of anthraquinone derivatives can be accommodated between the successive base pairs of DNA in chromosomes [5].

Protein adduct formation – AN2-type interactions. Some anthraquinone derivatives, containing, e.g., ethyl or hydroxymethyl functionalities, apart from the above-mentioned mechanistic schemes resulting in chromosomal protein binding and CA, could undergo some interactions associated with covalent adducts formation. This is usually preceded by metabolic activation with external S9 mix to the corresponding arenecarbonyl derivatives, prone to Schiff base-type formation with proteins (Fig. 5):



C. Salicylaldehydes

Schiff bases can be derived from salicylaldehydes with their interaction with lysine residues in proteins. Due to its intramolecular hydrogen bonding, o-hydroxy salicylidene-type Schiff bases exhibit two tautomeric forms such as enol-imine and keto-enamine species. A zwitterionic structure may also appear, due to a proton transfer involving the enol – imine and keto – amine tautomeric forms as shown in Fig. 6 below [34]:



Set of chemicals used for profile development	Arenecarbonyl Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Kovacic, P., Jacintho, J.D., Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. Curr. Med. Chem., 2001, 8(7), 773-796. 4. Do Céu Silva, M., Gaspar, J., Duarte Silva, I., Leão, D., Rueff, J., Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells. Mutagenesis, 2003, 18(6), 491-496. 5. Double, J. C., J. R. Brown, Evaluation of the Binding of Some Substituted Anthraquinones and Naphthacenequinones to DNA, Communications, J. Pharm. Pharmac. 1976, 28, 166 – 169. 6. Yordi, E. G., E. M. Perez, M. J. Matos, E.U. Villares, Structural Alerts for Predicting Clastogenic Activity of Pro-Oxidant Flavonoid Compounds: Quantitative Structure-Activity Relationship Study, J. Biomolecular Screening, 2012, 17(2), 216 – 224. 7. Enescu, M., Gardey, B., Mechanism of cysteine oxidation by a hydroxyl radical: a theoretical study. Chemphyschem., 2006, 7(4), 912-919. 8. Resende, Fl. A., W. Vileges, L. C. dos Santos, E. A. Varanda, Mutagenicity of Flavonoids Assayed by Bacterial Reverse Mutation (Ames) Test, Molecules, 2012, 17, 5255 – 5268. 9. Spencer, J. P. E., G. G. C. Kunhle, R. J. Williams, C. R. Evans, Intracellular Metabolism and Bioactivity of Quercetin and Its In Vivo Metabolites, Biochem. J. 2003, 372, 173 – 181. 10. Award, H. M., Studies on the pro-oxidant chemistry of flavonoids, Thesis, Wageningen University, 2002;

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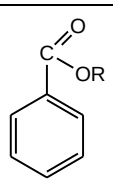
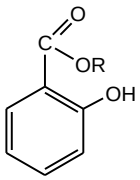
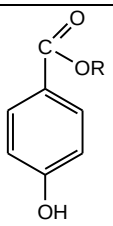
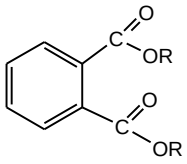
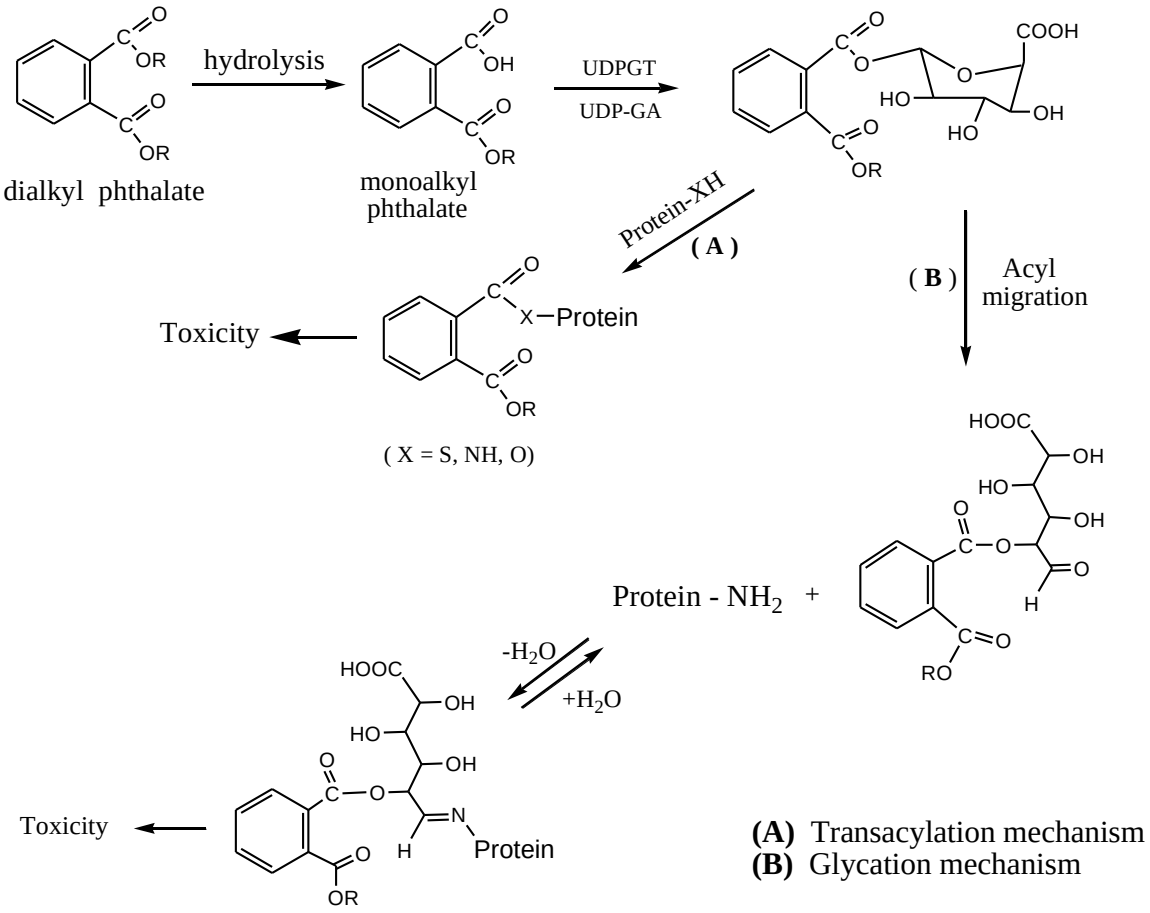
Individual profile/alert	
Name	Arenecarboxylic Acid Esters
Type of profile	Structural alert

Description/applicability domain	 Alkyl benzoates  Alkyl salicylates  Alkyl p-hydroxy-benzoates  Dialkyl phthalates R = -CH_3; -C_2H_5; -CH_2-CH_2-CH_3; -$CH(CH_3)_2$ -CH_2-CH_2-CH_2-CH_3; (methyl) (ethyl) (n-propyl) (i-propyl) (n-butyl) -CH_2-$CH(CH_3)_2$; -CH_2-CH_2-CH_2-CH_2-CH_2-CH_3; -CH_2-$CH(C_2H_5)$-CH_2-CH_2-CH_2-CH_3 (i-butyl) (n-hexyl) (2-ethylhexyl)
Mechanism	Acylation, Mechanistic Alert: Acylation involving an activated (glucuronidated) ester group

Many authors were established that all of phthalate esters were rapidly hydrolyzed in vivo to the corresponding acids, monoesters and/or their glucuronide conjugates [9-14]. It is believed that the acute toxicity is mainly due to the acids and monoesters or their conjugates [10,11,13]. For example, the salicyl conjugates have been detected and estimated in the plasma of normal subjects [9]. Fennell et al. [6] were established the presence of monobutyl phthalate glucuronide in plasma of pregnant rats as a single peak, but in amniotic fluid it was observed as several peaks. They refer these peaks to the rearranged forms of acyl glucuronide (i.e 1-O-β-glucuronide undergoes pH-dependent migration to the 2-O, 3-O, and 4-O positions).

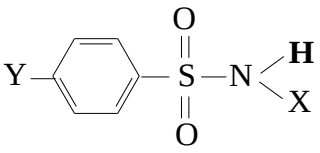
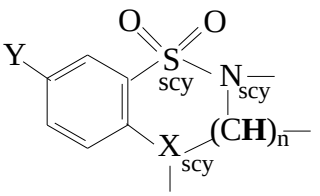
Bearing in mind these facts, it can be assumed that acyl glucuronides of hydrolyzed arenecarboxylic esters can form protein adducts by two mechanisms - transacylation mechanism and glycation mechanism (or Schiff's base mechanism) [6,15]):

Scheme 1



Set of chemicals used for profile development	Arenecarboxylic Acid Esters
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Branca, M., A. Garcovich, L. D. Linfante, A. Macri, A. Mantovani, G. Olivetti, and G. Salvatore, Cont. Derm. 1988, Vol. 19, pp. 320-334. 2. Soni, M. G., I. G. Carabin, and G. A. Burdock, Food Chem. Toxicol. 2005, Vol. 43, pp. 985-1015. 3. Nakagawa, Y., and G. Moore, Biochem. Pharmacol. 1999, Vol. 58, pp. 811-816. 4. Marsman, D., Toxic Rep. Ser. 1995, Vol. 30, pp. 1-G5. 5. Mylchreest, E., D. G. Wallace, R. C. Cattley, and P. M. D. Foster, Toxicol. Sci. 2000, Vol. 55, pp. 143-151. 6. Fennell, T. R., W. L. Krol, S. C. J. Sumner, and R. W. Snyder, Toxicol. Sci. 2004, Vol. 82, pp. 407-418. 7. Koch, H. M., R. Preuss, and J. Angerer, Int. J. Androl. 2006, Vol. 29, pp. 155-165.

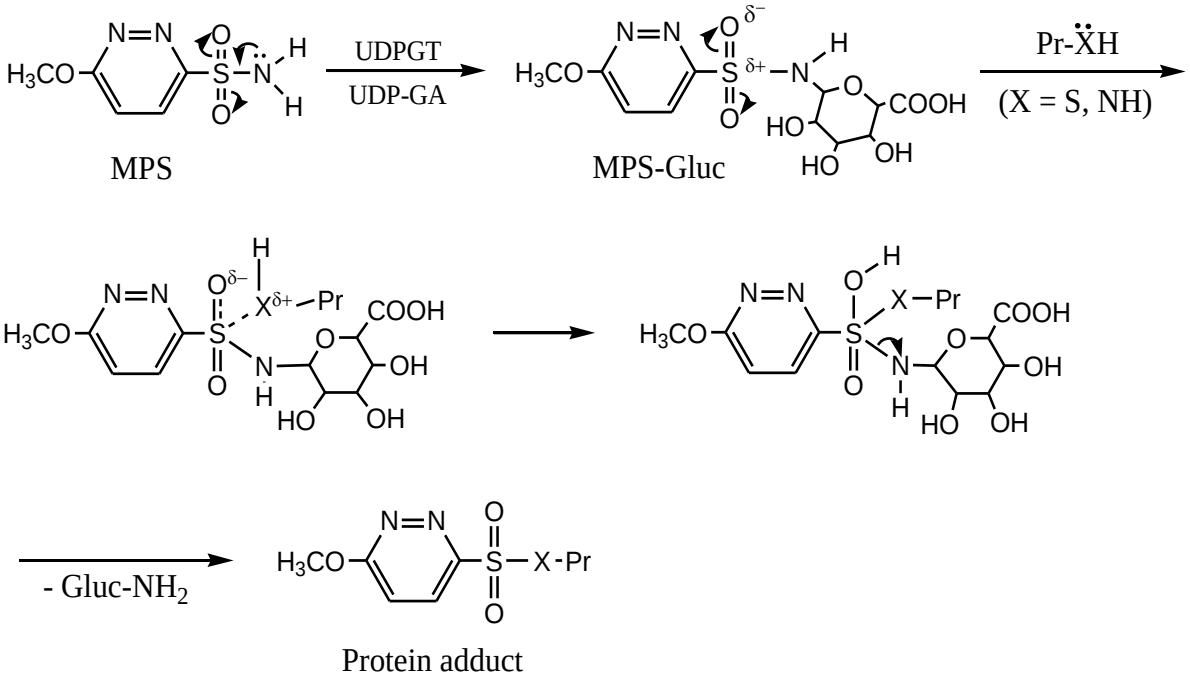
	8. Foster, P. M., Int. J. Androl. 2006, Vol. 29, pp. 140-147. 9. Schachter, D., and J. G. Manis, J. Clin. Invest. 1958, Vol. 37, pp. 800-807. 10. Morris, M. E., Drug Metab. Dispos. 1990, Vol. 18, pp. 809-811. 11. Foster, P. M., M. W. Cook, L. V. Thomas, D. G. Walters, and S. D. Gangolli, Drug Metab. Dispos. 1983, Vol. 11, pp. 59-61. 12. Lhuguenot, J. C., A. M. Mitchell, and C. R. Elcombe, Toxicol. Ind. Health 1988, Vol. 4, pp. 431-441. 13. Kambia, K., T. Dine, B. Gressier, T. Dupin-Spriet, M. Luyckx, and C. Brunet, Int. J. Artif. Organs 2004, Vol. 27, pp. 971-978. 14. Calafat, A. M., J. W. Brock, M. J. Silva, L. E. Gray Jr., J. A. Reidy, D. B. Barr, and L. L. Needham, Toxicology 2006, Vol. 217, pp.22-30. 15. Wang, M., and R. G. Dickinson, Drug Metab. Dispos. 1998, Vol. 26, pp. 98-104.

Individual profile/alert	
Name	Arenesulfonamides
Type of profile	Structural alert
Description/applicability domain	**Acyclic structure**  **X** = H, NH_2, Csp^2(aryl), Csp^2(heteroaryl - 5- or 6-membered), C(=O)N**H**Csp^3(acy,scy) **Y** = H, Hal (F, Cl, Br, I), OCsp^2(aryl), C(=O)Csp^3(acy), N**H**Csp^2(aryl), N**H**Csp^3-Csp^2(heteroaryl). **Cyclic structural fragment**  where **n** can be zero (5-membered ring) or 1 (6-membered ring); **X** is usually Nsp^3 atom or C=O group and **Y** is H atom, SO_2NH_2 or

	SO_2NH- groups.
Mechanism	Acylation (Ac-SN2 mechanism), Acylation involving an activated (glucuronidated) sulfonamide group AN2, Mechanistic Alert: Nucleophilic addition at polarized N-functional double bond

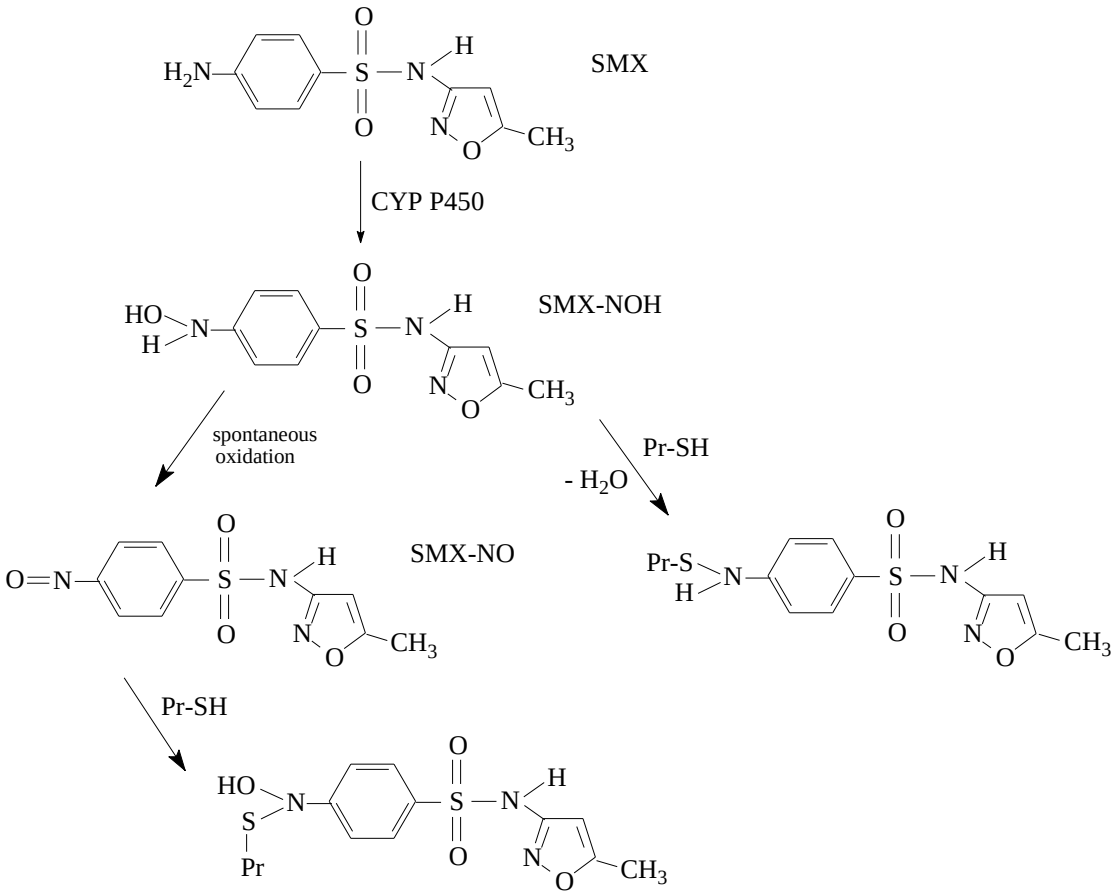
Chiu and Huskey [6] established that primary and secondary sulfonamides can undergo glucuronidation in vitro and in vivo in a large number of animal species and in humans. The bioactivation of sulfonamide non-antibiotics via N-glucuronidation results in the formation of more reactive intermediate [7] in which the sulfur atom is a better electrophilic center than in the initial sulfonamide. It may be haptenated to target proteins containing strong nucleophilic sites such as cysteine thiols, lysine amines, protein N-terminal amines, etc. The proposed mechanism for the protein binding of sulfonamide nonantibiotics is presented on Scheme 1. A transacylation reaction can

Scheme 1



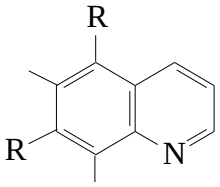
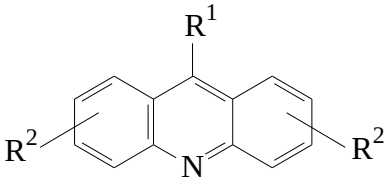
Sulfonamide antibiotics (antimicrobials) can be regarded as derivatives of the sulfanilamide having an aromatic amine group at the para-position towards SO2NH-group. The aromatic amine moiety is considered to be trigger for serious drug reactions which occur mainly in internal administration. For example, sulfamethoxazole (SMX), one of the most commonly used sulfonamide antibiotics (antimicrobials), is metabolized to the respective hydroxylamine and nitroso derivatives, resulting in the covalent adduct formation with intracellular proteins [8-10]. The oxidation of SMX to SMX-NOH arises via the CYP P450 monooxygenase system. Then SMX-NOH is spontaneously converted to nitroso SMX (SMX-NO) [8,11]. Incubation of cells with SMX-NOX and SMX-NO revealed more than 20 protein bands, which were sulfa-specific [8]. Proposed “bioactivation-dependent” pathway for the sulfonamide antimicrobial sulfamethoxazole was presented in Scheme 2.

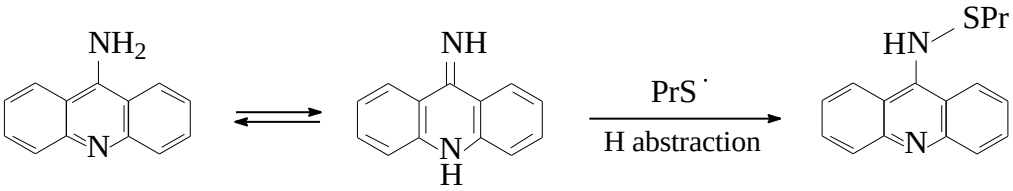
Scheme 2



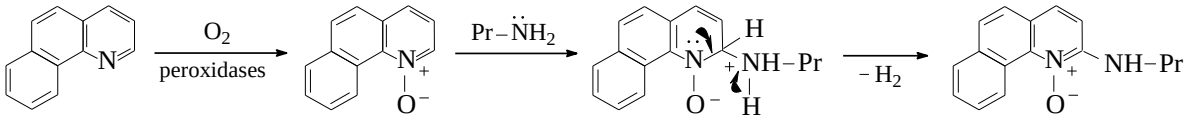
Set of chemicals used for profile development	Arenesulfonamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. S.R. Knowles, L.E. Shapiro, N.H. Shear, *Drug Saf.*, **2001**, 24(4), 239-247. 2. L.E. Shapiro, S.R. Knowles, E. Weber, M.G. Neuman, N.H. Shear, *Drug Saf.*, **2003,** 26(3), 187-195. 3. C.K. Svensson, E.W. Cowen, A.A. Gaspari, *Pharmacol. Rev.*, **2000**, 53(3), 357-379. 4. J. Clausen, *J. Pharmacol. Exp. Ther.*, **1966**, 153(1), 167-175. 5. M. Schafer-Korting, *Arzneimittelforschung*, **1985**, 35(12), 1828-1831. 6. S.-H. L. Chiu, S.-E. W. Huskey, *Drug Metab. Dispos.*, **1998**, 26(9), 838 – 847. 7. S. Zhou, E. Chan, W. Duan, M. Huang, Y.-Z. Chen, *Drug Metab. Rev.*, **2005**, 37(1), 41-213. 8. T. Manchandra, D.A. Hess, L. Dale, S.G. Ferguson, M.J.

	Rieder, *Mol. Pharmacol*., **2002**, 62(5), 1011-1026. 9. P.M. Vyas, S. Roychowdhury, C.K. Svensson, *Drug Metab. Dispos*., **2006**, 34(1), 16-18. 10. P. Bhaiya, S. Roychowdhury, P.M. Vyas, M.A. Doll, D.W. Hein, C.K. Svensson, *Toxicol. Appl. Pharmacol*., **2006**, 215(2), 158-167. 11. D.A. Hess, M.E. Sisson, H. Suria, J. Wijsman, R. Puvanesasingham, J. Madrenas, M.J. Rieder, *FASEB J*., **1999**, 13(13), 1688-1698. 12. G. Choquet-Kastylevsky, T. Vial, J. Descotes, *Curr. Allergy Asthma Rep*., **2002**, 2(1), 16-25.

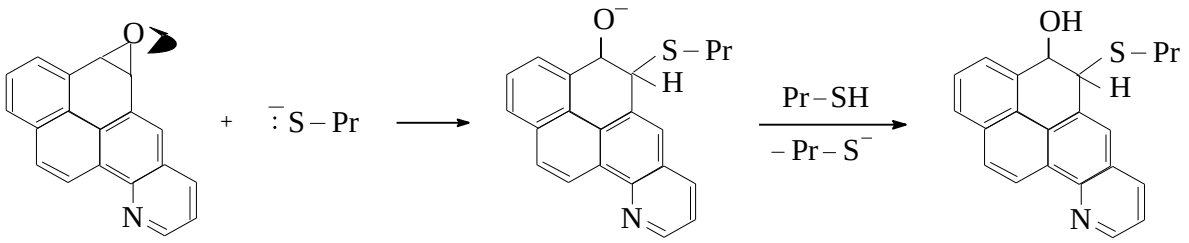
Individual profile/alert	
Name	Benzoquinoline and Acridine derivatives
Type of profile	Structural alert
Description/applicability domain	 **R** = Csp^2(aryl) – fused rings  **R^1** = –H, –Cl, –NH_2; **R2** = –H, –Cl, –NO_2, –OCH_3, –CF_3
Mechanism	A_R, Radical-type addition to imino tautomer of aminoacridines S_NAr, Nucleophilic substitution on activated Csp2-atoms in quinolines S_N2, Ring opening nucleophilic substitution involving arene oxide derivatives and proteins
9-Aminoacridine may exist in two tautomer forms, namely, amino and imino form. The latter has a reactive imino group, which is able to associate with protein thiol radicals via radical version of Michael addition, as is assumed by Aptula *et al*. [5]. The possible mechanism of protein binding of 9-aminoacridine is presented in Scheme 1.	



Nonsubstituted benzoquinolines are able to cause chromosomal damage in Chinese hamster lung cells without metabolic activation. This could be due to the possibility of peroxidase-dependent *N*-oxidation of their pyridine ring in the presence of hydrogen peroxide. It is well known that positions 2 and 4 in *N*-oxidized quinolines are activated due to the strong electron-withdrawing effect of *N*-oxide moiety [6]. Thus, C2- and C4-atoms are highly electrophilic (C2 being more positive than C4) and can undergo nucleophilic substitution reactions involving proteins as shown in Scheme 2.

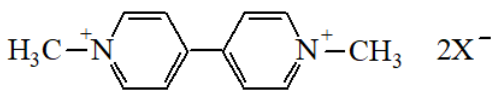


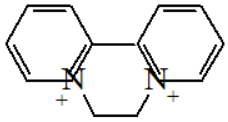
Pyrenoline 4,5-oxide has an epoxide ring in the molecule and is a direct acting mutagen as the bay region epoxides are the active metabolites of aza-PAHs *in vivo* [7,8]. Cleavage of the epoxide ring by various nucleophiles, such as amino and sulfhydryl groups, is one of the most frequently encountered behaviors of this system, both biologically and synthetically. The ring opening S_N2 mechanism has been suggested to be responsible for the nucleic acids and protein reactivity of pyrenoline 4,5-oxide as shown in Scheme 3 [1,7-10].



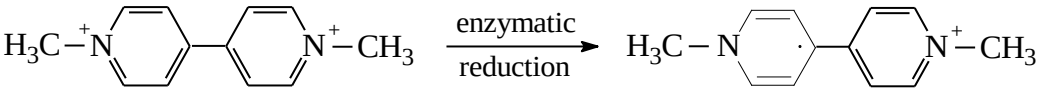
Set of chemicals used for profile development	Benzoquinoline and Acridine derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. E.A. Bleeker, S. Wiegman, P. De Voogt, M. Kraak, H.A. Leslie, E. De Haas, W. Admiraal, Toxicity of azaarenes. *Rev. Environ. Contam. Toxicol*., **2002**, 173, 39-83. 2. A. Matsuoka, K. Shudo, Y. Saito, T. Sofuni, M. Ishidate Jr, Clastogenic potential of heavy oil extracts and some azaarenes in Chinese hamster cells in culture. *Mutat. Res.*, **1982**, 102(3), 275-283.

	3. M. Ishidate Jr, M.C. Harnois, T. Sofuni, A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 4. E.A.J. Bleeker, H.J. Van Der Geest, H.J.C. Klamer, P. De Voogt, E. Wind, M.H.S. Kraak, Toxic and genotoxic effects of azaarenes: Isomers and metabolites. *Polycycl. Aromat. Comp*., **1999**, 13(3), 191-203. 5. A.O. Aptula, S.J. Enoch, D.W. Roberts, Chemical mechanisms for skin sensitization by aromatic compounds with hydroxy and amino groups. *Chem. Res. Toxicol.*, **2009**, 22(9), 1541-1547. 6. R. Alajarin, C. Burgos, Six-membered heterocycles: Quinoline and isoquinoline *In* Modern Heterocyclic Chemistry. J. Alvarez-Builla, J.J. Vaquero, J. Barluenga (Eds.), Wiley-VCH Verlag, **2011**, p. 1529. 7. D. Warshawsky, G. Talaska, W. Xue, J. Schneider, Comparative carcinogenicity, metabolism, mutagenicity, and DNA binding of 7H-dibenzo[c,g]carbazole and dibenzo[a,j]acridine. *Crit. Rev. Toxicol.*, **1996**, 26(2), 213-249. 8. W. Xue, D. Warshawsky, Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage. *Toxicol. Appl. Pharmacol*., **2005**, 206(1), 73-93. 9. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.T.D. Cronin, A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol.*, **2011**, 41(9), 783-802. 10. S.S. Murphree, Three-membered heterocycles. Structure and reactivity: *In* Modern Heterocyclic Chemistry. J. Alvarez-Builla, J.J. Vaquero, J. Barluenga (Eds.), Wiley-VCH Verlag, **2011**, 92-95.

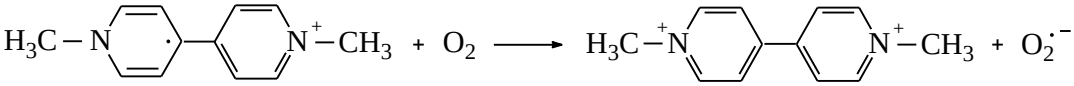
Individual profile/alert	
Name	Bipyridilium Herbicides
Type of profile	Structural alert
Description/applicability domain	 paraquat

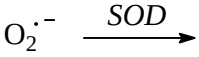
	 $2X^-$ diquat where X = Cl^-, Br^-, I^-, $^-OSO_2OCH_3$, etc.
Mechanism	Radical mechanism, ROS generation and protein carbonylation A_N2, Nucleophilic addition to monopyridone moiety of paraquot or diquat

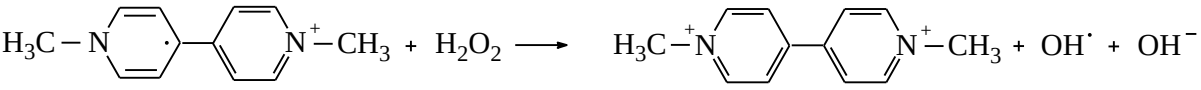
The herbicidal action and toxicity of PQ and DQ are thought to result from the generation of reactive oxygen species (ROS) through redox cycling [Bus et al., 1976, 1984; Winterbourn, 1981; Cohen and Doherty, 1987; Peng et al., 2004; Wang et al., 2007]. During redox cycling PQ and DQ undergo a single electron reduction to form a PQ and DQ cation radicals by several enzymes including peroxidases, NADPH-cytochrome P450 reductase, NADH dehydrogenase, UV irradiation, etc. The free radicals formed can react rapidly with oxygen forming superoxide radical anion ($O2^{\cdot-}$) and the initial compounds, which can then be reduced again at the expense of cellular reductases. Under anaerobic conditions, however, PQ radical was shown to be stable [Winterbourn, 1981]. The presence of this radical was confirmed experimentally [Black et al., 2008]. Subsequent reduction of superoxide radical anion ($O2^{\cdot-}$) under the influence of the superoxide dismutase (SOD) generates H2O2 and highly reactive hydroxyl radicals (·OH). The cyclic reduction-oxidation of PQ is presented in Scheme 1.



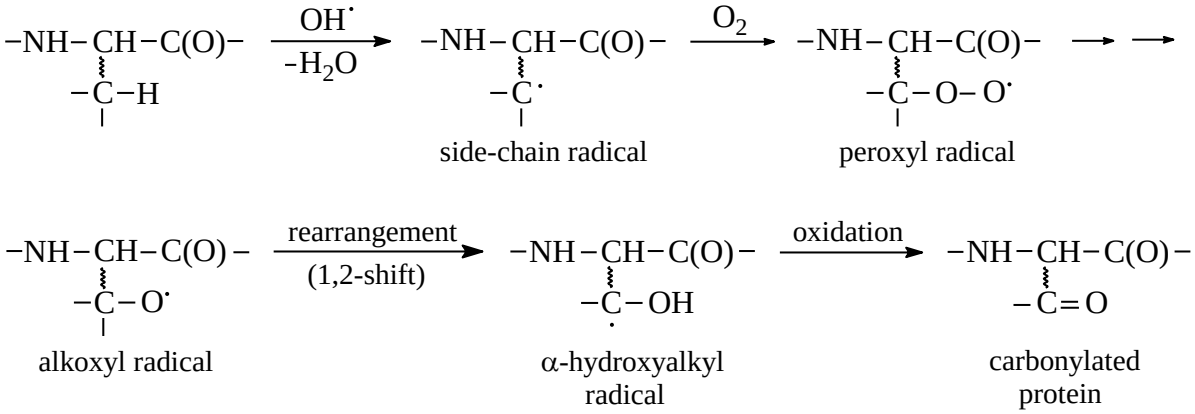
paraquat — paraquat radical



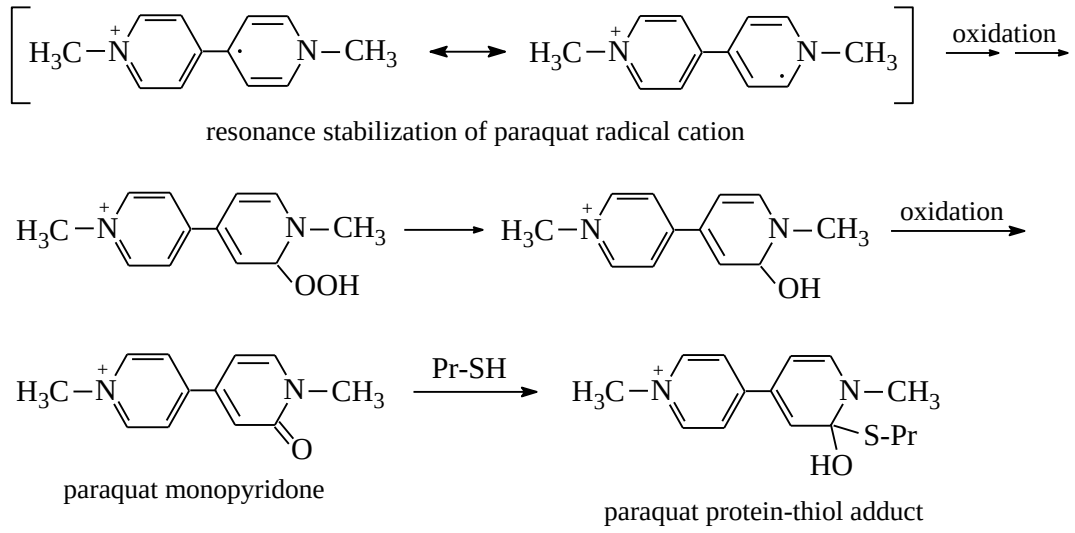




An excess of ROS ($O2^{\cdot-}$, H2O2, ·OH) causes oxidative damage to cellular macromolecules including proteins, nucleic acids and lipids. This results in many adverse effects such as the formation of mutagenic lesions, altered enzyme function, lipid peroxidation and inappropriate cell signaling [Black et al., 2008]. The oxidation of proteins is known to be an important marker of cellular oxidative stress. Four different categories of amino acids side-chains such as aliphatic, aromatic, cysteine and cystine residues, and methionine residues can undergo oxidative damage [Davies, 2005]. Carbonylation is one of the major reaction of protein aliphatic side-chain oxidation processes, which can occur in the presence of ROS [Davies, 2005; Dean et al., 1997; Wong et al., 2008].



In addition, PQ degraded rapidly in aqueous solutions when exposed to UV-light. Minimal photodegradation of PQ in aqueous solution occurred when exposed to natural light. One of the main metabolites of PQ under visible light in water medium is PQ monopyridone [Paraquat Explanation, 2004]. Then, it can be assumed that the oxidation of PQ radical cation to PQ monopyridone may occur in a manner described above for the oxidation of protein side-chains. Diquat monopyridone and diquat dipyridone were also found as oxidative products in biological materials [Fuke et al., 2002]. The PQ and DQ pyridones are able to react with protein nucleophiles (Pr-SH, Pr-NH2) via a nucleophilic addition reaction (Scheme 3).



Set of chemicals used for profile development	Bipyridilium Herbicides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Y.Q. Wang, H.M. Zhang, G.C. Zhang, S.X. Liu, Q.H. Zhou, Z.H. Fei, Z.T. Liu, Studies of the interaction between paraquat and bovine hemoglobin. *Int. J. Biol. Macromol.*, **2007**, 41(3), 243-250. 2. A.T. Black, J.P. Gray, M.P. Shakarjian, D.L.

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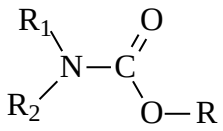
14. J. Peng, X.O. Mao, F.F. Stevenson, M. Hsu, J.K. Andersen, The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway. *J. Biol. Chem*.,

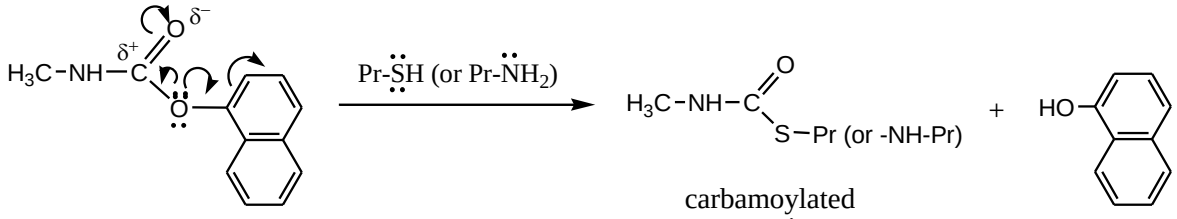
	2004, 279(31), 32626-32632. 15. M.J. Davies, The oxidative environment and protein damage. *Biochim. Biophys. Acta*, **2005**, 1703(2), 93-109. 16. R.T. Dean, S. Fu, R. Stocker, M.J. Davies, Biochemistry and pathology of radical-mediated protein oxidation. *Biochem. J*., **1997**, 324(Pt 1), 1-18. 17. C.M. Wong, A.K. Cheema, L. Zhang, Y.J. Suzuki, Protein carbonylation as a novel mechanism in redox signaling. *Circ. Res*., **2008**, 102(3), 310-318. 18. E.R. Stadtman, L. Levine, Protein oxidation. *Ann. NY Acad. Sci*., **2000**, 899, 191-208. 19. S.N. Giri, P. Lunsman, Binding of [methyl-^{3}H]paraquat to rat, rabbit, hamster, mouse and quinea pig lung proteins, in vitro. *Toxicol. Lett*., **1981**, 9(2), 93-100. 20. T.M. Sullivan, M.R. Montgomery, The relationship between paraquat accumulation and covalent binding in rat lung slices. *Drug Metab. Dispos*., **1983**, 11(6), 526-530. 21. Y. Yamada, Paraquat Explanation *In* Pesticide residues in food 2004, Evaluations - Part I: Residues. FAO and WHO, **2005**, pp. 533-698. 22. C. Fuke, T. Arao, Y. Morinaga, H. Takaesu, K. Ameno, T. Miyazaki, Analysis of paraquat, diquat and two diquat metabolites in biological materials by high-performance liquid chromatography. *Legal Med*., **2002**, 4(3), 156-163. 23. C.C. Winterbourn, The reaction of hemoglobin with paraquat radicals in the presence and absence of O_2. *Biochem. Int*., **1983**, 7(1), 1-8. 24. 24. R.M. LoPachin, T. Gavin, Response to "Paraquat: The red herring of Parkinson's disease research". *Toxicol. Sci*., **2008**, 103(1), 219-221.

Individual profile/alert	
Name	Carbamates
Type of profile	Structural alert
Description/applicability domain	 R can be C{ar} (benzenoid fragment)) or C{sp2} or C{sp3} or C{sp2} or azomethyne group (-N=C<); R1, R2 can be H, C{sp3}, C{sp2}, C{ar} or combinations

	thereof
Mechanism	Acylation, Ester aminolysis or thiolysis

The primary insecticidal effect of carbamates is elicited by interaction with acetylcholinesterase enzyme, (AChE), resulting in acute cholinergic poisoning. The inhibition of AChE by carbamate esters is considered to involve formation of a reversible enzyme-substrate complex, followed by conversion of the latter to carbamoylated enzyme protein adduct [15].

Covalent binding of carbamoyl moiety from carbaryl and other carbamates was also suggested [16]. The reaction takes place as a bimolecular nucleophilic substitution, which is enhanced if the ester moiety is aromatic. During carbamoylation of proteins (including chromosomal ones) by carbaryl and other carbamates, containing aryl ester moiety, phenolic metabolites could be also formed as shown below



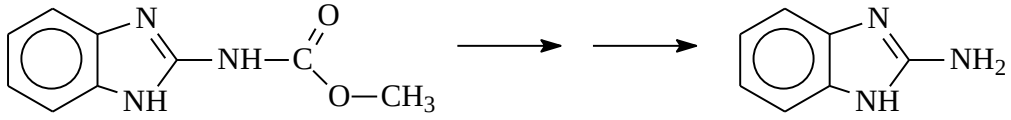
carbaryl

carbamoylated protein

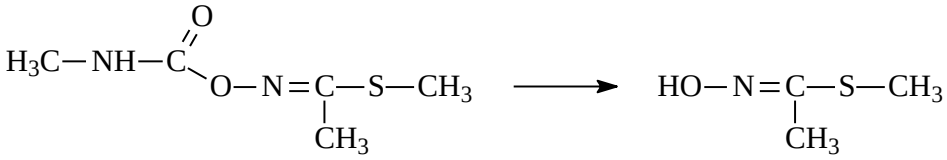
α-naphthol

(Pr-NH2: chromosomal protein with lysine side primary amino groups); Pr-SH: chromosomal protein with cysteine side thiol groups)

Additionally, other non-aryl carbamate esters could act by the above-described acylation mechanism (Table 1, Chemicals 4, 8 – 10). For these chemicals, however, in vitro microsomal/S9 metabolic activation may result in formation of mutagenic and/or clastogenic metabolites, acting by other genotoxicity molecular mechanisms:

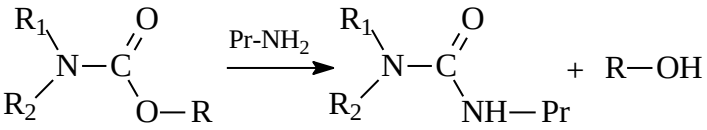


(Assumed potentially active metabolite: heterocyclic amine)



(Assumed potentially active metabolite: oxime)

Generally, if ester aminolysis is accepted as principal molecular mechanism, associated with in vitro clastogenicity by formation of chromosomal protein adducts with carbamates via direct SN2 acylation, the following mechanistic scheme can be proposed:

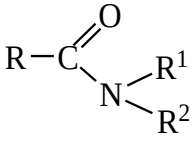


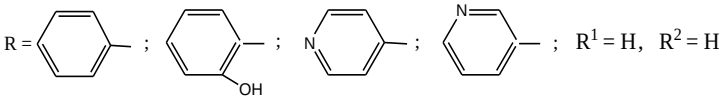
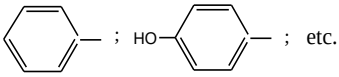
(Chromosomal protein adduct)

It should be noted that the above mechanistic scheme is believed to be best applied when R is electron-wthdrawing benzenoid-type aromatic or azomethyne ester moiety.

Set of chemicals used for profile development	Carbamates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Wei, L.Y., Chao, J.S., Hong, C.C., Assessment of the ability of propoxur, methomyl, and aldicarb, three carbamate insecticides, to induce micronuclei in vitro in cultured Chinese hamster ovary cells and in vivo in BALB/c mice, Environ. Mol. Mutagen., 1997, 29(4), 386-393. 4. Carbaryl, EHC No. 153. World Health Organization, Geneva, 1994. 5. Carbamate pesticides: a general introduction, EHC No. 64, World Health Organization, Geneva, 1986. 6. Naravaneni, R., K. Jamil, Cytogenetic Biomarkers of Carbofuran Toxicity Utilizing Human Lymphocyte Cultures In Vitro, Drug Chem. Toxicol. 2005, 28(3), 359 – 372. 7. Soloneski, S., M. L. Larramendy, Genetic Toxicological Profile of Carbofuran and Pirimicarb Carbamic Insecticides, 2012; http://cdn.intechweb.org/pdfs/28277.pdf. Last visited: July, 2021. 8. Sofuni, T. 1998. Data Book of Chromosomal Aberrations Test In Vitro, Revised Edition, Live-Science Information Center, Tokyo, Japan.

	9. Murakami, M., Fukami, J.I., Uptake of benzo[a]pyrene, carbaryl, DDT and parathion in cultured human cells: re-evaluation, Bull. Environ. Contam. Toxicol., 1979, 21(1), 478 – 482. 10. Metcalf, R.L., Structure-activity relationships for insecticidal carbamates, Bull. World Health Organ., 1971, 44(1-3), 43 - 78. 11. Aldridge, W.N., Nature of reaction of organophosphorus compounds & carbamates with esterases, Bull. World Health Organ., 1971, 44(1-3), 25 - 30. 12. Pipy, B., Gaillard, D., Derache, R., Enzymatic activities of liver serine esterases during the reticuleondothelial system phagocytosis blockade by carbaryl, an anticholinesterasic insecticide, Toxicol. Appl. Pharmacol. 1982, 62(1), 11 - 18. 13. Murakami, M., Fukami, J.I., Incorporation of labeled pesticides and environmental chemicals into nuclear fraction of cultured human cells, Bull. Environ. Contam. Toxicol., 1980, 24(1), 27 - 30. 14. Murakami, M., Fukami, J.I., Carbaryl binds to proteins in human cells in culture but chlorinated organic chenicals do not, Bull. Environ. Contam. Toxicol., 1982, 28(4), 500 - 503. 15. Davies, J.H., Campbell, W.R., Kearns, Inhibition of fly head acetylcholinesterase by bis-(m-hydroxyphenyl)-trimethylammonium iodide) esters of polymethylenedicarbamic acids, C.W., Biochem. J., 1970, 117(2), 221 - 230. 16. Krug, H.F., Hamm, U., Berndt, J., Mechanism of inhibition of cyclo-oxygenase in human blood platelets by carbamate insecticides, Biochem. J., 1988, 250(1), 103 - 110.

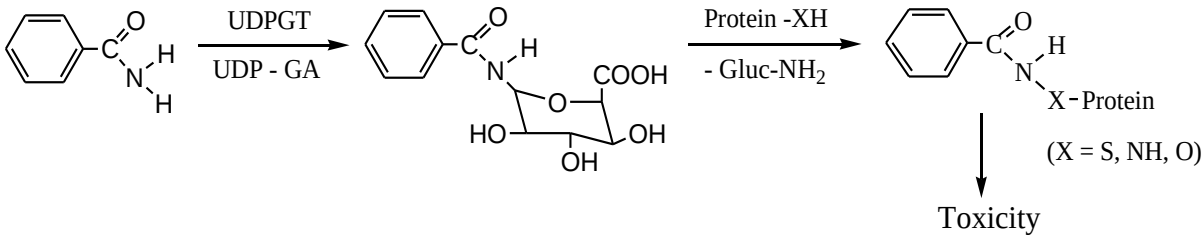
Individual profile/alert	
Name	Carboxylic Acid Amides
Type of profile	Structural alert
Description/applicability domain	

	where **Ist group:** R =  ; R^1 = H, R^2 = H **IInd group:** R = Alkyl (-CH_3, -C_2H_5, etc); R^1 = H; R^2 =  ; etc.
Mechanism	Acylation, Acylation involving an activated (glucuronidated) carboxamide group Acylation, Direct acylation involving a leaving group Michael addition, Quinone type compounds

Acylation involving an activated (glucuronidated) carboxamide group

It is well known that an amide is hydrolyzed to yield an amine and carboxylic acid under strong acidic or basic conditions. Obviously, amide hydrolyzation is not possible under physiological conditions. Then, it may be assumed that one of the way for bioactivation of primary aryl amides is N-glucuronidation. Several reports suggested formation of amide N-glucuronides [6,13], which were resistant to hydrolysis at pH levels of 3.0, 7.4, 9.0 and by bacterial β-glucuronidases [13]. Since amide N-glucuronides are more reactive toward nucleophilic attack than the amides itself, it would be possible to form protein adducts according to Scheme 1.

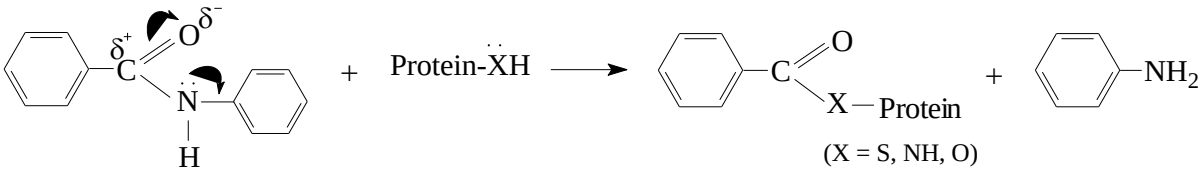
Scheme 1



Direct acylation involving a leaving group

Some of the N-aryl amides can refer to proteins as acyl transfer agents since their carbon atom possesses enhanced electropyilicity [14]. For example, tetrachlorosalicylanilide, a suspected immunotoxin and known contact photoallergen can form protein adducts with serum albumin according to the proposed mechanism in Scheme 2 [14-18].

Scheme 2

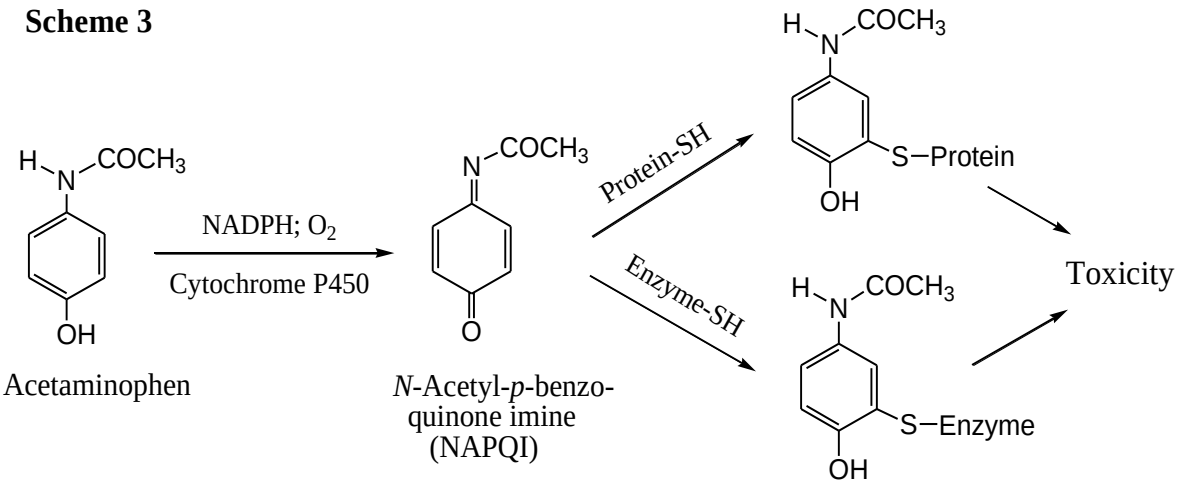


Quinone type compounds

Acetaminophen is one of these secondary N-aryl amides which toxicity is related with its initial metabolism. It is metabolically activated by CYP450 to form a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [8-11]. In overdose, conjugation of this reactive metabolite with GSH

leads to the GSH depletion and NAPQI (a soft electrophile) covalently binds to cysteine residues on proteins to form acetaminophen adducts [11]. Covalent binding of NAPQI to proteins is thought to be a critical step in the development of hepatotoxicity [8]. It was also reported that NAPQI is a topoisomerase II poison [10]. It induces DNA strand breaks, chromosomal aberrations, and sister chromatid exchanges in a variety of mammalian cells [7,19]. DNA cleavage in the presence of NAPQI is mediated by topoisomerase IIα. The binding of NAPQI to cellular proteins and to cellular enzymes is an excellent correlate of acetaminophen toxicity [9,11] (Scheme 3).

Scheme 3

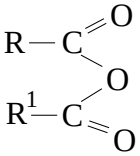


Acetaminophen

N-Acetyl-*p*-benzo-quinone imine (NAPQI)

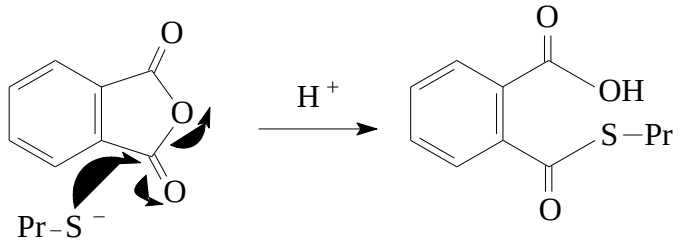
Set of chemicals used for profile development	Carboxylic Acid Amides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. C.S. Song, N.A. Gelb, S.M. Wolff, *J. Clin. Invest*. 1972, **51**, 2959-2966. 2. M.E. Morris, G. Levy, *J. Pharm. Sci*. 1983, **72**, 612-617. 3. X. Xu, B.K. Tang, K.S. Pang, *J. Pharmacol. Exp. Ther*. 1990, **253**, 965-973. 4. R.G. Tirona, K.S. Pang, *Drug Metab. Dispos.* 1996, **24**, 821-833. 5. Final report of the safety assessment of niacinamide and niacin, *Int. J. Toxicol.* 2005, **24,** 1-31. 6. A.G. Staines, M.W.H. Coughtrie, B. Burchell, *J. Pharmacol. Exp. Ther.* 2004, **311,** 1131-1137. 7. K. Bergmen, L. Müller, S.W. Teigen, *Mutat. Res.* 1996, **349**, 263-288. 8. K.L. Muldrew, L.P. James, L. Coop, S.S. McCullough, H.P. Hendrickson, J.A. Hinson, P.R. Mayeux, *Drug Metab. Dispos.* 2002, **30,** 446-451. 9. L. P. James, P. R. Mayeux, J. A. Hinson, *Drug Metab. Dispos.* 2003, **31,** 1499-1506. 10. R.P. Bender, R.H. Lindsey, Jr., D.A. Burden, N. Osheroff,

	Biochemistry 2004, **43**, 3731-3739. 11. A.B. Reid, R.C. Kurten, S.S. McCullough, R.W. Brock, J.A. Hinson, *J. Pharmacol. Exp. Ther.* 2005, **312**, 509-516. 12. D.J. Naisbitt, M. Britschgi, G. Wong, J. Farrell, J.P.H. Depta, D.W. Chadwick, W.J. Pichler, M. Pirmohamed, B.K. Park, *Mol. Pharmacol.* 2003, **63**, 732-741. 13. D. Zhang, W. Zhao, V.A. Roongta, J.G. Mitroka, L.J. Klunk, M. Zhu, *Drug Metab. Dispos.* 2004, **32,** 545-551. 14. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, *Chem. Res. Toxicol.* 2007, **20**, 1019–1030. 15. Toxic substances – Focus on Children; Developing a Canadian List of Substances of Concern to Children's Health, June 2004, p. A-64. 16. E.W. Scholes, D.A. Basketter, W.W. Lovell, A.E. Sarll, R.U. Pendlington, *Photodermatol. Photoimmunol. Photomed.* 1991, **8**, 249-254. 17. G.F. Gerberick, C.A. Ryan, E.C. Von Bargen, S.B. Stuard, G.M. Ridder, *J. Invest. Dermatol.* 1991, **97**, 210–218. 18. H. Spielmann, L. Müller, D. Averbeck, M. Balls, S. Brendler-Schwaab, J.V. Castell, R. Curren, O. de Silva, N.K. Gibbs, M. Liebsch, W.W. Lovell, H.F. Merk, J.F. Nash, N.J. Neumann, W.J. Pape, P. Ulrich, H.W. Vohr, *ATLA* 2000, **28**, 777-814. 19. E. Dybing, J.A. Holme, W.P. Gordon, E.J. Soderlund, D.C. Dahlin, S.D. Nelson, *Mutat. Res.* 1984, **138,** 21-32.

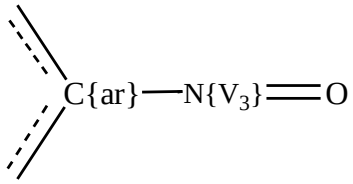
Individual profile/alert	
Name	Carboxylic Acid Anhydrides
Type of profile	Structural alert
Description/applicability domain	 R = R^1 = Csp^2 (scy), Csp^2 (aryl), Csp^2 (aryl fused)
Mechanism	SN2 Acylation, Ring opening acylation reaction

Maleic anhydride (MA), 2-acetoxybenzoic anhydride did induce chromosomal aberrations in cultured Chinese hamster lung (CHL) cells in the absence of exogenous metabolic activation and phthalic anhydride in cultured Chinese hamster ovary (CHO) cells in the absence of exogenous metabolic activation [2,3].

Ring opening acylation reaction for cyclic anhydrides with protein-nucleophile is shown in Scheme 1 [4].



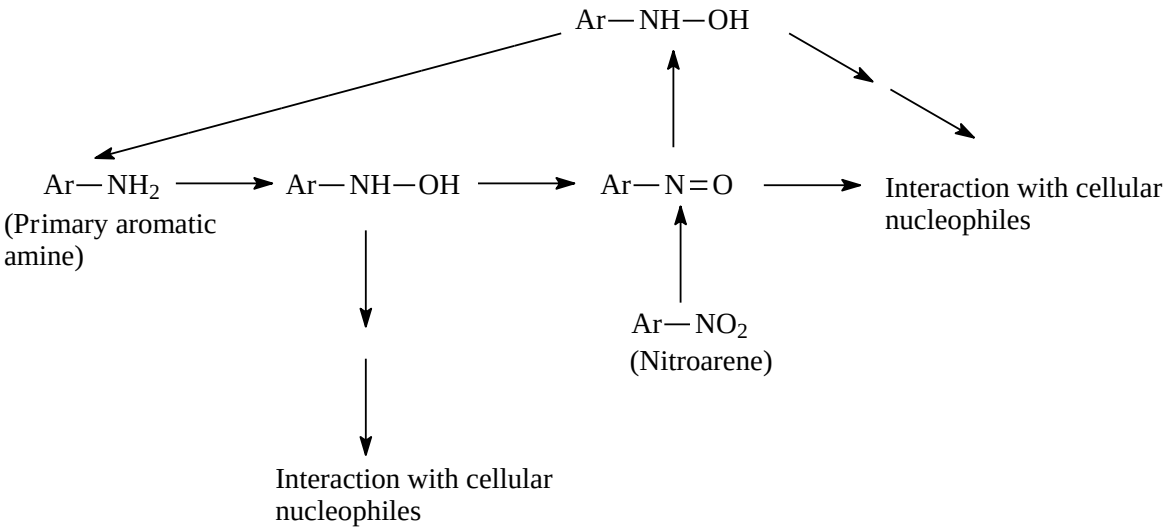
Set of chemicals used for profile development	Carboxylic Acid Anhydride
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kim, J. H., Gibb, H.J., Iannucci, A., Cyclic acid anhydrides: Human health aspects. IPCS, Concise International Chemical Assessment Document 75. Sciences International Inc., **2009**, p.4. 2. Ishidate, M. Jr., Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 3. Phthalic anhydride, CAS No. 85-44-9, OECD SIDS Initial Assessment Report for SIAM 20, UNEP Publications, **2005**, p.5. 4. Enoch, S.J., Ellison, C.M., Schultz, T.W., Cronin M.T.D., A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol.*, **2011**, 41(9), 783-802.

Individual profile/alert	
	C-Nitroso compounds protein binding
Type of profile	Structural alert
Description/applicability domain	
Mechanism	AN2, Nucleophilic addition at polarized N-functional double bond

A number of chemicals, which show positive in vitro CA effects after S9 metabolic activation to C-nitroso compounds belong to the sub-class of aromatic amines, and, to a lesser extent, to that of nitroarenes. Aromatic amines exert their toxic effects usually after oxidative biotransformation, primarily in liver. As a result, aromatic N-hydroxylamines are generated, which can further undergo

oxidative activation by two-electron oxidation to nitrosoarenes. Nitrosoarenes can also be formed by reduction of nitroarenes [3].

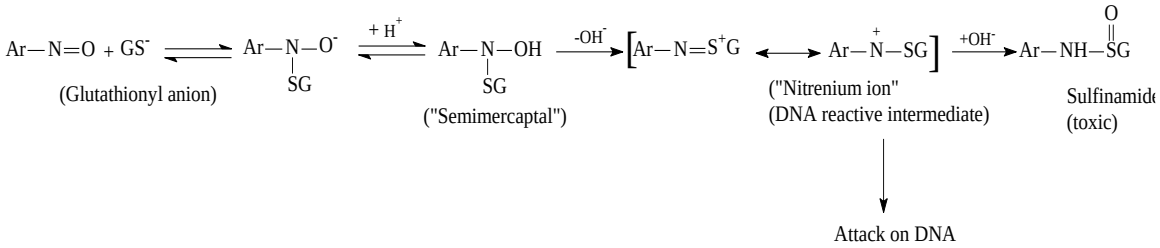
These processes may occur, according to the following oversimplified scheme [4, 5]:



Such metabolic activation, with the contribution of nitroreductase, CYP isoenzymes and other enzymatic systems may produce electrophilic intermediates, responsible for toxic, allergic, mutagenic, and carcinogenic effects.

Nitrosoarenes and some other C-nitroso compounds may exert their toxic effects through their thiol reactivity. The nitroso group is strongly electron-withdrawing and bears some similarity to the carbonyl group C=O. Due to the strong polarization and the similarity of nitroso-group to the carbonyl one, C-nitroso compounds are capable of undergoing the characteristic addition (AN2) reactions with nucleophiles such as thiols. The reactions of thiols with nitrosoarenes are complex, and product formation is dependent on thiol concentration, pH, and substituent effects. Examples of some toxicologic implications of the interactions of nitroso compounds with thiols can be found mainly for nirosoarenes, and, to the lesser extent, for nitrosoimidazoles, heterocyclic nitroso compounds, etc. These data indicate that interactions of activated arylamines with thiols can be regarded as bioactivation, rather than detoxification reaction.

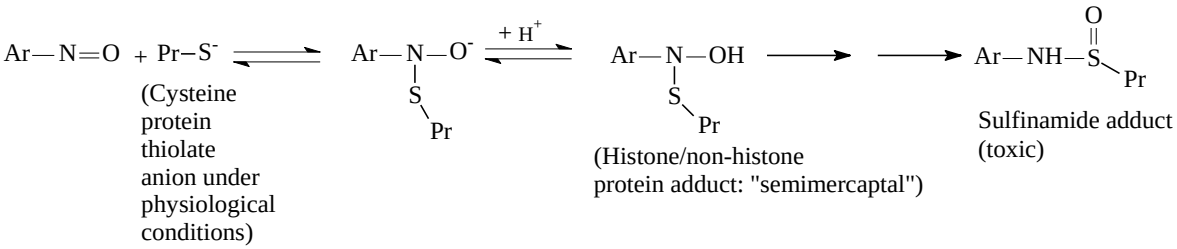
Formation of products of interaction of C-nitroso compounds with thiols such as glutathione (GSH) has been proposed for chemicals such as Nitrosobenzene, 4-Nitrosophenetol, 4-Nitroso-N,N-dimethylaniline, some nitrosoheterocyclic compounds such as nitroso-substituted pyridoindoles and pyridoimidazoles, 4-Nitrosochlorobenzene, etc. Mechanistic pathway involving formation of N-hydroxylamine sulfenamide (“semimercaptal”) and sulfinamide products of interaction with glutathione has been proposed (Scheme 2 below) [3]:



Other chemicals from the C-nitroso sub-class such as 2-Nitrosofluorene and 1-Methyl-2-Nirosoimidazole were also reported to be cytotoxic and mutagenic. These data suggest that GSH interferes with metabolically formed reactive species, probably by scavenging the nitrosoarene via initial AN2-type interactions.

Covalent binding to proteins can inactivate vital enzymes and may lead to haptenization, followed by an immune response and skin sensitization [3]. Moreover, formation of protein adducts, derived from N-hydroxylamine metabolites oxidized to nitrosoarenes in erythrocytes, has been proposed for 2-Nitrotoluene, 2,4-Dinitrotoluene and 2,6-Dinitrotoluene in rats. Again, N-hydroxylamine sulfenamide ("semimercaptal") and sulfinamide intermediates were suggested as active species in the mechanistic pathway [6].

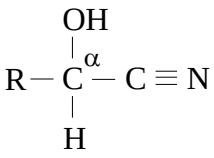
Based on the possible nitrosoarene metabolites of a number of aromatic amines and nitroarenes with positive in vitro CA results after S9 metabolic activation, and their cysteine protein thiol reactivity, the following mechanistic scheme of interaction with chromosomal proteins is expertly proposed:



Set of chemicals used for profile development	C-Nitroso compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Eyer, P., Reactions of Oxidatively Activated Arylamines with Thiols: Reaction Mechanisms and Biological Implications. An Overview, Environ. Health Persp. 1994, 102, Suppl. 6, 123 – 132. 4. Kalgutkar, A. S., I. Gardner, R. S. Obach, C. L. Shaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O, Donnell, J. Boer, S. P. Harriman, A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups, Current Drug Metabol., 2005, 6, 161 – 225. 5. Shamovsky, I., L. Ripa, L. Borjesson, Chr. Mee, B. Norden, P.

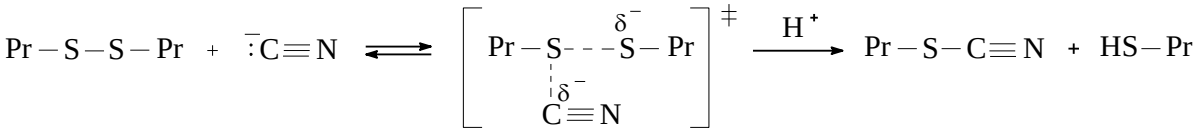
Hansen, C. Hasselgren, M. O, Donovan, P. Sjo, Explanation for Main Features of Structure-Genotoxicity Relationships of Aromatic Amines by Theoretical Studies of Their Activation Pathways in CYP1A2, JACS, 2011, 133, 16168 – 16185.

6. Sabbioni, G., Chr. R. Jones, O. Sepai, et al. Biomarkers of Exposure, Effect, and Susceptibility in Workers Exposed to Nitrotoluenes, Cancer Epidemiol Biomarkers Prev. 2006, 15(3), 559 – 566.

Individual profile/alert	
Name	Cyanohydrins
Type of profile	Structural alert
Description/applicability domain	 R = $(Csp^3)n$, where "n" represents up to 7 carbon atoms.
Mechanism	SN2, Thiocyanate formation via the nucleophilic-type substitution at the disulfide bond of proteins and enzymes SN2, yanoalkylation of proteins via the nucleophilic substitution at sp3-carbon atom of cyanohydrins

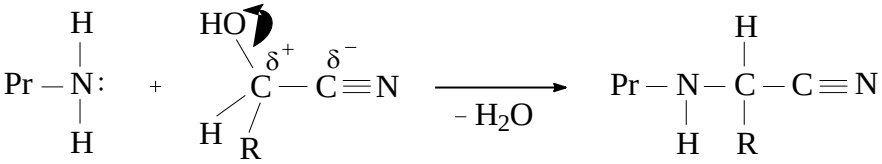
While many chemical forms of cyanide are used in industrial application or are present in the environment, the cyanide anion is the primary toxic agent, regardless of its origin and quantity in the respective system [6]. So, the toxicity of cyanohydrins is believed to be predominantly attributable to dissociation products, i.e., the rapid release of cyanide ions rather than the parent compound. Once absorbed, cyanide can inhibit approximately 40 enzymes, including a number of metalloenzymes, containing iron, copper, molybdenum, disulfide enzymes such as catalase, peroxidase, as well as enzymes containing Schiff base intermediates (e.g. 2-oxo-4-hydroxyglutarate aldolase), etc. Cyanide ion may be bound to nonhematin metal containing enzymes, like tyrosinase, xanthine oxidase, amino acid oxidase and various phosphates, cystine and methionine residues in proteins [4,6,7].

The cleavage of disulfide bonds in proteins and certain enzymes involving cyanide anions could be presented as follows (Scheme 2):



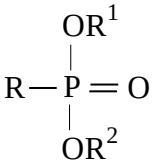
As the process of cyanide release is reversible and pH dependent, it would be expected that under physiological conditions (pH 7.2–7.4) there will be a certain amount of parent cyanohydrin. According to Horvath et al. [8], cyanohydrins may react with basic amino groups of proteins under

physiological conditions, which leads to formation of the corresponding cyanoalkyl derivatives. This reaction proceeds as nucleophilic substitution of the hydroxyl group at the expence of increased electrophilicity of alpha-carbon atom, adjacent to the electron-withdrawing cyano group (Scheme 3).

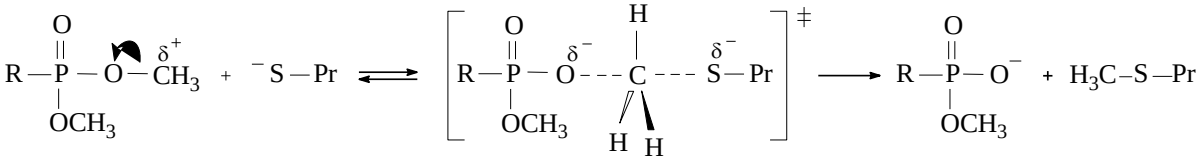


Set of chemicals used for profile development	Cyanohidrins
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 2-Hydroxypropanenitrile: SIDS Initial Assessment Report for SIAM 2, July **1994**, pp. 3, 6, 11. 2. H. Kusakabe, K. Yamakage, S. Wakuri, K. Sasaki, Y. Nakagawa, M. Watanabe, M. Hayashi, T. Sofuni, H. Ono, N. Tanaka, Relevance of chemical structure and cytotoxicity to the induction of chromosome aberrations based on the testing results of 98 high production volume industrial chemicals. *Mutat. Res.*, **2002**, 517(1-2), 187-198. 3. T. Frisch, B.L. Møller, Possible evolution of alliarinoside biosynthesis from the glucosinolate pathway in *Alliaria petiolata*. *FEBS J*., **2012**, 279(9), 1545-1562. 4. Acetone Cyanohydrin: *In* Acute Exposure Guideline Levels for Selected Airborne Chemicals, vol. 7, pp. 13-47, National Academies Press, Washington, D.C., **2009**. 5. P.V. Kaplita, R.P. Smith, Pathways for the bioactivation of aliphatic nitriles to free cyanide in mice. *Toxicol. Appl. Pharmacol.*, **1986**, 84(3), 533-540. 6. F.P. Simeonova, L. Fishbein, Hydrogen cyanide and cyanides: Human Health Aspects. Concise International Chemical Assessment Document, CICAD 61, WHO **2004**. 7. Cyanogenic glycosides in cassava and bamboo shoots, Technical Report Series No. 28. Food

	Standards Australia New Zealand, July **2004**. 8. V. Horváth, L. Trézl, T. Szarvas, J. Pipek, C. Vida, K. Bauer, Investigation of cyano-methylation reaction by cyanohydrin and its determination in tobacco-smoke. (Strecker-reactions). *Period. Polytech. Chem. Eng.*, **1992**, 36(3), 209-218.

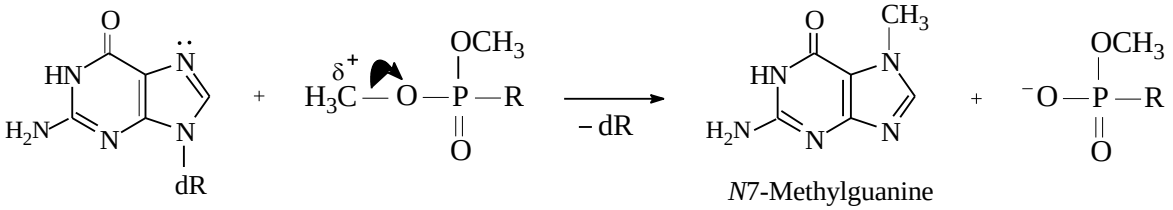
Individual profile/alert	
Name	Dialkyl Alkylphosphonates
Type of profile	Structural alert
Description/applicability domain	 R, R^1, R^2 are Csp^3-atoms and R = R^1 = R^2 or R is different from R^1 and R^2
Mechanism	SN2, Protein and/ or DNA alkylation

Dialkyl alkylphosphonates containing methoxy groups and shorter carbon-chain alkoxy groups possess electrophilic carbon atoms, capable of interacting with nucleophilic sites of biomolecules by alkylation. The negative mutagenicity data in Ames S. typhimurium test suggest the lack of a possibility of DNA alkylation. However, the positive in vitro clastogenicity in CHO cells without S9 activation implies some alkylating ability towards protein amine and protein thiol groups. The SN2 reaction mechanism between dialkyl alkylphosphonates and protein-thiolate ion is shown in Scheme 1.



where R = Csp^3-atoms

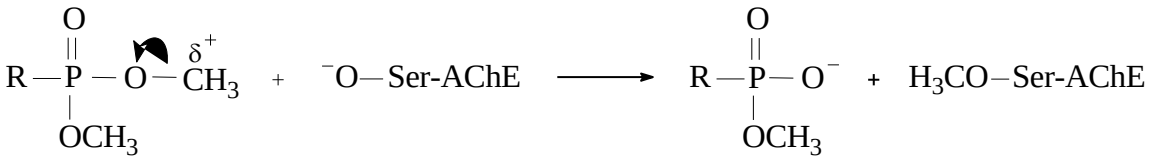
Trichlorfon and the other phosphonate esters with electron-withdrawing substituents possess higher electrophilicity on carbon atoms of the methoxy groups, compared to the non-substituted phosphonates. It was established that trichlorfon has a DNA-alkylating properties and may react with DNA *in vitro* to cause depurination and excision [12]. In the *in vivo* studies *N*7-methylguanine adducts were found to be formed involving trichlorfon [13].



*N*7-Methylguanine

where R = Cl_3C-CH(OH)– or HOH_2C-NH-C(O)-$(CH_2)_2$–

The powerful acute toxicity of organophosphorus poisons is primarily due to the fact that they are potent irreversible inhibitors of AChE, forming a covalent bond with a serine residue at the active site of the enzyme [16,17]. Cleavage of organophosphonates or organophosphates by AChE leaves a phosphoryl group in the esteratic site which is slow to be hydrolyzed and can become covalently bound. Then, the alkylation of AChE-Ser-O^- fragment by the phosphonate esters could be suggested to occur via a nucleophilic substitution reaction as shown in Scheme 3.

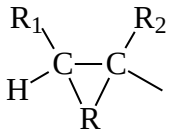


where R = Cl_3C-CH(OH)– or HOH_2C-NH-C(O)-$(CH_2)_2$–

Set of chemicals used for profile development	Dialkyl Alkylphosphonates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. T.C. Wang, C.M. Lin, L.W. Lo, Genotoxicity of methoxyphosphinyl insecticide in mammalian cells. *Zool. Stud.*, **2003**, 42(3), 462-469. 2. T. Feng, Z.B. Li, X.Q. Guo, J.P. Guo, Effects of trichlorfon and sodium dodecyl sulphate on antioxidant defense system and acetylcholinesterase of *Tilapia nilotica* in vitro. *Pest. Biochem. Physiol.*, **2008**, 92(3), 107-113. 3. G. Klopman, R. Contreras, H.S. Rosenkranz, M.D. Waters, Structure-genotoxic activity relationships of pesticides: comparison of the results from several short-term assays. *Mutat. Res.*, **1985**, 147(6), 343-356. 4. NTP Toxicology and Carcinogenesis Studies of Dimethyl Methylphosphonate (CAS No. 756-79-6) in F344/N rats and B6C3F1 Mice (Gavage Studies). *Natl. Toxicol. Program Tech. Rep. Ser.*, **1987**, NTP TR 323, pp. 1-172.

5. Toxicological Profile for Diisopropyl Methylphosphonate. U.S. Department of Health and Human Services, August **1998**.
6. S.M. Galloway, D.A. Deasy, C.L. Bean, A.R. Kraynak, M.J. Armstrong, M.O. Bradley, Effects of high osmotic strength on chromosome aberrations, sister chromatid exchanges and DNA strand breaks, and the relation to toxicity. *Mutat. Res*., **1987**, 189(1), 15-25.
7. M. Ishidate Jr, K. Yoshikawa, Chromosome aberration tests with Chinese hamster cells *in vitro* with and without metabolic activation. *Arch. Toxicol.*, **1980**, Suppl. 4, 41-44.
8. Trichlorfon: *In* IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 30, **1983**, pp. 207-231.
9. K. Baetcke, I.S. Rodgers, L. Tahan, G. Hard, R. McGaughy, *alpha*-2u-Globulin: Association with chemically-induced renal toxicity and neoplasia in the male rat. U.S. Environmental Protection Agency, February 1991.
10. J.A. Swenberg, α_{2u}-Globulin nephropathy: Review of the cellular and molecular mechanisms involved and their implications for human risk assessment. *Environ. Health Perspect*., **1993**, 101(Suppl. 6), 39-44.
11. K. Blumbach, A. Pähler, H.M. Deger, W. Dekant, Biotransformation and male rat-specific renal toxicity of diethyl ethyl- and dimethyl methylphosphonate. *Toxicol. Sci*., **2000**, 53(1), 24-32.
12. H.S. Rosenkranz, S. Rosenkranz, Reaction of DNA with phosphoric acid esters: gasoline additives and insecticides. *Experientia (Basel)*, **1972**, 28(4), 386-387.
13. W. Dedek, K. Lohs, G.W. Fischer, R. Schmidt, Alkylation of guanine in mice in vivo by organophosphorus insecticides. I. Trichlorphone and butonate. *Pest. Biochem. Physiol*., **1976**, 6(2), 101-110.
14. J.C. Sanchez-Hernandez, C.H. Walker, *In vitro* and *in vivo* cholinesterase inhibition in Lacertides by phosphonate- and phosphorothioate-type organophosphates. *Pestic. Biochem. Physiol*., **2000**, 67(1), 1-12.
15. T. Feng, Z.B. Li, X.Q. Guo, J.P. Guo, Effects of trichlorfon and sodium dodecyl sulphate on

	antioxidant defense system and acetylcholinesterase of *Tilapia nilotica* in vitro. *Pest. Biochem. Physiol*., **2008**, 92(3), 107-113. 16. D.M. Quinn, Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states. *Chem. Rev*., **1987**, 87(5), 955-979. 17. H. Dvir, I. Silman, M. Harel, T.L. Rosenberry, J.L. Sussman, Acetylcholinesterase: From 3D structure to function. *Chem.-Biol. Interact*., **2010**, 187(1-3), 10-22.

Individual profile/alert	
	Epoxides, Aziridines and Sulfuranes
Type of profile	Structural alert
Description/applicability domain	 (R_1 is –H, -CH_2-O-C, -CH_2OH, -CH_2F, -CH_2Cl, -CH_2Br, -C(=O)OC (carboxylic ester group), C{ar} (aromatic carbon), C{sp3}{scy}(cycloalkyl carbon), C{sp2}{scy}(cycloalkenyl or carbonyl carbon); R_2 is –H, -CH_2-O-C, -CH_2OH, -CH_2F, -CH_2Cl, -CH_2Br, -C(=O)OC, C{ar}, C{sp2}{scy})
Mechanism	SN2, Ring opening SN2 reaction

Epoxides, aziridines and thiiranes are electrophiles, acting predominantly by similar, SN2 mechanisms. For instance, the reactivity of epoxides as DNA/protein alkylating agents is affected by the degree of substitution at the carbon center, where the attack takes place, and by the type of substituents:

- Primary, mono-substituted, terminal epoxides, with -CH2- group are more reactive than secondary ones;

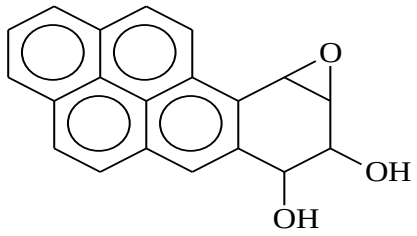
- Electron-withdrawing groups that stabilize the negative charge on the oxygen atom as the ring opens can also enhance reactivity;

- Covalent binding at nucleophilic protein sites such as –NH2, -SH, etc. constitutes the chemical basis determining the toxicity of epoxides, thiiranes and aziridines [3, 4, 6].

- According to some authors, the key importance of protein/peptide binding is a process that can be modeled by combining, for example, reactivity and hydrophobicity parameters [5].

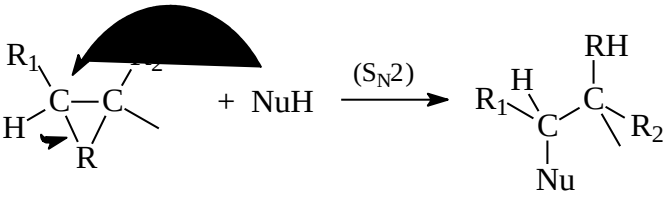
An example can be presented, which indicates the positive in vitro CA results of epoxides, which are mainly determined by covalent protein binding. The majority of the mode of binding a characteristic

benzo[a] pyrene metabolite to nuclear macromolecules, which occurs in intact hamster embryo cells was reported to be due to formation of adducts with various classes of nuclear proteins. Thus benzopyrene diol epoxide:



as active metabolite of benzo[a]pyrene was found to induce in vitro CA [7, 8].

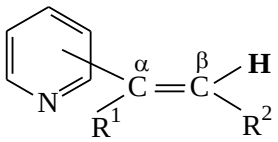
On the basis of the above discussions, and the fact that the cyclic carbon in the three-membered heterocycles may interact as both the “soft” and “hard” electrophile, the following SN2-type reaction mechanistic schemes which may elicit CA by protein binding can be expertly proposed [3, 5, 9]:



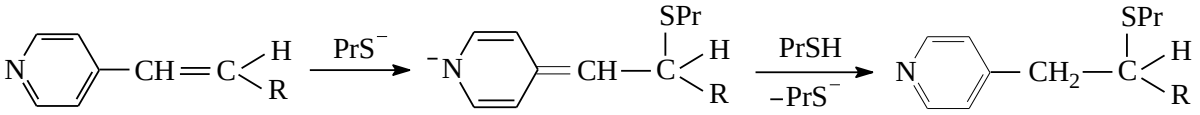
(NuH may correspond to Pr-NH2 (chromosomal protein with lysine side primary amino groups) or to Pr-SH (chromosomal protein with cysteine side thiol groups))

Set of chemicals used for profile development	Epoxides, Aziridines and Thiiranes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Roberts, D. W., A. M. Api, R. J. Safford, J. F. Lalko, Principles for identification of High Potency Category Chemicals for which the Dermal Sensitisation Threshold (DST) approach should not be applied, Regulatory Toxicology and Pharmacology, 2015, 72, 683 – 693. 4. Schramm, Fr., A. Muller, H. Hammer, A. Paschke, G.

	Schuurmann, Epoxide and Thiirane Toxicity In vitro with the Ciliates Tetrahymena pyriformis: Structural Alerts Indicating Excess Toxicity, Environ. Sci. Technol. 2011, 45, 5812 – 5819. 5. Roberts, D. W., Gr. Patlewitz, P. S. Kern, Fr. Gerberick, I. Kimber, R. Dearmann, C. A. Ryan, D. Basketter, A. O. Aptula, Mechanistic Applicability Domain Classification of a Local Lymph Node Assay Dataset for Skin Sensitization, Chem. Res. Toxicol. 2007, 20, 1019 – 1030. 6. Buback, V., M. Mladenovic, B. Engels, T. Schirmeister, Rational Design of Improved Aziridine-Based Inhibitors of Cysteine Proteases, J. Phys. Chem. B 2009, 113, 5282 –5289. 7. McLeod, M. C., A. Kootstra, B. K. Mansfield, T. J. Slaga, J. K. Selkirk, Specificity in interaction of benzo[a]pyrene with nuclear macromolecules: Implication of derivatives of two dihydrodiols in protein binding, Proc. Natl. Acad. Sci (USA), 1980, 77(11), 6396 – 6400. 8. Wei, Q., J. Gu, L. Cheng, M. L. Bondy, H. Jiano, W. K. Hong, M. R. Spitz, Benzo[a]pyrene Diol Epoxide Induced Chromosomal Aberrationas and Risk of Lung Cancer, Canc. Res. 1996, 56, 3975 – 3979. 9. Enoch, S. J., C. M. Ellison, T. W. Schultz, M. T. D. Cronin, A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity, Crit. Rev. Toxicol. 2011, 41(9), 783 – 802.

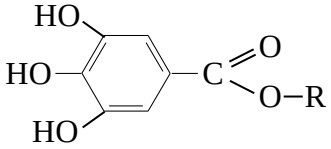
Individual profile/alert	
Name	Ethenyl Pyridines
Type of profile	Structural alert
Description/applicability domain	 (mainly in *o*- or *p*-positions relative to a *N*-atom) R^1 and R^2 = any carbon or hydrogen atoms and also R^1 and R^2 can be located in *Z*- or *E*-positions to one another.
Mechanism	AN2, Michael-type addition to activated double bonds in vinyl pyridines

Vinyl pyridine isomers (ortho- and para-) are typical Michael acceptors due to the presence of a polarized double bond under the influence of electron-withdrawing effect of the nitrogen atom [7,8]. It has been suggested that di-substitution at the β-carbon atom of the alkene moiety sterically hinders the Michael reaction [7,9]. The mechanism of reaction is considered to involve predominantly the attack of protein thiolate ions to β-carbon atom [2,7,10], as shown in Scheme 1.



The vinyl group in para-position was found to be more reactive to biological macromolecules than the vinyl group in ortho-position. This is probably due to the difference in stability of the corresponding intermediates with para- or ortho-quinoid structures, the first one being more stable than the latter.

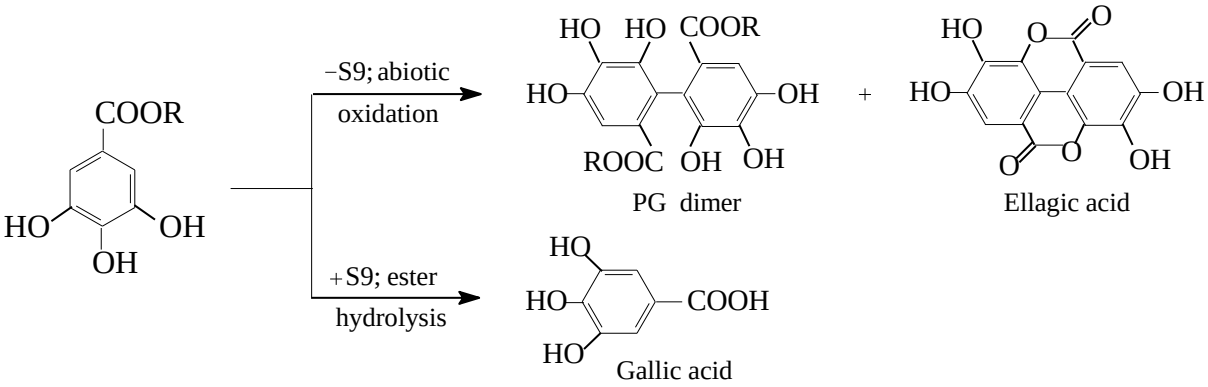
Set of chemicals used for profile development	Ethenyl Pyridines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. D. Sesseville, A. Balbul, P. Kwong, K. Yu, *Contact Dermatitis*, **1996**, 35(2), 100-101. 2. O. Bergendorff, J. Wallengren, *Contact Dermatitis*, **1999**, 40(5), 280-281. 3. O.W. Griffith, *Anal. Biochem.* **1980**, 106(1), 207-212. 4. V.S. Sharov, E.S. Dremina, N.A. Galeva, T.D. Williams, C. Schöneich, *Biochem. J.*, **2006**, 394 (Pt 3), 605-615. 5. K.D. Brunnemann, A. Rivenson, S.C. Cheng, V. Saa, D. Hoffmann, *Cancer Lett.*, **1992**, 65(2), 107-113. 6. D.W. Bombick, D.J. Doolittle, *In Vitro Toxicol.*, **1995**, 8(4), 349-356. 7. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, *Chem. Res. Toxicol.*, **2007**, 20(7), 1019-1030. 8. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.T.D. Cronin, *Crit. Rev. Toxicol.*, **2011**, 41(9), 783-802. 9. G.Y. Patlewicz, Z.M. Wright, D.A. Basketter, C.K. Pease, J.P. Lepoittevin, E.G. Arnau, *Contact Dermatitis*, **2002**, 47(4), 219–226. 10. T.W. Schultz, J.W. Yarbrough, R.S. Hunter, A.O. Aptula, *Chem. Res. Toxicol.*, **2007**, 20(9), 1359-1363.

Individual profile/alert	
Name	Gallic Acid Esters
Type of profile	Structural alert
Description/applicability domain	

	R can be H, Csp^3(alkyl, cycloalkyl), Csp^2(aryl), etc.
Mechanism	AN2, Michael-type addition to quinoid structures

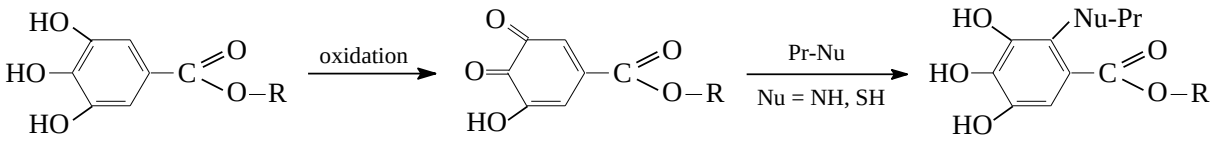
Gallate esters are used as synthetic antioxidants. Despite their presumed low toxicity, many authors were reported that some of linear alkyl gallates had many adverse effects. The cytotoxic effects of methyl, ethyl, propyl, butyl, octyl and dodecyl gallates have been studied by Nakagawa and Tayama [1,2]. It was found that they cause concentration-dependent losses of intracellular ATP, GSH and protein thiol levels [1]. Propyl gallate was positive in in vitro chromosome aberration (CA) tests [3,4], and was both positive and negative in the sister-chromatid test [4]. The effects of propyl gallate on carcinogenesis and mutagenesis have been reported to be both enhancing and suppressing [2].

The adverse effects of gallates may be related to their antioxidizing effect, which follows from the corresponding autoxidation. For example, when propyl gallate (PG) is autoxidized in the test media of CHO cells in the absence of metabolic activation (S9 fraction), it is converted via a PG radical into PG dimer and finally into ellagic acid [2]. The authors established that the medium change from clear red to dark brown. But when PG is metabolized in the presence of S9 fraction, it is converted mainly to gallic acid (GA) which is also autoxidized as indicated by the changing of color medium (Scheme 1). It was also found that the oxidative enzymes superoxide dismutase and superoxide dismutase plus catalase increased PG cytotoxicity [2].



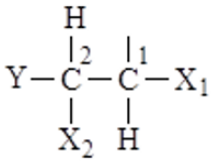
During the oxidation of GA, the consumption of oxygen was higher than in the case of PG oxidation. It was observed that intra- and extra-cellular H2O2 was generated by GA autoxidation, and that the H2O2 may played a role in the toxic effects of GA [2].

Bearing in mind the presence of catechol moiety in gallates and their dimers, they are able to be converted to Michael acceptors by abiotic and/or enzymatic oxidation to the corresponding quinoid structures [2,5]. Upon oxidation to an o-quinone, a Michael-type addition reaction could take place as shown in Scheme 2.



The reactivity of gallate esters toward proteins depends on the relative elongation of alkyl side-chains which determine their hydrophobicity. It was established that butyl gallate, octyl gallate and dodecyl

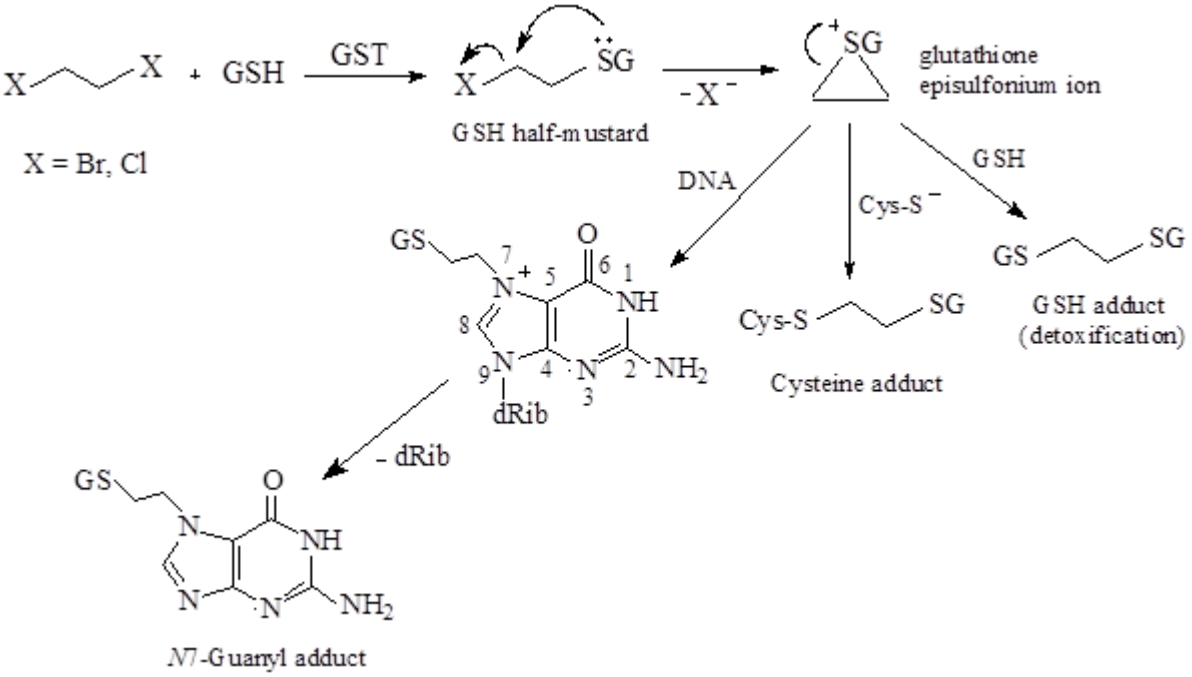
gallate are more cytotoxic than propyl, ethyl, and methyl gallate [1].	
Set of chemicals used for profile development	Gallic Acid Esters
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Y. Nakagawa, S. Tayama, Cytotoxicity of propyl gallate and related compounds in rat hepatocytes. *Arch. Toxicol*., **1995**, 69(3), 204-208. 2. S. Tayama, Y. Nakagawa, Cytogenic effects of propyl gallate in CHO-K1 cells. *Mutat. Res*., **2001**, 498(1-2), 117-127. 3. M. Ishidate Jr, T. Sofuni, K. Yoshikawa, M. Hayashi, T. Nohmi, M. Sawada, A. Matsuoka, Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol*., **1984**, 22(8), 623-636. 4. D.K. Gulati, K. Witt, B. Anderson, E. Zeiger, M.D. Shelby, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. III. Results with 27 chemicals. *Environ. Mol. Mutagen*., **1989**, 13(2), 133-193. 5. G. Patlewicz, D.W. Roberts, E. Uriarte, Skin sensitization: A comparison of reactivity schemes for the prediction skin sensitization potential. *Chem. Res. Toxicol*., **2008**, 21(2), 521-541.

Individual profile/alert	
Name	Halogenated Vicinal Hydrocarbons
Type of profile	Structural alert
Description/applicability domain	 where: X_1 and X_2 = Br, Cl, I; C_1 and C_2 = Csp^3 (acy or scy); Y = H, Csp^3 (acy or scy), OH, O-P^{+5} and S^{+2}.
Mechanism	SN2, Nucleophilic type substitution together with ring-opening of

	an episulfonium ion intermediate

➢ Non-alkylated vicinal haloalkanes

Vicinal dihaloalkanes such as 1,2-dichloroethane, 1,2-dibromoethane, and the mixed 1-bromo-2-chloroethane can be activated to electrophilic species by either oxidative metabolism or conjugation with glutathione [1-3]. Although GSH-conjugation is generally a route of detoxification, in this case it leads to genetic damage. 1,2-Dibromoethane has been shown to induce DNA adduct formation as a result of GSH-dependent bioactivation [7]. The major DNA adduct formed from 1,2-dibromoethane in vitro has been identified as S-[2-(N7-guanyl)ethyl]glutathione, which is believed to arise via GSH half-mustard (GS-CH2-CH2-X) [1,3]. The mechanism of alkylation is associated with an episulfonium (tiiranium) ion formation involving GSH half-mustard and the subsequent binding with the N7-position of guanine to yield a bulky DNA adduct via the depurination reaction. Cysteine thiol groups of proteins can also be alkylated. The GSH-dependent activation pathway of vicinal dihaloalkanes is depicted in Scheme 1.

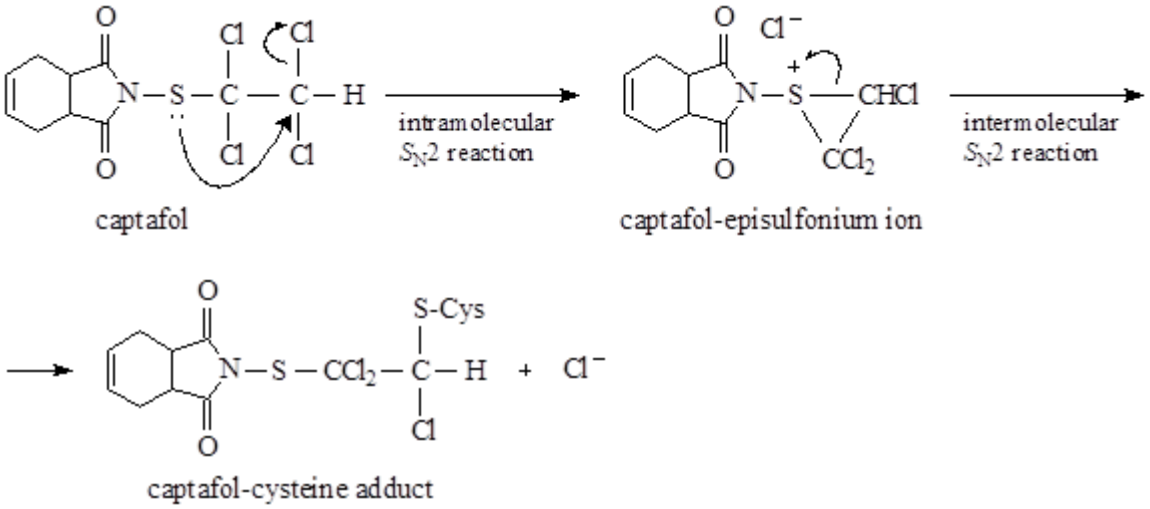


The other experimentally found DNA adducts of GSH-activated 1,2-dibromoethane are N2- and O6-guanyl derivatives. However, only with the N7-guanyl adduct depurination occurs, which could result in respective mutations [1]. For the series of 10 direct alkylating halogenated hydrocarbons a positive relationship between carcinogenicity and the initial ratios of O6/N7-alkylguanine formed with double-stranded DNA was found in vitro [8].

In vitro evidence for some DNA adduct formation via the GSH-conjugation pathway could be obtained for 1,2-dibromo-3-chloropropane, 2,3-dibromo-1-propanol and tris(2,3-dibromopropyl)phosphate [1], although the contribution of the oxidative pathways seems to be more important [1,9]. The reactivity is dependent upon the leaving group ability of the halogen substituent with the following order of decreased activity: I > Br > Cl, and benzyl-Br > alkyl-Br [10].

The fungicide captafol differs structurally from vicinal dihaloalkanes having tetrachloroethylthio side chain moiety. This difference confers profound effect on its biological activity. Captafol can act

directly as a clastogen without the need for GSH-dependent activation [11]. Captafol's tetrachloroethylthio side chain is able to form an intramolecular episulfonium ion due to the nucleophilicity of the sulfur atom. The opening of this intermediate involving cysteine thiolate residues of proteins is an intermolecular SN2 reaction. It is believed to proceed very fast since positively charged sulfur atom is a good leaving group and moreover, the strain in the three-membered ring is also released [12]. The mechanism of captafol's clastogenicity is shown in Scheme 2.



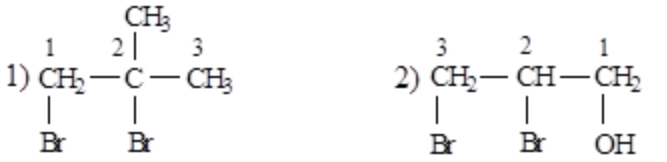
captafol | captafol-episulfonium ion

captafol-cysteine adduct

- Alkylated and C1-O-Arylated vicinal haloalkanes

  - Alkylated vicinal haloalkanes

Alkyl substitution at the halogen-bearing C1 and C2 carbon atoms reduces both glutathione transferase activity and mutagenicity of vicinal dihalogenated compounds [10]. In all cases, methylation decreased mutagenicity relative to the parent compound, but the degree of reduced mutagenicity varied considerably depending on the position of the methyl substitution [13]. For example, l,2-dibromo-2-methylpropane (1) is not mutagenic, while 2,3-dibromopropan-l-ol (2) is mutagenic [10].

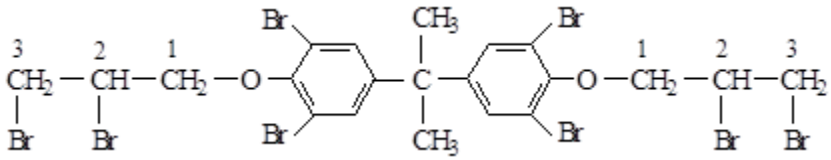


1,2-dibromo-2-methylpropane (non-mutagenic) | 2,3-dibromopropan-1-ol (mutagenic)

The first compound, having two electron-donating methyl groups bonded to C2 carbon atom, do not show any mutagenicity. This is probably related to the reduced electrophilicity of the C2 carbon and somewhat to the steric hindrance under the influence of the methyl groups. The second compound contains an electron-withdrawing hydroxymethyl group suggesting the presence of a greater partial positive charge at the C2 atom and a possibility for the formation of reactive episulfonium ion. Based on these observations might be assumed that the lack of mutagenicity of the methylated compounds could be determined by both electronic and steric effects of the substituents.

- C1-O-Arylated vicinal haloalkanes

In general, the genotoxic potential of halogenated compounds is dependent not only on the nature, number, and position of halogens, but also on the molecular size of the compound [14,15]. For example, the large molecule of tetrabromobisphenol A bis(2,3-dibromopropyl) ether (TBBPA bis(2,3-dibromopropyl) ether) is expected to be a poor alkylating agent in in vitro mammalian cells. This could be due to both low water solubility (~1 mg/L), i.e. high lipophilicity of TBBPA bis(2,3-dibromopropyl) ether and a reduced ability to form a stable episulfonium ion due to steric and electronic effects of a bulky O-aryl moiety.



TBBPA bis(2,3-dibromopropyl) ether

It was found that TBBPA bis(2,3-dibromopropyl) ether did not cause chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary (CHO) cells in vitro, was negative in an in vivo micronucleus assay in mice and did not produce unscheduled DNA synthesis in rats [16]. Moreover, in the in vitro experiments utilizing hepatocytes or liver microsomal protein, no detectable metabolism of TBBPA bis(2,3-dibromopropyl) ether occurred [17].

Set of chemicals used for profile development	Halogenated Vicinal Hydrocarbons
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Guengerich, F.P., Activation of dihaloalkanes by thiol-dependent mechanisms. *J. Biochem. Mol. Biol*., **2003**, 36(1), 20-27. 2. Tezuka, H., Ando, N., Suzuki, R., Terahata, M., Moriya, M., Shirasu, Y., Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. *Mutat. Res*., **1980**, 78(2), 177-191. 3. Guengerich, F.P., Peterson, L.A., Cmarik, J.L., Koga, N., Inskeep, P.B., Activation of dihaloalkanes by glutathione conjugation and formation of DNA adducts. *Environ. Health Perspect*., **1987**, 76, 15-18. 4. van Esch, G.J., Tris(2,3-dibromopropyl)phosphate and bis(2,3-dibromopropyl) phosphate. International Programme on Chemical Safety, Environmental Health Criteria 173. World Health Organization, Geneva, **1995**, pp. 18-21. 5. Nakamura, A., Noriyuki, T., Kojima, S., Kaniwa, M., Kawamura, T., The mutagenicity of halogenated alkanols and

their phosphoric acid esters for *Salmonella typhimurium*. *Mutat. Res.*, **1979**, 66(4), 373-380.
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10. van Bladeren, P.J., Breimer, D.D., Rotteveel-Smijs, G.M., de Knijff, P., Mohn, G.R., van Meeteren-Wälchli, B., Buijs, W., van der Gen, A., The relation between the structure of vicinal dihalogen compounds and their mutagenic activation via conjugation to glutathione. *Carcinogenesis*, **1981**, 2(6), 499-505.
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13. Omichinski, J.G., Søderlund, E.J., Bausano, J.A., Dybing, E., Nelson, S.D., Synthesis and mutagenicity of selectively methylated analogs of tris(2,3-dibromopropyl)phosphate and 1,2-dibromo-3-chloropropane. *Mutagenesis*, **1987**, 2(4), 287-292.
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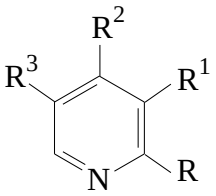
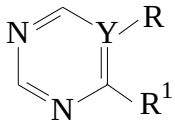
15. Perez-Garrido, A., Giron-Rodriguez, F., Morales Helguera, A., Borges, F., Combes, R.D., Topological structural alerts modifications of mammalian cell mutagenicity for halogenated derivatives. *SAR QSAR Environ. Res.*, **2013**; doi: 10.1080/1062936X.2013.820791.
16. Flame Retardant Alternatives for Hexabromocyclododecane (HBCD). Final Report, EPA Publication 740R14001, June **2014**.
17. Knudsen, G.A., Jacobs, L.M., Kuester, R.K., Sipes, I.G.,

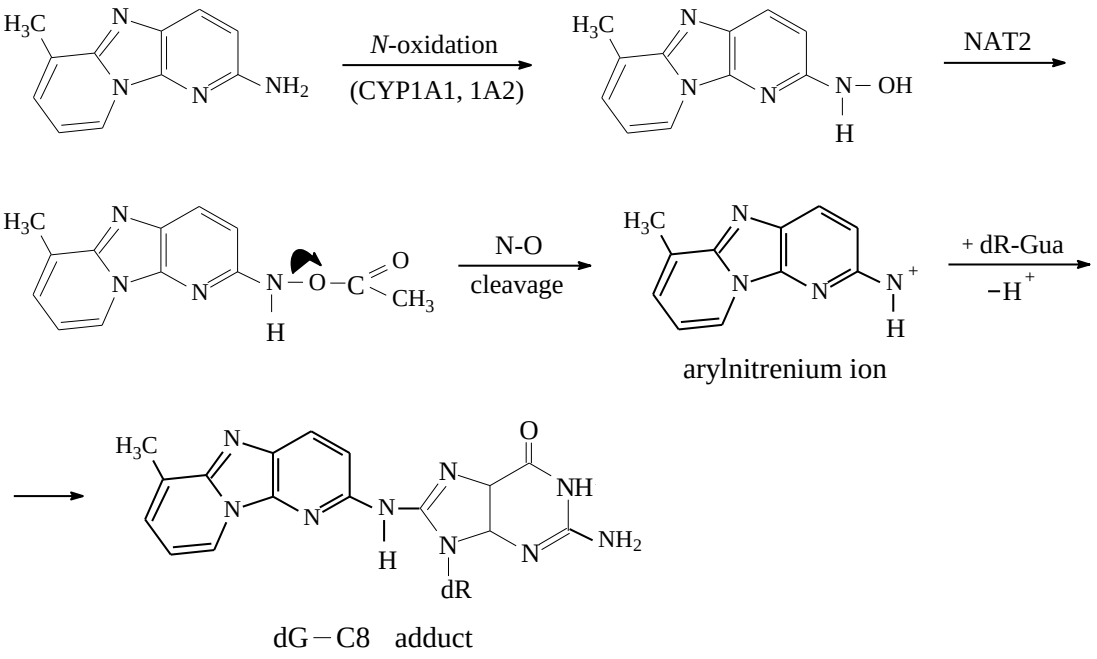
	Absorption, distribution, metabolism and excretion of intravenously and orally administered tetrabromobisphenol A [2,3-dibromopropyl ether] in male Fischer-344 rats. *Toxicology*, **2007**, 237(1-3), 158-167. 18. McKee, R.H., Phillips, R.D., Traul, K.A., The genetic toxicity of 1,2-dibromo-3-chloropropane, 1,2-dibromo-3-chloro-2-methylpropane, and 1,2,3-tribromo-2-methylpropane. *Cell Biol. Toxicol.*, **1987**, 3(4), 391-406. 19. Låg, M., Omichinski, J.G., Dybing, E., Nelson, S.D., Søderlund, E.J., Mutagenic activity of halogenated propanes and propenes: effect of bromine and chlorine positioning. *Chem. Biol. Interact.*, **1994**, 93(1), 73-84.

Individual profile/alert	
Name	Heterocyclic Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	 **R** and **R²** can be Csp^2(aryl) and Nsp^3(scy); **R¹** and **R³** can be Csp^2(aryl) or Nsp^2(scy) in five- or six-membered fused rings  Y = Nsp2(scy), if R1 ≠ H Y = Csp2(aryl), if R = R1 = Nsp2(scy)
Mechanism	**S_E reaction (CYP450-activated heterocyclic amines)**, Direct attack of arylnitrenium cation to the C8 position of nucleoside base **S_R reaction (peroxidase-activated heterocyclic amines)**, Direct attack of arylnitrenium radical to the C8 position of nucleoside base **Radical mechanism**, ROS generation and direct attack of hydroxyl radical to the C8 position of nucleoside base **A_N2**, Nucleophilic addition to pyridonimine tautomer of aminopyridoindoles or aminopyridoimidazoles (hypothesized)
➢ S_E reaction (CYP450-activated heterocyclic amines), Direct attack of arylnitrenium cation to the	

C8 position of nucleoside base

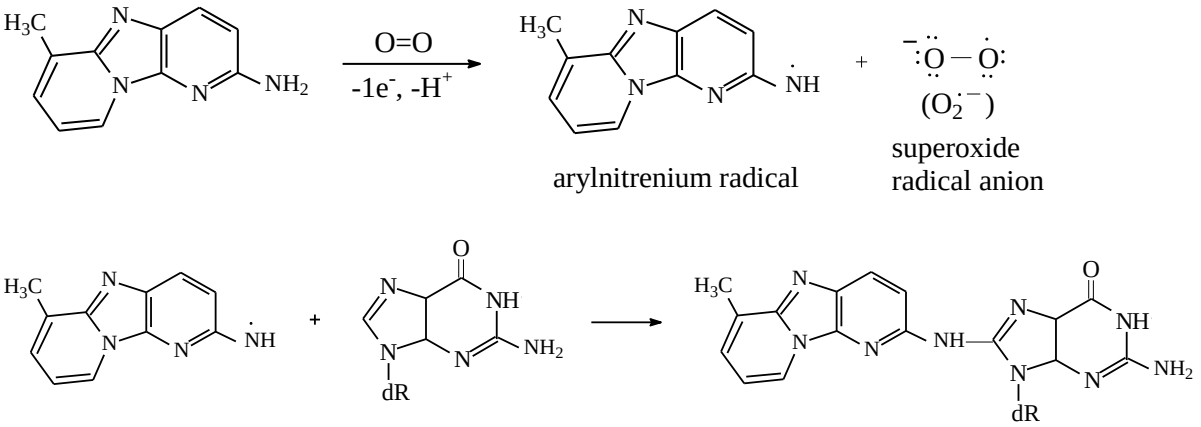
Heterocyclic aromatic amines (HCAs) and aromatic amines are structurally related classes of mutagens that can be formed during the high-temperature cooking of meats or during the combustion of tobacco [2]. Both classes of procarcinogens undergo metabolic activation in vivo and in vitro by N-hydroxylation of the exocyclic amine group to form a common proposed intermediate, the arylnitrenium ion. N-hydroxylation and arylnitrenium ion formation are catalyzed mainly by cytochrome P450 isoenzymes 1A1, 1A2 and N-acetyltransferase (NAT2). Arylnitrenium ion is the critical metabolite implicated in toxicity and DNA damage [2,7,8].

The major DNA adducts formed by activated HCAs in vivo and in vitro have been identified as N-(deoxyguanosyl-8-yl)-HCA (dG-C8) adducts and 5-(deoxyguanosyl-N2-yl)-HCA (dG-N2) adducts [1,2,9]. The level of dG-C8 adducts was much greater than the amount of dG-N2 adducts formed from the reaction of dR-Gua with N-acetoxy derivatives of aminoimidazoquinoline (IQ) and aminomethylimidazoquinoxaline (MeIQx) [2]. For HCAs Glu-P-1 and Trp-P-2, however, only C8-guanine adducts have been identified after metabolic activation (Scheme 1).



- S_R reaction (peroxidase-activated heterocyclic amines) , Direct attack of arylnitrenium radical to the C8 position of nucleoside base

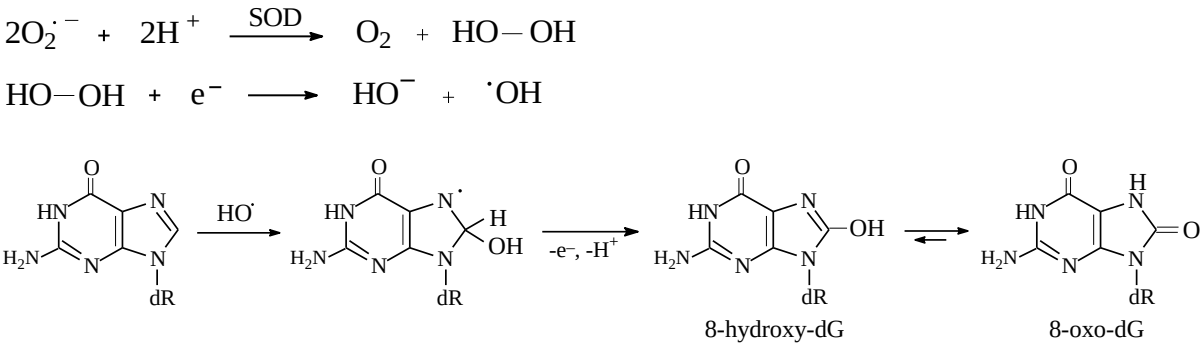
The genotoxicity of HCAs in Chinese hamster cells in the absence of metabolic activation (CYP450 and acetyltransferases) could hardly be explained by the formation of arylnitreniun ion intermediate. According to some authors [2,11,12], HCAs can be suitable cosubstrates for peroxidases in the cells, thereby undergoing one-electron oxidation that leads to the formation of HCA free-radical metabolites producing DNA adducts (Scheme 2).



N-Hydroxy intermediates do not appear to be involved in the metabolism by peroxidases including prostaglandin H synthase [2]. Thus, the N-centered free radicals of HCAs are believed to play a crucial role in an extrahepatic pathway proving that this metabolic pathway proceeds via a one-electron mechanism. Moonen et al. [11] were used an indirect method, previously described, to establish the formation of HCAs free radicals. It was based on the fact that these radicals were reduced by GSH, while, in turn, glutathione was oxidized to form a thiyl radical.

- Radical mechanism, ROS generation and direct attack of hydroxyl radical to the C8 position of nucleoside base

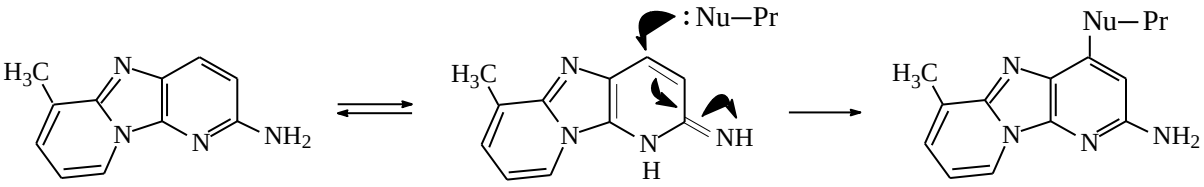
During mitochondrial oxidative metabolism, the majority of oxygen is reduced to water; however, an estimated 4% to 5% is converted to reactive oxygen species, primarily the superoxide radical anion ($O2^{\cdot -}$). Dismutation by superoxide dismutase (SOD) reduces $O2^{\cdot -}$ to H2O2 which is then converted to hydroxyl free radicals ($HO^{\cdot}$) [13]. Hydroxyl radicals are the much stronger oxidants than superoxide radicals. They react with most biological molecules such as DNA at or near diffusion-controlled rates, causing damage to the heterocyclic DNA bases and the sugar moiety by a variety of mechanisms [14]. Three major intermediates are known that result from hydroxyl radical attack at a guanine nucleobase, the C4, C5 and C8 adducts [12]. The major pathway under oxidative conditions yields 7,8-dihydro-8-oxoguanosine (8-oxo-dG) together with its minor tautomer 8-hydroxyguanosine (Scheme 3) [12-15].



- A_N2, Nucleophilic addition to pyridonimine tautomer of aminopyridoindoles or aminopyridoimidazoles (hypothesized)

The reactivity of 2- and 4-aminopyridoindoles or pyridoimidazoles could also be explained by the possibility such compounds to form two tautomeric forms, the first one (aminopyridine form) being

more stable. The second form, pyridonimine tautomer, may act as a Michael acceptor and can bind to protein nucleophiles according to Scheme 4.



aminopyridine derivative pyridonimine derivative HCA– Protein adduct

Set of chemicals used for profile development	Heterocyclic Aromatic Amines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. H.A.J. Schut, E.G. Snyderwine, DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. *Carcinogenesis*, **1999**, 20(3), 353-368. 2. R.J. Turesky, L.L. Marchand, Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. *Chem. Res. Toxicol.*, **2011**, 24(8), 1169-1214. 3. H. Frederiksen, Two food-borne heterocyclic amines: Metabolism and DNA adduct formation of amino-*α*-carbolines. *Mol. Nutr. Food Res.*, **2005**, 49(3), 263-273. 4. Y.F. Sasaki, H. Yamada, K. Shimoi, N. Kinae, I. Tomita, H. Matsumura, T. Ohta, Y. Shirasu, Enhancing effects of heterocyclic amines and *beta*-carbolines on the induction of chromosome aberrations in cultured mammalian cells. *Mutat. Res.*, **1992**, 269(1), 79-95. 5. M. Ishidate, Jr, S. Odashima, Chromosome tests with 134 compounds on Chinese hamster cells *in vitro* - a screening for chemical carcinogens. *Mutat. Res.*, **1977**, 48(3-4), 337-353. 6. M. Ishidate, Jr, K.F. Miura, T. Sofuni, Chromosome aberration assays in genetic toxicology testing *in vitro*. *Mutat. Res.*, **1998**, 404(1-2), 167-172. 7. Y. Yanagawa, M. Sawada, T. Deguchi, F.J. Gonzalez, T. Kamataki, Stable expression of human CYP1A2 and *N*-acetyltransferases in Chinese

hamster CHL cells: Mutagenic action of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline. *Cancer Res*., **1994**, 34(13), 3422-3427.

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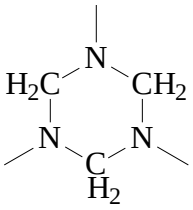
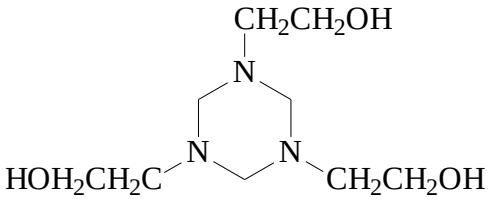
14. M. Dizdaroglu, Oxidatively induced DNA damage: Mechanisms, repair and disease. *Cancer lett*., **2012**, 327(1-2), 26-47.
15. P. Møller, H. Wallin, U. Vogel, H. Aytrup, L. Risom, M.T. Hald, B. Daneshvar, L.O. Dragsted, H.E. Poulsen, S. Loft, Mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline in colon and liver of Big Blue rats: role of DNA adducts, strand breaks, DNA repair and oxidative stress. *Carcinogenesis*, **2002**, 23(8), 1379-1385.

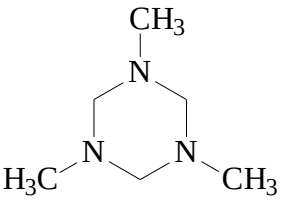
Individual profile/alert

Name	Hexahydrotriazine Derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	AN2, Formaldehyde release (abiotic)

Representative chemicals:



Hexahydro-1,3,5-Tris(hydroxyethyl) Triazine (HHT, CAS No. 4719-04-4)

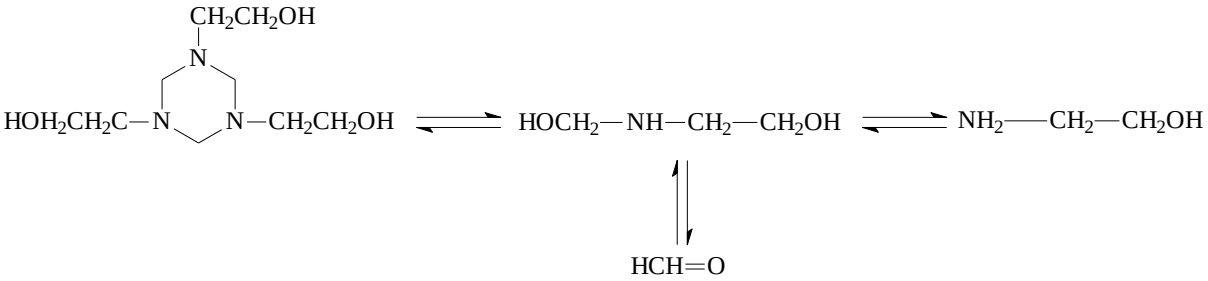


Hexahydro-1,3,5-Trimethyl-1,3,5-Triazine (CAS No. 108-74-7)

HHT is evaluated as a formaldehyde releaser. The hydrolysis half-life of HHT is 50 days for pH 7, which means release of very small amounts of formaldehyde under the conditions of in vitro incubation with eukaryotic cells during the chromosome aberration (CA) test. The released small amounts of formaldehyde, which is both the clastogen and aneugen could be the reason for the in vitro positive CA test results of the chemical [1, 2].

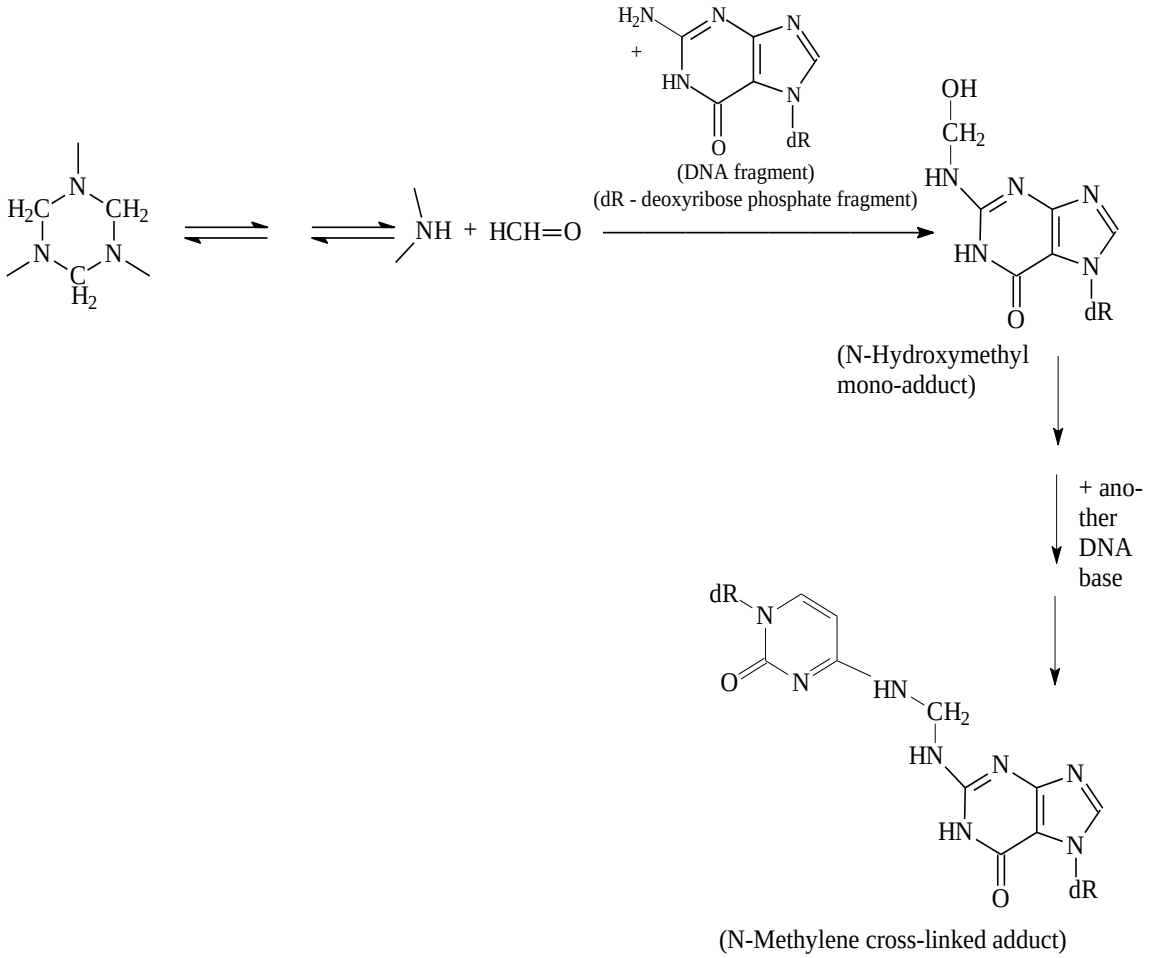
Positive results for another structurally similar chemical, hexahydro-1,3,5-trimethyl-1,3,5-triazine, have also been reported [3].

One of the slow-hydrolysis products of HHT was found to be monoethanolamine, and the other is presumably formaldehyde [4]. An equilibrium, indicating the abiotic hydrolytic formaldehyde release, causing clastogenicity can be established at pH 5 – 7 [5]:



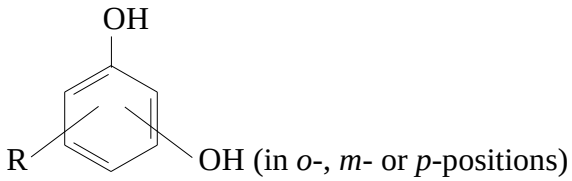
The released formaldehyde, which is genotoxic substance induces DNA-DNA and DNA-protein cross-links as the primary DNA lesions. This is related to the cytotoxicity and clastogenicity (chromosomal aberrations) [6]. Formaldehyde induces mainly N-hydroxymethyl mono-adducts on guanine, adenine and cytosine, and N-methylene cross-links between adjacent purines in DNA [7].

Thus one of the possible general general mechanistic schemes for eliciting DNA damage and in vitro chromosomal aberrations for hexahydrotriazine derivatives can be expressed as follows:



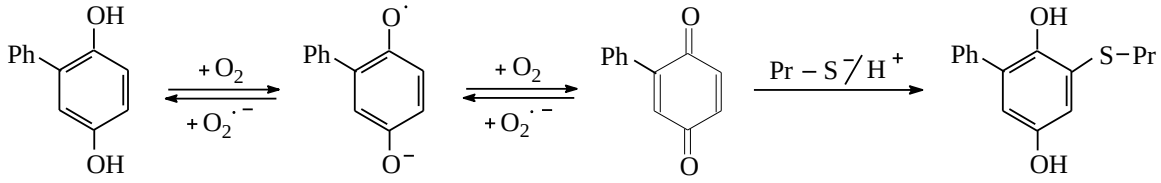
Set of chemicals used for profile development	Hexahydrotriazine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Reregistration Eligibility Decision (RED) for Crotan (HHT)*, US EPA, OPP, June 27, 2008. Speit, G., *Genotoxicity of Formaldehyde In Vitro and In Vivo*, CEFIC 2012 (Presentation); https://www.scribd.com/document/91977532/Genotoxicity-of-Formaldehyde-in-Vitro-and-in-Vivo-by-Gunter-Speit. Last visited: July, 2021. 2. *Hexahydro-1,3,5-Trimethyl-1,3,5-Triazine*, Exp Key Genetic Toxicity In Vitro.002; https://echa.europa.eu/registration-dossier/-/registered-dossier/13293/7/7/2/?documentUUID=8822835a-905f-46dc-9489-09c35a57989b. Last visited: July, 2021. 3. Bakke, J. M., J. Buhaug, J. Riha, *Hydrolysis of 1,3,5-Tris(2-Hydroxyethyl)Hexahydro-s-Triazine and Its Reaction with H_2S*, Ind. Eng. Chem. Res. **40** (2001), 6051 – 6054. 5. Rossmoore, H. W., M. Sondossi, *Applications and Mode of Action of Formaldehyde Condensate Biocides*, Adv. Appl. Microbiol.

	33 (1988), 223 – 277. 6. Speit, G., O. Merk, *Evaluation of Mutagenic Effects of Formaldehyde In Vitro: Detection of Crosslinks and Mutations in Mouse Lymphoma Cells*, Mutagen. **17**(3) (2002), 183 – 187. 7. Kawanishi, M., T. Matsuda, T. Yagi, *Genotoxicity of Formaldehyde: Molecular Basis of DNA Damage and Mutation*, Froniers in Environmental Science **2** (2014), 1 – 8.

Individual profile/alert	
Name	Hydroxylated Phenols
Type of profile	Structural alert
Description/applicability domain	 R = OH; Csp^3(acy, scy) where Csp^3(acy) involves linear or branched chains, containing between one and four carbons; Csp^2(aryl); Csp^2(vinyl), etc.
Mechanism	AN2, Mchael-type addition to quinoid structures

The substituted catechols and hydroquinones are able to be oxidized to the corresponding benzoquinones under the influence of the endogenously expressed cell enzymes (peroxidases). Nonenzymatic oxidative pathways might also take place with catecholamines, catechins, gallic acid esters, etc. [2-4]. As reported by many authors, their effects associated with oxidative damage of cellular macromolecules were due to the formation of benzoquinones [5-7].

For example, as a result of phenylhydroquinone (PHQ) autoxidation, the phenylbenzoquinone is formed. It behaves as very reactive Michael acceptor against protein nucleophiles (mainly protein thiols) yielding the corresponding protein adducts (Scheme 1).



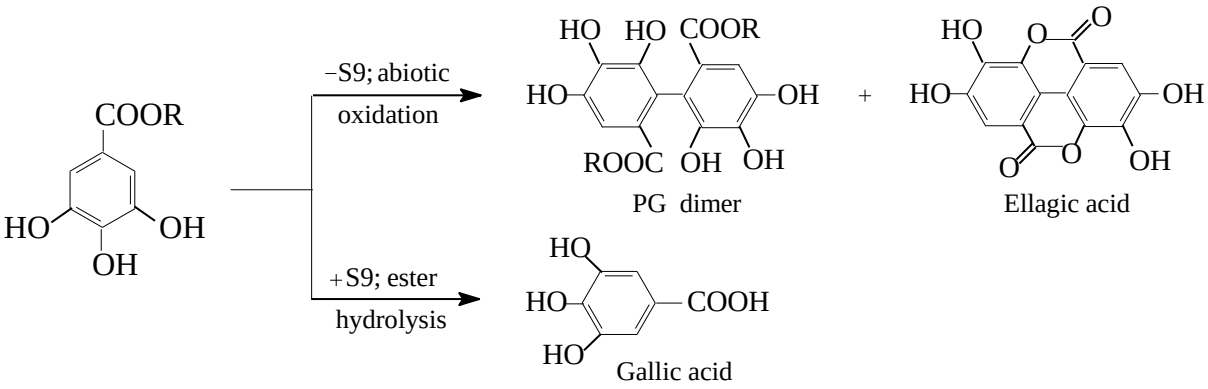
phenylhydroquinone | phenylsemiquinone anion-radical | phenylbenzoquinone | protein-adduct formation

According to Zhao et al. [8], phenylbenzoquinone can also covalently bind to nucleophilic sites on DNA in vitro. The proposed mechanism involves nucleophilic attack of the exocyclic amine nitrogen of deoxyquanosine in position 2 (N2) on the electrophilic quinone carbon. Different types of DNA adducts were characterized by spectral analysis [8]

Gallate esters are used as synthetic antioxidants. Despite their presumed low toxicity, many authors were reported that some of linear alkyl gallates had many adverse effects. Propyl gallate was positive in in vitro chromosomal aberration tests [10,11], and was both positive and negative in the sister-

chromatid test [11]. The effects of propyl gallate on carcinogenesis and mutagenesis have been reported to be both enhancing and suppressing [12].

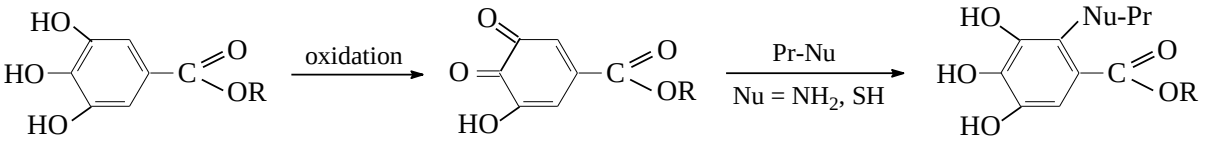
The adverse effects of gallates may be related to their antioxidizing potential. For example, when propyl gallate (PG) is autoxidized in the test media of CHO cells in the absence of metabolic activation, it is converted via a PG radical into PG dimer and finally into ellagic acid [13]. The authors established that the medium was changed from clear red to dark brown. But when PG is metabolized in the presence of S9 fraction, it is converted mainly to gallic acid which is also autoxidized as indicated by the changing of color medium (Scheme 2).



It was also found that the oxidative enzymes superoxide dismutase and superoxide dismutase plus catalase increased propyl gallate cytotoxicity [13].

During the oxidation of gallic acid, the consumption of oxygen was higher than in the case of propyl gallate oxidation. It was observed that intra- and extra-cellular H2O2 was generated by gallic acid autoxidation, and that the H2O2 could play a role in its toxic effects [13].

Bearing in mind the presence of catechol moiety in gallates and their dimers, they are able to be converted to Michael acceptors by abiotic and/or enzymatic oxidation to the corresponding quinoid structures [13,14]. Upon oxidation to an o-quinone, a Michael-type addition reaction could take place as shown in Scheme 3.

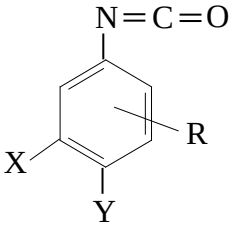


The reactivity of gallate esters toward proteins depends on the relative elongation of alkyl side-chains which determine their hydrophobicity. It was established that butyl gallate, octyl gallate and dodecyl gallate are more cytotoxic than propyl, ethyl, and methyl gallate [13].

Set of chemicals used for profile development	Hydroxylated Phenols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.

References	1. Ishidate, M. Jr., Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 2. Bindoli, A., Rigobello, M.P., Deeble, D.J., Biochemical and toxicological properties of the oxidation products of catecholamins. *Free Radic. Biol. Med*., **1992**, 13(4), 391-405. 3. Gaspar, J., Rodrigues, A., Laires, A., Silva, F., Costa, S., Monteiro, M.J., Monteiro, C., Rueff, J., On the mechanisms of genotoxicity and metabolism of quercetin. *Mutagenesis*, **1994**, 9(5), 445-449. 4. Brunmark, A., Cadenas, E., Redox and addition chemistry of quinoid compounds and its biological implications. *Free Radic. Biol. Med*., **1989**, 7(4), 435-477. 5. Bradley, M.O., Bhuyan, B., Francis, M.C., Langenbach, R., Peterson, A., Huberman, E., Mutagenesis by chemical agents in V79 Chinese hamster cells: A review and analysis of the literature. *Mutat. Res.*, **1981**, 87(2), 81-142. 6. Aptula, A.O., Patlewicz, G., Roberts, D.W., Skin sensitization: reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol.*, **2005**, 18(9), 1420-1426. 7. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol.*, **2007**, 20(1), 44-60. 8. Zhao, S., Narang, A., Gierthy, J., Eadon, G., Detection and characterization of DNA adducts formed from metabolites of the fungicite *ortho*-phenylphenol. *J. Agric. Food Chem*., **2002**, 50(11), 3351-3358. 9. Takumi-Kobayashi, A., Ogura, R., Morita, O., Nishiyama, N., Kasamatsu, T., Involvement of hydrogen peroxide in chromosomal aberrations induced by green tea catechins in vitro and implications for risk assessment. *Mutat. Res*., **2008**, 657(1), 13-18. 10. Ishidate, M. Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol*., **1984**, 22(8), 623-636. 11. Gulati, D.K., Witt, K., Anderson, B., Zeiger, E., Shelby, M.D., Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. III. Results with 27 chemicals. *Environ. Mol. Mutagen*., **1989**, 13(2), 133-193. 12. Tayama, S., Nakagawa, Y., Cytogenic effects of propyl gallate in CHO-K1 cells. *Mutat. Res*., **2001**, 498(1-2), 117-

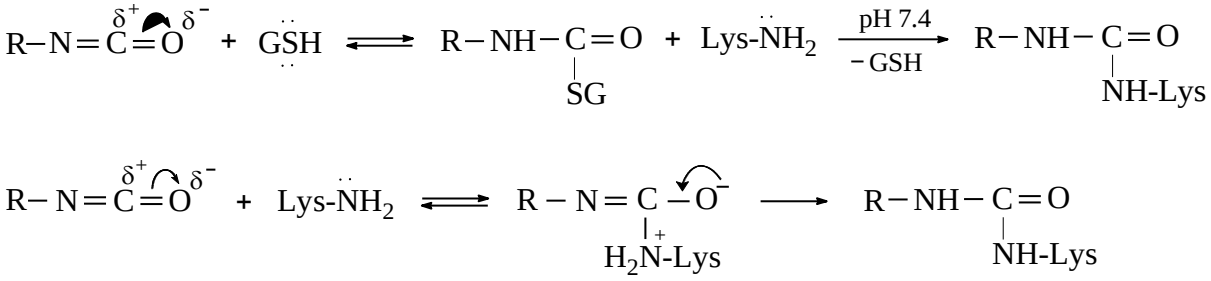
	127. 13. Nakagawa, Y., Tayama, S., Cytotoxicity of propyl gallate and related compounds in rat hepatocytes. *Arch. Toxicol*., **1995**, 69(3), 204-208. 14. Patlewicz, G., Roberts, D.W., Uriarte, E., Skin sensitization: A comparison of reactivity schemes for the prediction skin sensitization potential. *Chem. Res. Toxicol*., **2008**, 21(2), 521-541.

Individual profile/alert	
Name	Isocyanates and Diisocyanates
Type of profile	Structural alert
Description/applicability domain	Isocyanates are regarded as chemicals containing one, two or more isocyanate (-N=C=O) functional groups. They can be classified as aliphatic (acyclic or alicyclic) isocyanates and the aromatic isocyanates. The main structures of aliphatic and aromatic iso- and diisocyanates can be presented as follows: $(Csp^3)n-N=C=O$ where n = 1–4 at Csp^3 (acyclic) and n = 5–7 at Csp^3 (alicyclic). Arene iso- and diisocyanates structure for the compounds with positive effect in the chromosomal aberration test without metabolic activation is given below:  R = H, Csp3(acy); X = H, Csp2(aryl), N = C = O group; Y = H, Csp2(aryl).
Mechanism	Acylation, Acyl transfer via nucleophilic addition reaction
The electrophilic isocyanate moiety of iso- and diisocyanates is capable of undergoing nucleophilic addition with a variety of active hydrogen species including amines, alcohols, phenols and thiols [2]. The isocyanates can also react with water to form amines. In addition, the isocyanates may react reversibly with sulfhydryl groups of GSH, and, in the presence of appropriate nucleophiles, GSH-isocyanate adducts could react further to yield transcarbamoylating products [8]. These reversible thiocarbamates might shelter iso- and diisocyanates from hydrolysis thereby allowing further penetration into the body and increasing toxicity and allergenicity under physiological conditions [10]. The preferred reaction of isocyanates with GSH and their subsequent transfer to nucleophilic sites of peptides and proteins is favored under physiological conditions, i.e. at pH ~ 7.2-7.4. It is well known that carbamoylated organic isocyanates are stable at acidic pH conditions but not at	

physiological pH [9].

On the other hand, iso- and diisocyanates were observed to react mainly with primary amines such as the N-terminal α-NH2 of valine and the ε-NH2 of the side chain of lysine residues [2, 9]. The N-terminal groups of peptides and proteins were found to react about 100 times faster than ε-amino groups of lysine. Albumin has been identified as a major reaction target for TDI, MDI and HDI in vivo [10, 11]. TDI, conjugated to albumin reactive lysine residues, results in stable, covalently bonded species.

Thus, as highly reactive electrophiles, iso- and diisocyanates can readily undergo nucleophilic addition reactions (AN) with GSH and the adducts formed can participate in carbamoylation of protein amines. It may also be assumed the direct carbamoylation of protein amines to occur (Scheme 1).



The nature of the substituents will affect the reactivity of isocyanate group. MDI and TDI react with a maximum of 20 and 37 residues of human albumin, respectively, i.e. MDI is less reactive than TDI. These results cannot be explained on the basis of simple sterics or hydrophobicity, but rather on the basis of increased reactivity of one TDI isocyanate moiety due to electron withdrawing character of the second isocyanate moiety. Furthermore, p-tolyl isocyanate, a structural monoisocyanate analog of TDI, showed similar reactivity to MDI, rather than TDI. The electron withdrawing character of the second N=C=O group on the aromatic ring of TDI significantly increases its reactivity toward nucleophilic sites of proteins. In contrast, the reactivity of the isocyanate functional group(s) on MDI is lower because the p-[(4-isocyanatophenyl)methyl] substituent is less electron-withdrawing than isocyanate group itself. p-Tolyl isocyanate lacks the second electron-withdrawing functional group and its reactivity toward albumin more closely resembles that of MDI [11].

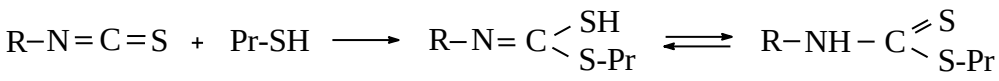
Set of chemicals used for profile development	Isocyanates and Diisocyanates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. A. Pronk, L. Preller, M. Raulf-Heimsoth, I.C.L. Lonkers, J.-W. Lammers, I.M. Wouters, G. Doekes, A.V. Wisnewski, D. Heederik, Respiratory symptoms, sensitization, and exposure-response relationships in spray painters exposed to isocyanates. *Am. J. Respir. Crit. Care Med.*, **2007**, 176(11), 1090-1097. 2. J.M. Hettick, T.B. Ruwona, P.D. Siegel, Structural elucidation of isocyanate-peptide adducts using tandem mass spectrometry.

	J. Am. Soc. Mass Spectrom., **2009**, 20(8), 1567-1575. 3. A.V. Wisnewski, L. Hu, E. Robinson, J. Liu, C.A. Redlich, C.A. Herrick, Immune sensitization to methylene diphenyl diisocyanate (MDI) resulting from skin exposure: albumin as a carrier protein connecting skin exposure to subsequent respiratory responses. *J. Occup. Med. Toxicol.*, **2011**, 6(6), 1-12. 4. J.M. Hettick, P.D. Siegel, B.J. Green, J. Liu, A.V. Wisnewski, Vapor conjugation of toluene diisocyanate to specific lysines of human albumin. *Anal. Biochem.*, **2012**, 421(2),706-711. 5. M.D. Shelby, J.W. Allen, W.J. Caspary, S. Haworth, J. Ivett, A. Kligerman, C.A. Luke, J.M. Mason, B. Myhr, R.R. Tice, R. Valencia, E. Zeiger, Results of *in vitro* and *in vivo* genetic toxicity tests on methyl isocyanate. *Environ. Health Perspect.*, **1987**, 72, 183-187. 6. J. Mäki-Paakkanen, H. Norppa, Chromosome aberrations and sister-chromatid exchanges induced by technical grade toluene diisocyanate and methylenediphenyl diisocyanate in cultured human lymphocytes. *Toxicol. Lett.*, **1987**, 36(1), 37-43. 7. K. Seel, U. Walber, B. Herbold, R. Kopp, Chemical behaviour of seven aromatic diisocyanates (toluene diisocyanates and diphenylmethane diisocyanates) under in vitro conditions in relationship to their results in the Salmonella/microsome test. *Mutat. Res.*, **1999**, 438(2), 109-123. 8. B.W. Day, R. Jin, D.M. Basalyga, J.A. Kramarik, M.H. Karol, Formation, solvolysis, and transcarbamoylation reactions of bis(*S*-glutathionyl) adducts of 2,4- and 2,6-diisocyanatotoluene. *Chem. Res. Toxicol.*, **1997**, 10(4), 424-431. 9. J. Mráz, Š. Boušková, 2,4-Toluenediisocyanate and hexamethylenediisocyanate adducts with blood proteins: assessment of reactivity of amino acid residues in vitro. *Chem.-Biol. Interact.*, **1999**, 117(2), 173-186. 10. A.V. Wisnewski, J.M. Hettick, P.D. Siegel, Toluene diisocyanate reactivity with glutathione across a vapor/liquid interface and subsequent transcarbamoylation of human albumin. *Chem. Res. Toxicol.*, **2011**, 24(10), 1686-1693. 11. J.M. Hettick, P.D. Siegel, Comparative analysis of aromatic diisocyanate conjugation to human albumin utilizing multiplexed tandem mass spectrometry. *Int. J. Mass Spectrom.*, **2012**, 309(1), 168-175.

Individual profile/alert	
Name	Isothiocyanates
Type of profile	Structural alert
Description/applicability	R—N═C═S

domain	where R can be Csp3 (alkyl, cycloalkyl), Csp2 (aryl), excluding vinyl group.
Mechanism	Acylation, Acyl transfer via nucleophilic addition reaction

Isothiocyanates may react with nucleophilic amino acid residues in proteins including thiol-containing cysteine, amine-containing lysines, arginine, proline and hydroxyl-containing serines, threonine and tyrosine. Among these sites, cysteines, especially the ionized forms (thiolate) represent the most likely binding sites of ITCs [7, 9]. The carbon atom of the isothiocyanate moiety is highly electrophilic and reacts with biological nucleophiles and especially with protein thiols as presented in Scheme 1.

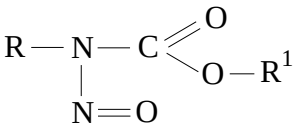


protein thiocarbamoylation

The genotoxicity of the ITCs should be carefully considered. Some but not all ITCs actually possess a genotoxic activity. Dietary consumption levels of ITCs appear to be several orders of magnitude lower than the doses used in the genotoxicity studies and thus it is highly unlikely that such toxicities would occur in humans. While there is a value in elucidating the genotoxic effects of ITCs on the in vitro cell systems, there is a need to examine the genotoxic effects of more ITCs in vivo, not only for some of them such as methyl isothiocyanate, allyl isothiocyanate, benzyl isothiocyanate and phenethyl isothiocyanate [4].

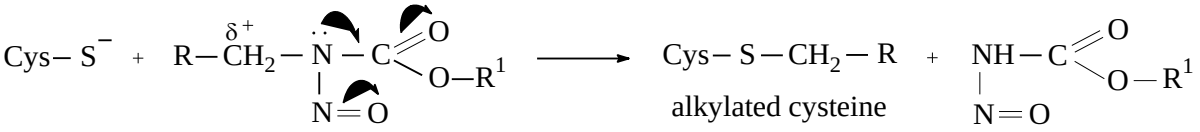
Set of chemicals used for profile development	Isothiocyanates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. F. Kassie, B. Pool-Zobel, W. Parzefall, S. Knasmuller, Genotoxic effects of benzyl isothiocyanate, a natural chemopreventive agent. *Mutagenesis*, **1999**, 14(6), 595-603. 2. B.E. Cavell, S.S. Syed Alwi, A. Donlevy, G. Packham, Anti-angiogenic effects of dietary isothiocyanates: Mechanisms of action and implications for human health. *Biochem. Pharmacol*., **2011**, 81(3), 327-336. 3. Y. Peng, C. Bao-An, L. De-Long, Anticancer mechanisms and researchers of isothiocyanates. *Chin. J. Nat. Med*., **2008**, 6(5), 325-332. 4. C. Fimognary, E. Turrini, L. Ferruzzi, M. Lenzi, P. Hrelia, Natural isothiocyanates: Genotoxic potential *versus* chemoprevention. *Mutat. Res*., **2012**, 750(2), 107-131. 5. F. Kassie, S. Knasmuller, Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC). *Chem. Biol. Interact*., **2000**, 127(2), 173-180. 6. F. Kassie, B. Laky, E. Nobis, M. Kundi, S. Knasmuller, Genotoxic effects of methyl isothiocyanate. *Mutat. Res*., **2001**, 490(10, 1-9. 7. L. Mi, Z. Xiao, T.D. Veenstra, F.L. Chung, Proteomic identification of binding targets of isothiocyanates: A

perspective on techniques. *J. Proteomics*, **2011**, 74(7), 1036-1044.
8. Y. Zhang, E.C. Callaway, High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate. *Biochem. J.*, **2002**, 364(Pt 1), 301-307.
9. K.K. Brown, M.B. Hampton, Biological targets of isothiocyanates. *Biochim. Biophys. Acta*, **2011**, 1810(9), 888-894.

Individual profile/alert	
Name	N-Alkyl-N-nitrosocarbamates
Type of profile	Structural alert
Description/applicability domain	 R = (Csp3)n – acy at n ≥ 1, preferably linear or branched C1–C5 alkyl groups; R may also include an acyl group as C(=O)Csp3; R1 = (Csp3)n – acy at n ≥ 1; Csp2 (aryl), such as phenyl, alpha-naphthyl, 7-benzofuranyl, etc.
Mechanism	SN2, Protein alkylation via direct attack at the N-alkyl group SN2, Protein nitrosylation via direct attack at the nitroso group SN1 and SN2, DNA and protein alkylation via direct attack at carbonyl carbon atom and the formation of alkyldiazonium ion

- SN2, Protein alkylation via direct attack at the N-alkyl group

N-Nitrosocarbamates were found to react with cysteine and glutathione at room temperature and at neutral pH [7]. There appear to be different possibilities for the reaction of protein thiolate ion with N-alkyl-N-nitrosocarbamates [1,8]. Such a possibility is associated with direct attack of the cysteine nucleophile on the N-alkyl group which has an electrophilic carbon, resulting from p,π-conjugation between lone-pair electrons at nitrogen atom and π-electrons of N=O and C=O groups (Scheme 1).

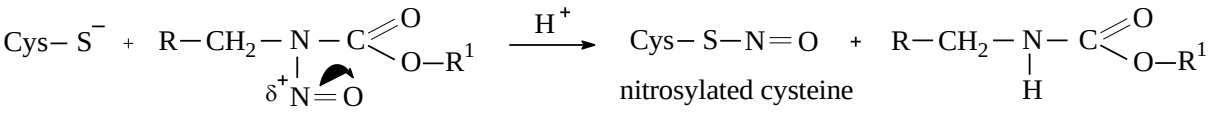


It was established that the relationship between the length of the N-alkyl chain and the mutagenicity

of N-nitrosonaphthylcarbamates was inversely proportional [6]. For example, an increase of the N-alkyl chain length from methyl to ethyl reduced the mutagenicity 5.2-fold, from ethyl to propyl 2.8-fold, and from propyl to butyl 1.4 fold.

- SN2, Protein nitrosylation via direct attack at the nitroso group

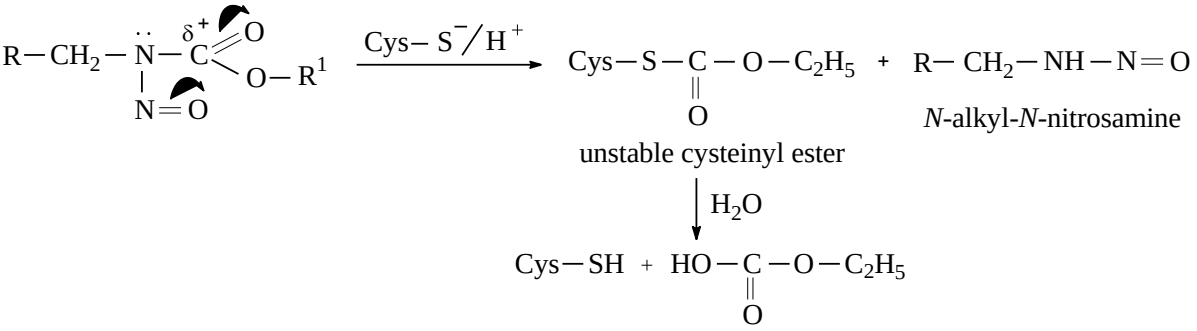
Another pathway involves nucleophilic attack of cysteine thiolate ion at the nitrogen of the nitroso group, leading to nitrosylated protein [1,8]. Thus, N-nitrosocarbamates can be used as N=O donors for the formation of S-nitrosothiols [1] (Scheme 2):

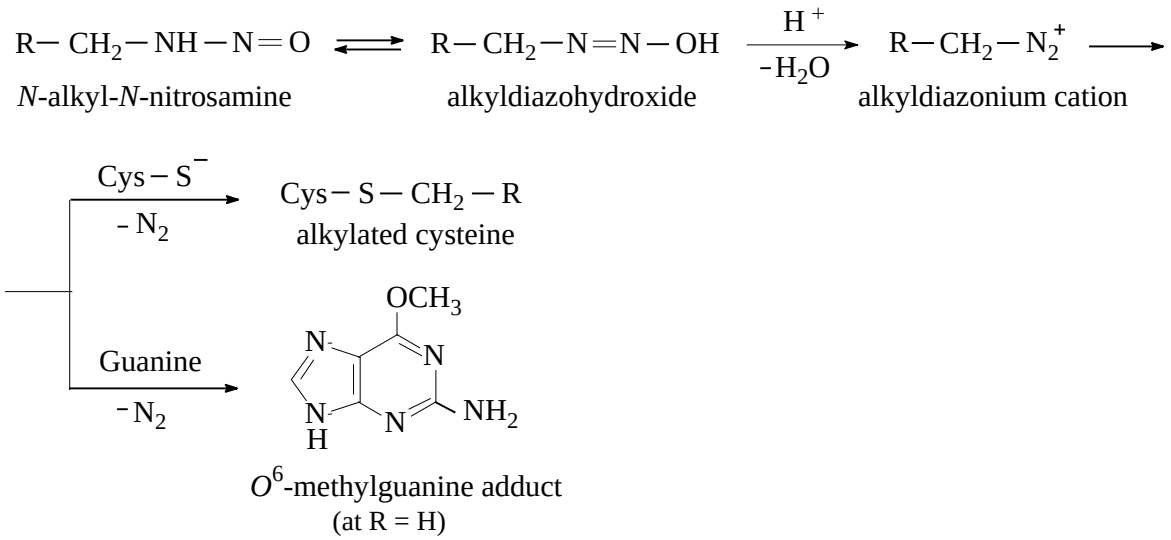


- SN1 and SN2, DNA and protein alkylation via direct attack at carbonyl carbon atom and the formation of alkyldiazonium ion

N-Alkyl-N-Nitrosocarbamates are potent direct acting mutagens and carcinogens and can be considered as SN1 and SN2 type alkylating agent [4,5]. Results from chromosome aberration and hprt gene mutation indicated the O6-methylguanine adduct (O6-MeG) is the major mutagenic base derivative formed in DNA on exposure of cells to DNA methylating agents. It is generally accepted that alkylation-induced cell killing is largely attributable to apoptosis and the O6-MeG acts as a trigger of this toxic response [5].

According to Schoental and Rive [7], in the presence of ionizable free thiol groups, N-alkyl-N-nitrosocarbamates decompose rapidly at neutral pH even in the dark, with evolution of nitrogen gas. The products formed with cysteine comprised the alkyl ester of substituted cysteine and N-alkyl-N-nitrosamine. The alkyl ester of cysteine was rather unstable and hydrolysed on standing at room temperature, even at neutral pH [7]. N-Alkyl-N-nitrosamine undergoes tautomerization to the corresponding alkyldiazohydroxide, which is able to form an alkyldiazonium ion as an ultimate electrophilic alkylating agent [8]. The corresponding reaction tranformations are shown in Scheme 3.

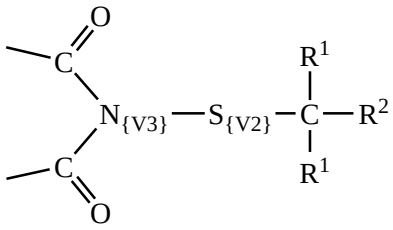
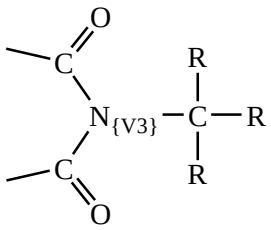
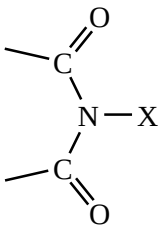


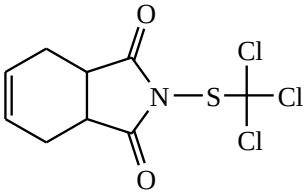


N-Alkyl-N-nitrosocarbamates as methylating agents are potent carcinogens that are mutagenic and cytotoxic towards bacteria and mammalian cells [2,5,9]. Their effects can be ascribed to an ability to modify DNA covalently.

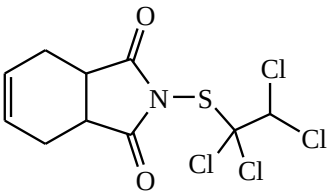
Set of chemicals used for profile development	N-Alkyl-N-nitrosocarbamates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Cai, T., Janczuk, A.J., Nitric oxide donors: chemical activities and biological applications. *Chem. Rev.*, **2002**, 102(4), 1091-1134. 2. Wang, T.C., Chiou, C.M., Chang, Y.L., Genetic toxicity of *N*-methylcarbamate insecticides and their *N*-nitroso derivatives. *Mutagenesis*, **1998**, 13(4), 405-408. 3. Lin, C.M., Wei, L.Y., Wang, T.C., The delayed genotoxic effect of *N*-nitroso *N*-propoxur insecticide in mammalian cells. *Food Chem. Toxicol.*, **2007**, 45(6), 928-934. 4. Wang, T.C., Chiou, J.M., Chang, Y.L., Hu, M.C., Genotoxicity of propoxur and its *N*-nitroso derivative in mammalian cells. *Carcinogenesis*, **1998**, 19(4), 623-629. 5. Yoon, J.Y., Oh, S.H., Yoo, S.M., Lee, S.J., Lee, H.S., Choi, S.J., Moon, C.K., Lee, B.H., N-Nitrosocarbofuran, but not carbofuran, induces apoptosis and cell cycle arrest in CHL cells. *Toxicology*, **2001**, 169(2), 153-161. 6. Eya, B.K., Talcott, R.E., Effect of *N*-alkyl chain length on the mutagenicity of *N*-nitrosated 1-naphthyl

	N-alkylcarbamates. *Environ. Mutagen*., **1980**, 2(3), 395-404. 7. Schoental, R., Rive, D.J., Interaction of N-alkyl-N-nitrosourethanes with thiols. *Biochem. J.*, **1965**, 97(2), 466-474. 8. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol*., **2007**, 20(1), 44-60. 9. Bignami, M., O'Driscoll, M., Aquilina, G., Karran, P., Unmusking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res*., **2000**, 462(2-3), 71-82.

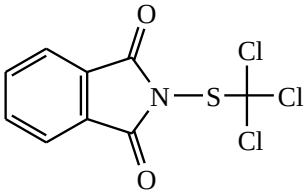
Individual profile/alert	
	N-Haloacylamides
Type of profile	Structural alert
Description/applicability domain	 R^1 = F, Cl, Br, I; R^2 = R^1 or $CH(R^1)_2$   R = F, Cl, Br, I; X = F, Cl, Br, I;
Mechanism	Acylation, Direct acylation involving a leaving group
Agricultural fungicides as captan, captafol and folpet were studied in vitro for induction of chromosomal damage in various types of Chinese hamster cells [1-3].	



Captan

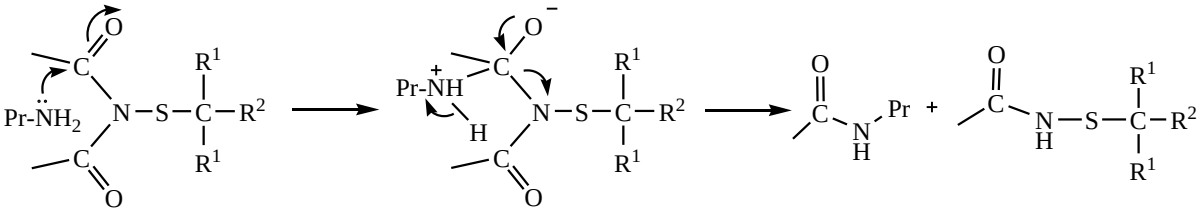


Captafol



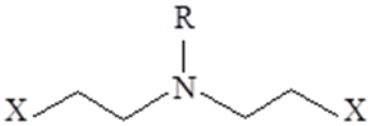
Folpet

The test results show positive responses in the absence of S9 and little or no activity when S9 is present [1]. These imides as acylating agents in vitro are considered to be hard electrophiles and could bind to Lysine residues in proteins (Pr-NH2) [4]. For imide structures such as RCO.NYCOR1 and YNHCOR1 which are not sufficiently acidic, when Y = H, but becomes more acidic and reactive, when Y is a strongly electronegative group (for example trichloromethylthio functional group SCCl3) an acylation mechanism is shown below:

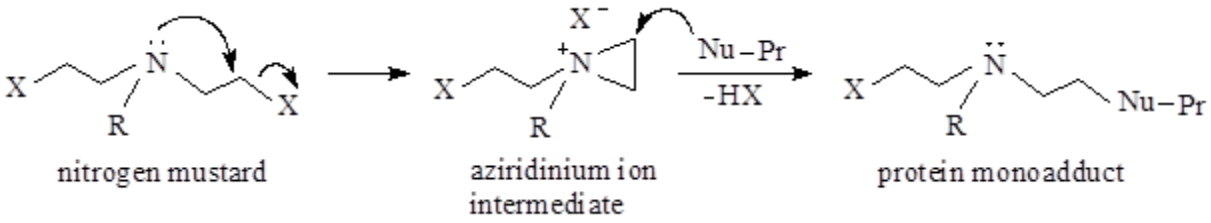


R1 = F, Cl, Br, I; R2 = R1 or CH(R1)2;

Set of chemicals used for profile development	N-Haloacylamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Arce, G.T., Gordon, E.B., Cohen, S.M., Singh, P., Genetic toxicology of folpet and captan. Crit. Rev. Toxicol., 2010, 40(6), 546–574. 2. Tezuka, H., Ando, N., Suzuki, R., Terahata, M., Moriya, M., Shirasu, Y., Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. Mutat. Res., 1980, 78(2), 177–191. 3. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutat. Res., 1988, 195(2), 151–213. 4. Aptula, A.O., Roberts, D.W., Mechanistic applicability domains for nonanimal-based prediction of toxicological end points: general principles and application to reactive toxicity. Chem. Res. Toxicol., 2006, 19(8), 1097–1105.

Individual profile/alert	
Name	Nitrogen mustards
Type of profile	Structural alert
Description/applicability domain	 X = Cl, Br, I; R = any atom/group
Mechanism	SN2, Nucleophilic ring opening on aziridinium ion intermediate of N-mustards

N-mustards are typical alkylating agents. In aqueous conditions they spontaneously form reactive aziridinium ion that can covalently bind to nucleophilic sites within proteins and other biomolecules. The initial formation of a reactive aziridinium intermediate is followed by covalent binding to protein-nucleophilic sites, such as cysteine thiols, lysine amines and histidine groups. A ring opening SN2-mechanism is shown in Scheme 1 [3,4].

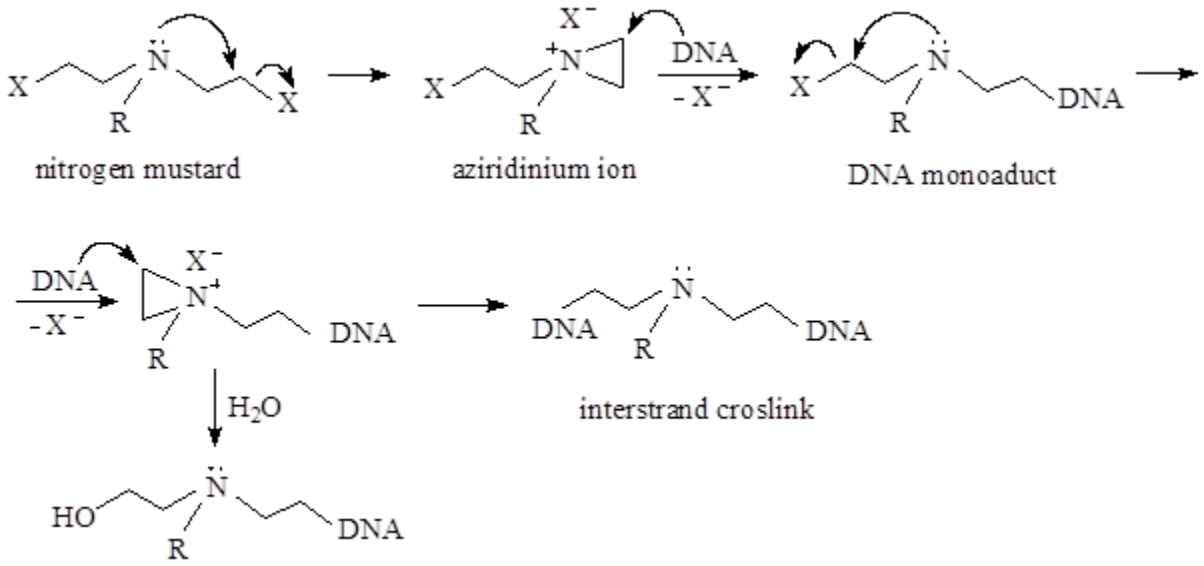


Nu = -SH (Cysteine residue), -NH_2 (Lysine residue), -NH (Histidine residue)

In the same manner N-mustard–protein monoaduct is able to bind another molecule of protein-nucleophile.

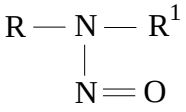
Nitrogen mustards are mutagenic in cultured mammalian cells. The aziridinium group can alkylate DNA mainly by attacking the N-7 nucleophilic center on the guanine base. The interstrand crosslinks can arise from the covalent binding of the alkylating agent to both strands of the double helix and it is considered to be the most toxic lesion. Monoalkylated adducts and intrastrand crosslinked products may be formed when the alkylating agent is smaller than the width of the minor groove of the DNA strand, while the interstrand product may be formed when the alkylating agent is longer than the width of the minor groove of the DNA strand.

Thus, the type of product that is formed depends on the nucleophiles present in the system as well as on the structure of the alkylating agent and DNA. The mechanism of DNA alkylation by N-mustards is shown in Scheme 2 [3].



Set of chemicals used for profile development	Nitrogen mustards
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. NAC/AEGL Committee. Acute exposure guideline levels for nitrogen mustards, **2007**, Interim1:11, p. 5. 2. R. Benigni, C. Bossa, Structure alerts for carcinogenicity, and the *Salmonella* assay system: A novel insight through the chemical relational databases technology. *Mutat. Res.*, **2008**, 659(3), 248-261. 3. V.R. Thompson, A.P. DeCaprio, Covalent adduction of nitrogen mustards to model protein nucleophiles. *Chem. Res. Toxicol.*, **2013**, 26(8), 1263-1271. 4. D. Florea-Wang, Reactions of chlorambucil and its main metabolite, phenylacetic acid mustard, with 2'-deoxyribonucleosides and calf thymus DNA. PhD Thesis, University of Turku, **2009**, 401A, pp. 14, 18.

Individual profile/alert	
Name	N-Nitrosoamine derivatives
Type of profile	Structural alert

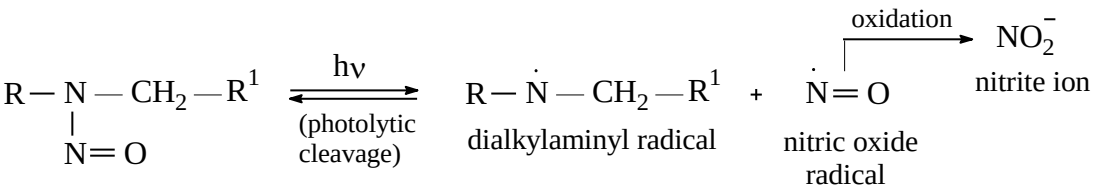
Description/applicability domain	 where: R = (Csp3)n acy at n ≥ 1; (Csp3)nacy–O– at n ≥ 1, preferably linear or branched short-chain alkyl groups with up to five carbons; (Csp3)acy–NH–; >Nacy–C(=O)–, etc. R1 = (Csp3)nacy at n ≥ 1, linear or branched short-chain alkyl groups with up to five carbons; (Csp3)nacy–O– at n ≥ 1; Csp2(aryl), such as phenyl, 1,3,5-triazinyl, etc.
Mechanism	S_N1 and S_N2 , DNA and protein alkylation via the formation of alkyldiazonium ion

The activation of N-nitrosoamine derivatives in the in vivo and in vitro systems is most frequently attributed to the cytochrome P450-dependent mixed function oxidases [2,7-9]. It proceeds via the formation of short-lived alpha-hydroxynitrosamines, which decompose into diazohydroxides and aldehydes [2]. However, the activation of nitrosamines into electrophilic or mutagenic species in cells, lacking CYP-450 activity (e.g., CHL and CHO cells), requires the action of either hydroxyl radicals or ultraviolet light [3,6,10-12].

For example, the irradiation of nitrosamines with near-UVA light results in homolytic N–N(O) bond cleavage, leading to the initial formation of nitric oxide radical (˙N=O) and extremely short-lived dialkylaminyl radical (R(R1CH2)N˙) (Scheme 1a). Subsequently, carbon-centered radicals are generated by rearrangement of the initially formed aminyl radical via homolytic cleavage of the α–C-H bond or by its disproportionation involving parent N-nitrosalkylamine [7,10] (Scheme 1b). Moreover, during the photolysis of nitrosamines, superoxide anions may be formed through reduction of oxygen by alkylaminyl radicals and may thus contribute to the production of α–carbon-centered free radicals [11].

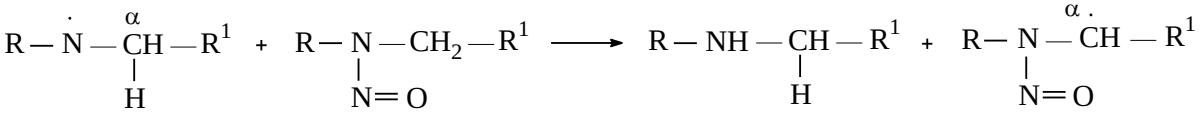
Scheme 1

a) homolytic cleavage of N–N(O) bond under the influence of ultraviolet light, sunlight, etc. and formation of a dialkylaminyl radical (the so called spontaneous denitrosation):



b) transformation of the short-lived dialkylaminyl radical to the corresponding alpha–carbon-centered alkylamine radical and alpha–carbon-centered N-nitrosamine radical:

dialkylaminyl radical — (rearrangement) → α–carbon-centered alkylamine radical

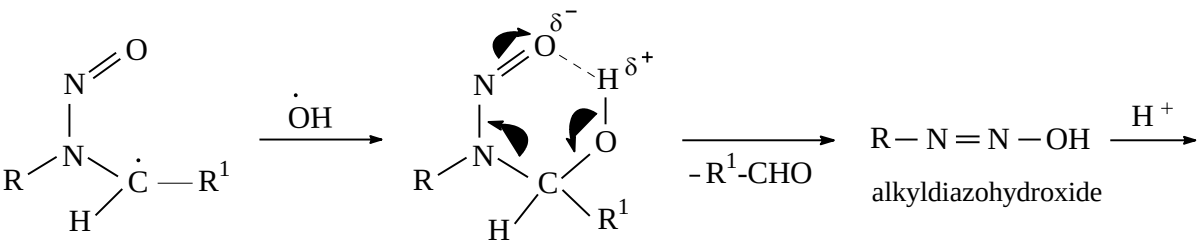


dialkylaminyl radical + *N*-nitrosodialkylamine → *N*,*N*-dialkylamine + α–carbon-centered *N*-nitrosamine radical

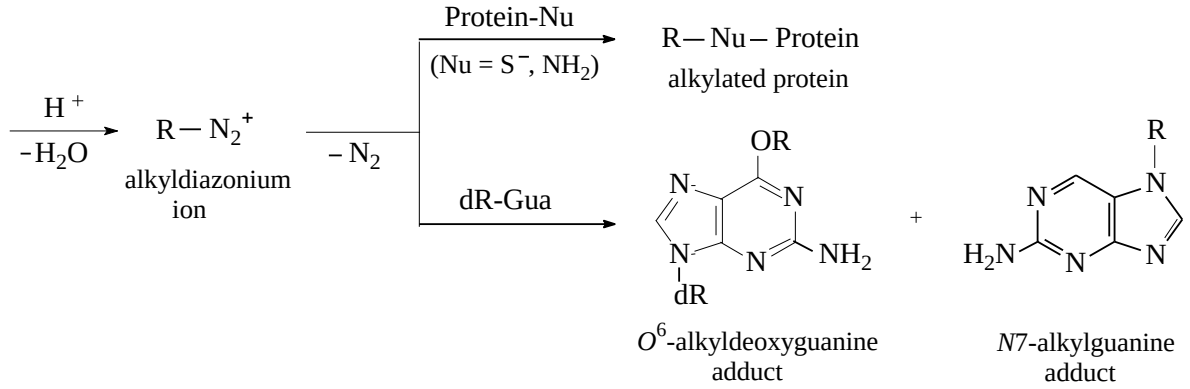
alpha–Carbon-centered N-nitrosamine radicals may undergo further transformations leading to the formation of the active alkylating species.

The rate-limiting step in the metabolism of N-nitrosamines involves the cleavage of alpha–C-H bond, followed by the interaction of alpha–carbon-centered radicals with reactive oxygen species such as ˙OH, H2O2, etc. (in the absence of CYP-450 activation). The hydroxylation usually occurs at longer alkyl chains or at benzyl moiety. The resulting short-lived α–hydroxylated derivatives are able to decompose into alkyldiazohydroxydes and aldehydes [1,10,11]. This decomposition may be achieved by a concerted pathway or by two-step mechanism [13]. In the case of concerted mechanism the reaction is able to be enhanced by the formation of an intramolecular hydrogen bond, as shown in Scheme 2.

The active alkylating species is believed to be the alkyldiazonium ion, formed after dissociation of the alkyldiazohydroxyde [5,7,13]. The most likely mechanism is associated with direct attack of alkyldiazonium ion on the nucleophilic sites of DNA and protein molecules [1]. The results indicate that the more nucleophilic sites on the DNA (i.e., N7-position of guanine) react with primary diazonium ions via an SN2 mechanism while the less nucleophilic sites (i.e., O6-position of guanine) react via an SN1 mechanism with the secondary carbenium ions produced from decomposition and subsequent rearrangement of the primary diazonium ion [14] (Scheme 2).



carbon-centered *N*-nitrosamine radical — ˙OH → short-lived *α*-hydroxy-*N*-nitrosamine derivative — -R¹-CHO → alkyldiazohydroxide — H⁺

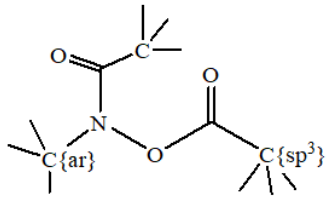
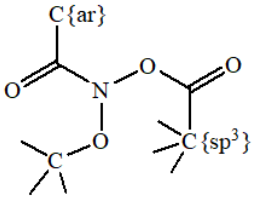


According to Arimoto-Kobayashi et al. [6], alkylation of DNA with UVA activated N-nitrosodimethylamine leads to the formation of N7-methylguanine adduct that is 40–70 times more than the respective O6-methyldeoxyguanine adduct.

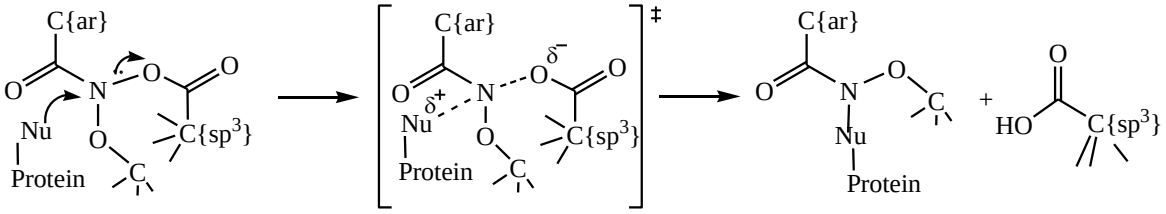
Overall, N-nitrosodialkylamines can cause chromosomal aberrations on irradiation with near-UV light. Bearing in mind that the irradiation used by many authors is much weaker than that of the sunlight [3,6], positive clastogenic effects could be expected for N-nitrosodialkylamines, which are not exposed to activation by CYP-450 enzymes.

Set of chemicals used for profile development	N-Nitrosoamine derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Guttenplan, J.B., *N*-Nitrosamines: bacterial mutagenesis and in vitro metabolism. *Mutat. Res*., **1987**, 186(2), 81-134. 2. Mochizuki, M., Anjo, T., Okada, M., Isolation and characterization of *N*-alkyl-*N*-(hydroxymethyl)nitrosamines from *N*-alkyl-*N*-(hydroperoxymethyl)nitrosamines by deoxygenation. *Tetrahedron Lett*., **1980**, 21(38), 3693-3696. 3. Yamashita, Y., Sumi, N., Arimoto, S., Hayatsu H., Synergistic action of N-nitrosodialkyl-amines and near-UV in the induction of chromosome aberrations in Chinese hamster lung fibroblasts in vitro. *Mutat. Res*., **1995**, 348(4), 163-168. 4. Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Cai, T., Janczuk, A.J., Nitric oxide donors: chemical activities and biological applications. *Chem. Rev*., **2002**, 102(4), 1091-1134.

5. Liu, Y.X., Guttenplan, J.B., Mutational specificities of *N*-nitrosoamines in a host-mediated assay: comparison with direct-acting *N*-nitroso compounds in vitro and an approach to deducing the nature of ultimate mutagens in vivo. *Mol. Carcinog*., **1992**, 6(4), 232-237.
6. Arimoto-Kobayashi, S., Kaji, K., Sweetman, G.M., Hayatsu, H., Mutation and formation of methyl- and hydroxylguanine adducts in DNA caused by *N*-nitrosodimethylamine and *N*-nitrosodiethylamine with UVA irradiation. *Carcinogenesis*, **1997**, 18(12), 2429-2433.
7. Hebels, D.G., Briedé, J.J., Khampang, R., Kleinjans, J.C., de Kok, T.M., Radical mechanisms in nitrosamine- and nitrosamide-induced whole-genome gene expression modulations in Caco-2 cells. *Toxicol. Sci*., **2010**, 116(1), 194-205.
8. Singer, B., Kuśmierek, J.T., Chemical mutagenesis. *Annu. Rev. Biochem*., **1982**, 51, 655-693.
9. Yoo, J.S., Yang, C.S., Enzyme specificity in the metabolic activation of *N*-nitrosodimethylamine to a mutagen for Chinese hamster V79 cells. *Cancer Res*., **1985**, 45(11 Pt 1), 5569-5574.
10. Grover, T.A., Ramseyer, J.A., Piette, L.H., Photolysis of nitrosamines and nitrosamides at neutral pH: A spin-trap study. *Free Radic. Biol. Med*., **1987**, 3(1), 27-32.
11. Bartsch, H., Hietanen, E., Malaveille, C., Carcinogenic nitrosamines: free radical aspects of their action. *Free Radic. Biol. Med*., **1989**, 7(6), 637-644.
12. Fujiwara, M., Honda, Y., Inoue, H., Hayatsu, H., Arimoto, S., Mutations and oxidative DNA damage in phage M13mp2 exposed to *N*-nitrosomorpholine plus near-ultraviolet light. *Carcinogenesis*, **1996**, 17(2), 213-218.
13. Andreozzi, P., Klopman, G., Hopfinger, A.J., Theoretical study of N-nitrosamines and their presumed proximate carcinogens. *Cancer Biochem. Biophys*., **1980**, 4(4), 209-220.
14. Spratt, T.E., Zydowsky, T.M., Floss, H.G., Stereochemistry of the *in vitro* and *in vivo* methylation of DNA by (*R*)- and (*S*)-*N*-[$^{2}H_{1}$,^{3}H]methyl-*N*-nitrosourea and (*R*)- and (*S*)-*N*-nitroso-*N*-[$^{2}H^{1}$,^{3}H]methyl-*N*-methylamine. *Chem. Res. Toxicol*., **1997**, 10(12), 1412-1419.

Individual profile/alert	
	N-Oxicarbonyl amides, N-Acyloxy-N-alkoxyamides
Type of profile	Structural alert
Description/applicability domain	  *N*-Oxycarbonyl amides *N*-Acyloxy-*N*-alkoxyamides
Mechanism	S_N2, Nucleophilic substitution at a Nitrogen atom

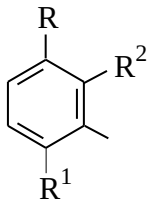
It was found that N-acyloxy-N-alkoxyamides are anomeric amide electrophiles that are capable of direct interaction with DNA, inducing DNA damage [3, 4]. The term "anomeric" is rather used to describe all systems, bearing two heteroatoms bound to the nitrogen that are thus capable of displaying anomeric effects. Amides, which are geminally substituted with two heteroatoms at the central nitrogen atom can support anomeric effects in much the same way as their carbon-containing analogues, e.g., acetals and aminals [4]. Based on their chemical electrophilicity in SN2 reactions with nitrogen and sulfur nucleophiles such as ammonia, N-methylaniline [3,5] and alkylthiols [4, 6], it is expertly assumed that N-acyloxy-N-alkoxyamides will be active in chromosomal aberration assay via covalent binding to lysine and cysteine residues in the chromosomal proteins. A common SN2 mechanism for N-acyloxy-N-alkoxyamides with nucleophilic groups of proteins is shown below:

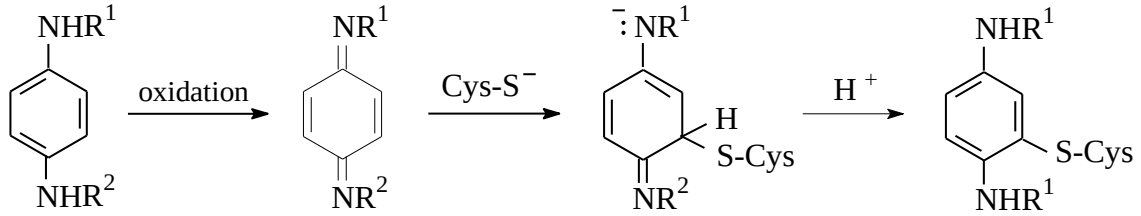


Nu = NH_2, SH

Set of chemicals used for profile development	N-Oxicarbonyl amides, N-Acyloxy-N-alkoxyamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. Chem. Res. Toxicol., 2007, 20(1), 44–60.

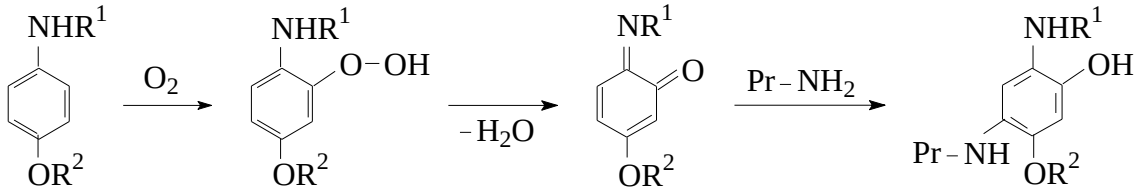
	2. Tates, A.D., Kriek, E., Induction of chromosomal aberrations and sister-chromatid exchanges in Chinese hamster cells in vitro by some proximate and ultimate carcinogenic arylamide derivatives. Mutat. Res., 1981, 88(4), 397–410. 3. Banks, T.M., Bonin, A.M., Glover, S.A., Prakash, A.S., Mutagenicity and DNA damage studies of N-acyloxy-N-alkoxyamides--the role of electrophilic nitrogen. Org. Biomol. Chem., 2003, 1(13), 2238–2246. 4. Glover, S.A., Adams, M., Reaction of N-acyloxy-N-alkoxyamides with biological thiols. Aust. J. Chem., 2011, 64(4), 443–453. 5. Glover, S.A., Anomeric amides—Structure, properties and reactivity. Tetrahedron, 1998, 54(26), 7229–7271. 6. Glover, S.A., Rosser, A.A., Heteroatom substitution at amide nitrogen-resonance reduction and HERON reactions of anomeric amides. Molecules, 2018, 23(11), pii: E2834. doi: 10.3390/molecules23112834.

Individual profile/alert	
Name	N-Substituted Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	 Where: R = NH2, NH-Csp3 (acy), NH-Csp2 (aryl) – fused or non-fused, O-Csp3 (acy); R1 = H, NH2, NH-Csp2(aryl); R2 = H, Csp2 (scy) – fused.
Mechanism	AN2, Michael addition to the quinoid type structures such as quinone-diimines, quinone-imines, etc.
N-Substituted para-phenylenediamines, alkoxyanilines and N-alkylated anilines are susceptible toward oxidation in the presence of air oxygen or peroxidases in the cellular systems [5,6]. For example, N,N'-diphenylamines can be oxidized to the corresponding electrophilic intermediates quinone-diimines. Then the quinone-diimines can bind to proteins mainly by the attack of protein-associated thiolate anion at a ring carbon atom (Scheme 1) [7].	



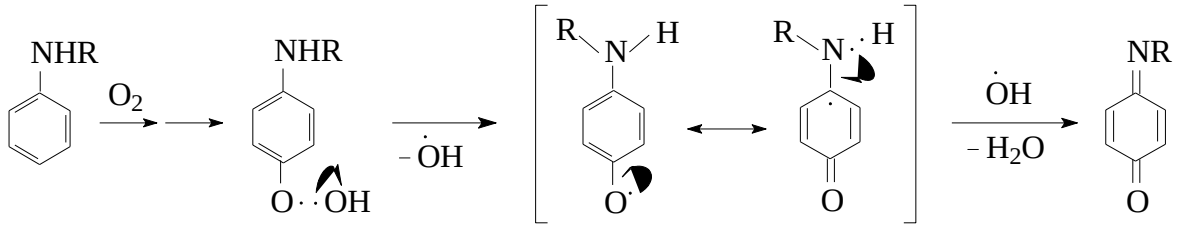
R^1 = H, Csp^3 (*iso*-propyl, 1,3-dimethylbutyl), Csp^2 (phenyl, 2-naphthyl); R^2 = H, Csp^2 (phenyl, 2-naphthyl).

On the other hand, the clastogenicity of para-alkoxyanilines could be explained by the possibility to undergo perhydroxylation which is analogous to that of meta-phenylenediamines [7]. The perhydroxylation is able to occur mainly in ortho-position toward amino group due to the strong stabilizing effect of nitrogen atom in the formation of free radicals. The ortho-quinone imine formed undergoes Michael-type addition reaction (Scheme 2).

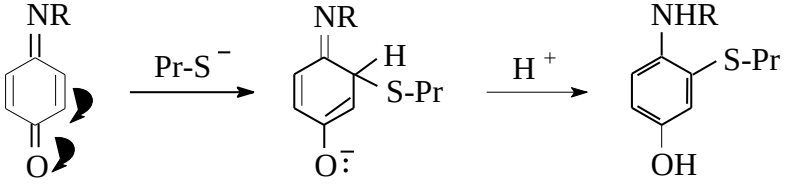


R1 = C6H5, p-C6H4OCH3; R2 = –CH3, –C2H5, –iso-C3H7, etc.

N-Alkylanilines such as N-methyl- and N-ethylaniline are susceptible to oxidation by a variety of reagents, including the oxygen in air [7-9]. The oxidation to the corresponding benzoquinone imine occurs in the ring preferably in para-position relative to the amino group. The possible mechanism is analogous to that of the oxidation of ring-alkylated anilines [8] and is associated with the preliminary formation of a phenyl hydroperoxide as shown in Scheme 3.



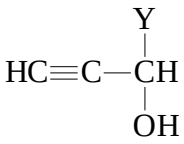
N-Alkylbenzoquinone imine thus obtained is able to undergo Michael-type addition reaction involving protein nucleophiles such as Pr-S⁻ or Pr-NH2 (Scheme 4).



R = Csp3 (acy) - CH3, C2H5, etc.

N-Ethylaniline induced chromosomal aberrations (25.0%) after 6-h treatment without S9 mix at the highest concentration of 9.1 mM. According to Morita et al. [10], the chromosomal aberrations observed might be due to high toxicity. However, there is no supporting evidence to reduce the level of concern and the minimal concern for its clastogenicity still exists.	
Set of chemicals used for profile development	N-Substituted Aromatic Amines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. T. Sofuni, A. Matsuoka, M. Sawada, M. Ishidate Jr, E. Zeiger, M.D. Shelby, A comparison of chromosome aberration induction by 25 compounds tested by two Chinese hamster cell (CHL and CHO) systems in culture. Mutat. Res., 1990, 241(2), 175-213. 2. S.M. Galloway, M.J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A.D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B.H. Margolin, M.A. Resnick, B. Anderson, E. Zeiger, Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ. Mol. Mutagen., 1987, 10 (Suppl. 10), 1-175. 3. N-Isopropyl-N'-phenyl-1,4-benzenediamine, CAS No. 101-72-4. Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257-8523, Japan. 4. N-Ethylaniline, CAS No. 103-69-5. Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257, Japan. 5. M. Uchimiya, A.T. Stone, Reversible redox chemistry of quinones: Impact on biogeochemical cycles. Chemosphere, 2009, 77(4), 451-458. 6. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, Mechanistic applicability domain classification of a Local Lymph Node Assay dataset for skin sensitization. Chem. Res. Toxicol., 2007, 20(7), 1019-1030. 7. A.O. Aptula, S.J Enoch, D.W. Roberts, Chemical mechanisms for skin sensitization by aromatic compounds with hydroxyl and amino groups. Chem. Res. Toxicol., 2009, 22(9), 1541-1547. 8. D.W. Roberts, G. Patlewicz, S.D. Dimitrov, L.K. Low, A.O. Aptula, P.S. Kern, G.D. Dimitrova, M.I.H.

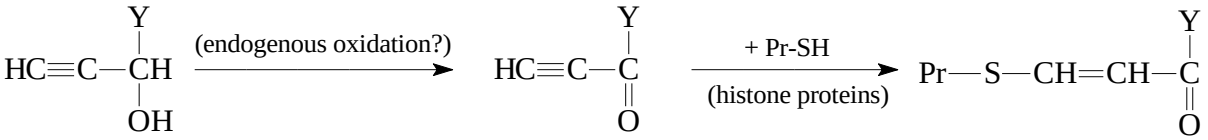
	Comber, R.D. Phillips, J. Niemelä, C. Madsen, E.B. Wedebye, P.T. Bailey, O.G. Mekenyan, TIMES-SS – A mechanistic evaluation of an external validation study using reaction chemistry principles. Chem. Res. Toxicol., 2007, 20(9), 1321-1330. 9. A. Brunmark, E. Cadenas, Redox and addition chemistry of quinoid compounds and its biological implications, Free Radic. Biol. Med., 1989, 7(4), 435-477. 10. T. Morita, M. Honma, K. Morikawa, Effect of reducing the top concentration used in the in vitro chromosomal aberration test in CHL cells on the evaluation of industrial chemical genotoxicity. Mutat. Res., 2012, 741(1-2), 32-56.

Individual profile/alert	
Name	Propargyl Alcohol Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is -H or -$(CH_2)_nH$ (n = 1 - 3)
Mechanism	A_N2, Nucleoophilic addition to alpha, beta - unsaturated carbonyl compounds

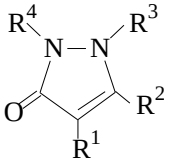
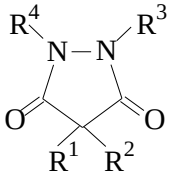
Propargyl alcohol was found to be clastogenic in vitro by inducing chromosomal aberrations in CHO cells with and without metabolic activation, while this chemical was not bacterial mutagen [1].

According to an evaluation report, in a chromosomal aberration (CA) test using CHO cells, cells collected 16 h following treatment with propargyl alcohol showed a small but statistically significant increase in chromosomal aberrations in the absence of metabolic activation. Although only the response at the highest dose was significantly higher than the control, the trend was positive. In the presence of exogenous metabolic activation, a larger, dose-related increase was induced. However, in cells after 10 h of treatment, there was no increase in CAs, either with or without metabolic activation [2].

One possible (and oversimplified) mechanistic scheme for in vitro bioactivation of propargyl alcohol and other short-chain derivatives, which could elicit clastogenicity by nucleophilic interaction with histone proteins, can be expressed as follows:



Set of chemicals used for profile development	Propargyl Alcohol Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Blakey, D. H., K.aus, R. bell, J. Bayley, G. R. Douglas, E. R. Nestmann, *Mutagenic Activity of 3 Industrial Chemicals in a Battery of In Vitro and In Vivo Tests*, Mutat. Res. **320** (1994), 273 – 283. 2. *Robust Summaries and Test Plan for Propargyl Alcohol (CAS No. 107-19-7)*, Final Revised Submission, High Production Volume Chemical Challenge Program, July 22, 2009.

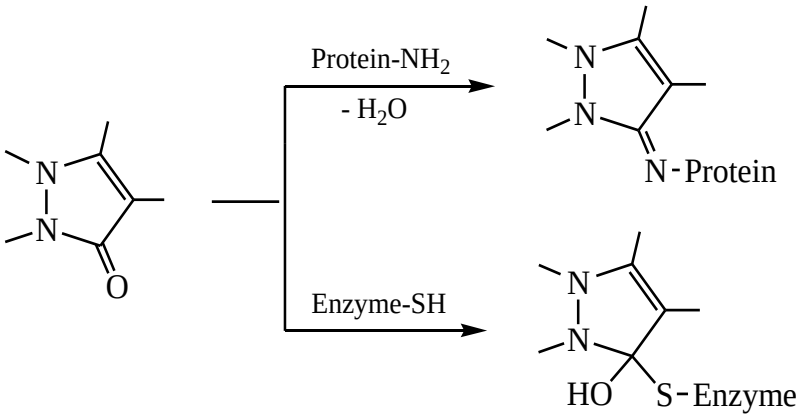
Individual profile/alert	
Name	Pyrazolone and Pyrazolidine-3,5-dione Derivatives
Type of profile	Structural alert
Description/applicability domain	**Pyrazolone derivatives**  R^1 = H, C_1-C_4 alkyl, allyl, propargyl, benzyl, dialkylamino, etc. R^2 = C_1-C_4 alkyl, benzyl, aryl, etc. R^3 = H or alkyl R^4 = C_4-alkyl, cycloalkyl, aryl, etc. **Pyrazolidine-3,5-dione derivatives**  R^1 = H, C_1-C_4 alkyl, cycloalkyl, allyl, benzyl, aryl, etc. R^2 = C_1-C_4 alkyl, allyl, benzyl, aryl, etc. R^3 = H, C_1-C_4 alkyl, acyl, carbamoyl, aryl, etc. R^4 = alkyl, cycloalkyl, aryl, etc.

Mechanism	A_N2, Michael addition to activated double bonds in heterocyclic ring systems A_N2, Shiff base formation with carbonyl compounds

- Shiff base formation with carbonyl compounds

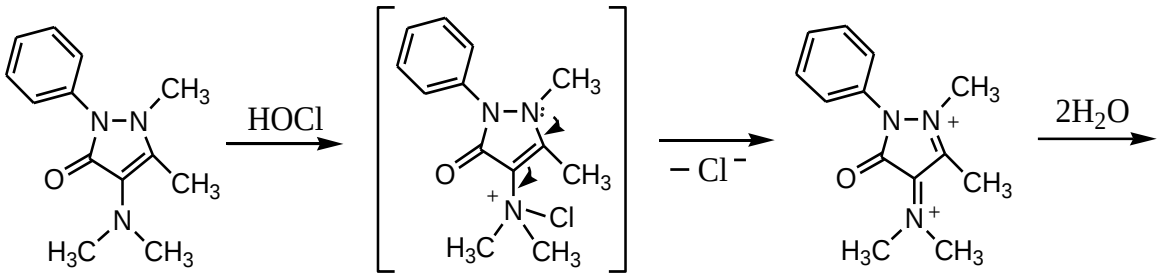
One of the reasons for the adverse effects of pyrazolone and pyrazolidine-3,5-dione derivatives should be the possibility to interact with different proteins. For example, antipyrine binds irreversibly to hepatic protein in vivo and in metabolizing liver microsomes [6], dipyrone and its metabolites bind to plasma protein [7], phenylbutazone and its hydroxylated metabolites bind covalently to plasma and human serum albumin [8,9]. All of these drugs and their metabolites contain carbonyl groups and it may be assumed that they react with the active sites of proteins or enzymes acting as Schiff base formers (Scheme 1):

Scheme 1

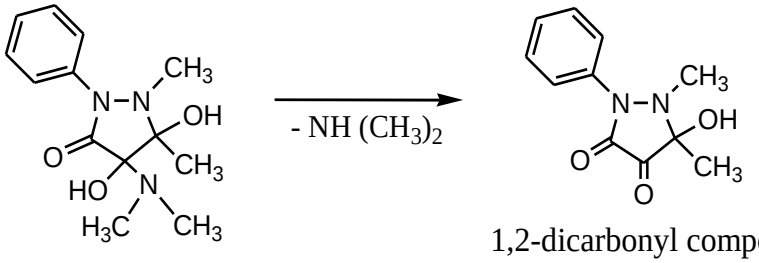


On the other hand, as a result of the bioactivation of aminopyrine derivatives, 1,2-dicarbonyl fragment can be form in the molecule under the influence of different oxidizing systems (myeloperoxidase, hydrogen peroxide, chloroperoxidase, hypochlorus acid, etc.), as it is shown in Scheme 2 [3,10].

Scheme 2



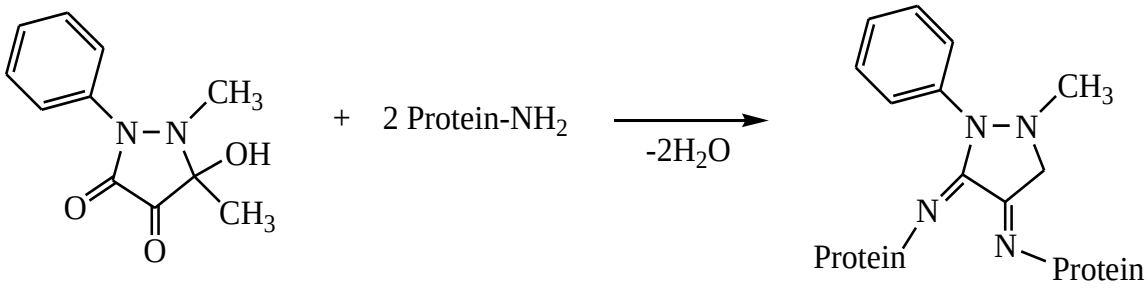
Aminopyrine *N*-chloroaminopyrine Reactive intermediate



1,2-dicarbonyl compound

The carbon atoms in 1,2-dicarbonyl compounds possess high electrophilicity because of the π,π-delocalization between two carbonyl groups. The following reaction scheme may be proposed as a result of the interaction with protein amines (Scheme 3).

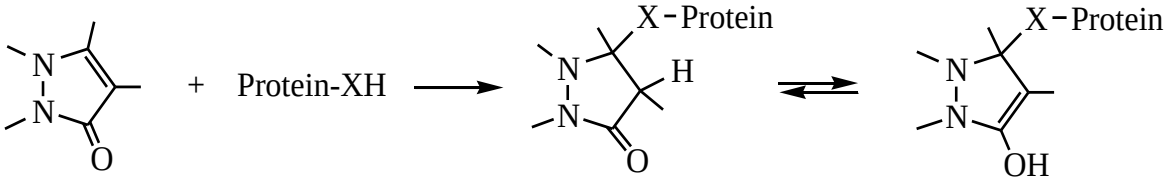
Scheme 3



- Michael addition to activated double bonds in heterocyclic ring systems

Pyrazolone derivatives may also undergo the Michael type addition reaction represented in Scheme 4.

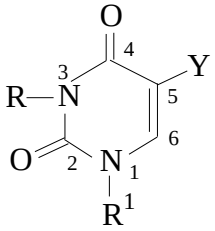
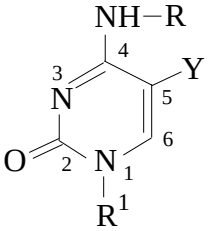
Scheme 4

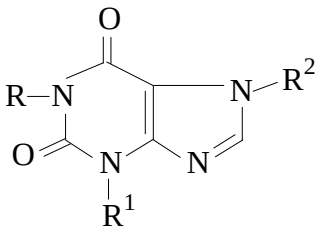
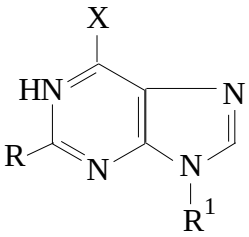


(X = S, NH, O)

Thus, the pyrazolone derivatives can bind covalently to proteins and enzymes both by Schiff base

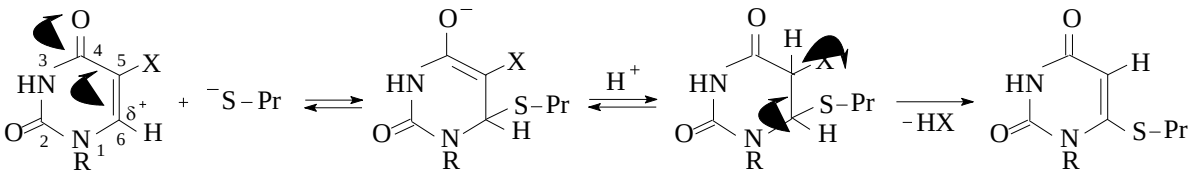
formation and/or Michael type addition reactions.	
Set of chemicals used for profile development	Pyrazolone and Pyrazolidine-3,5-dione Derivative
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. A.K. Giri, A. Mukhopadhyay, *Mutat. Res.*, **1998**, 420, 15-25. 2. R.N. Brogden, *Drugs*, **1986**, 32, 60-70. 3. A.S. Kalgutkar, D.K. Dalvie, J.P. O'Donnell, T.J. Taylor, D.C. Sahakian, *Current Drug Metab.*, **2002**, 3, 379-424. 4. Y. Bentur, O. Cohen, *J. Toxicol. Clin. Toxicol.*, **2004**, 42, 261-265. 5. S. Mao, S. Yang, D. Bi, *Biol. Pharm. Bull.*, **2006**, 29, 1355-1359. 6. S. Tarabelli-Poplawski, H. Uehleke, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1977**, 297, 105-110. 7. Zylber-Katz, L. Granit, M. Levy, *Eur. J. Clin. Pharmacol.*, **1985**, 29, 67-71. 8. W. Dieterle, J.W. Faigle, F. Fruh, H. Mory, W. Theoblad, K.O. Alt, W.J. Richter, *Arzneimittelforschung*, **1976**, 26, 572-577. 9. F. Chignell, *Mol. Pharmacol.*, **1969**, 5, 244-252. 10. J.P. Uetrecht, H.M. Ma, E. MacKnight, R. McClelland, *Chem. Res. Toxicol.*, **1995**, 8, 226-233.

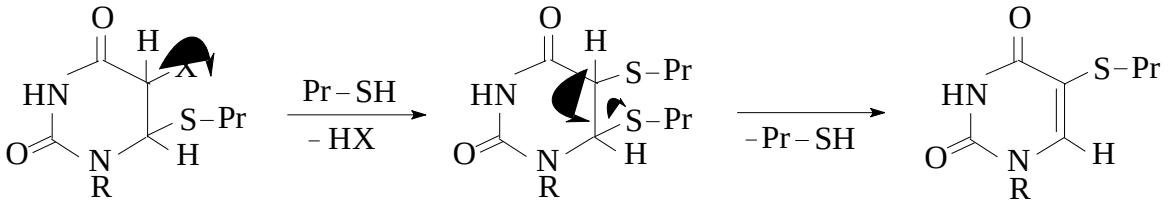
Individual profile/alert	
Name	Pyrimidines and Purines
Type of profile	Structural alert
Description/applicability domain	Pyrimidine derivatives, that are able to cause chromosomal aberrations in in vitro assays are presented with the following general structures: Structure 1  Structure 2  Structure 1: R = H or Csp3(scy), such as tetrahydrofuran-2-yl residue;

	R1 = H or Csp3(scy) such as tetrahydrofuran-2-yl residue, deoxyribose or ribose moieties, which are bound with nitrogen atom in position 2 of the five-membered heterocyclic ring; Y = F or Cl atoms. Structure 2: R = H or C(=O)-Csp3(acy) R1 = ribose or deoxyribose moieties; Y = H or Csp3(acy) short chains (CH3, C2H5). Purines, that possess clastogenic activity, may be presented with the following structures: Structure 1  Structure 2  Structure 1: R = R1 = CH3; R2 = H, CH3. Structure 2: X = OH, SH; R = H, NH2; R1 = H or ribose moiety, bound with nitrogen atom in position 2 of the five-membered heterocyclic ring.
Mechanism	A_N2, Michael-type addition reaction; Schiff base formation

Genotoxicity of pyrimidines and purines were studied by the reverse mutation assay in bacteria and the chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells. The chemicals such as fluorouracil, tegafur, caffeine, theophylline, 4-amino-1-pentofuranosyl-2(1H)-pyrimidinone, enocitabine, 6-mercaptopurine, disodium 5'-guanylate were found to induce chromosomal aberration in in vitro assay in CHL cells without metabolic activation [1,2].

Good leaving groups such as fluorine and chlorine atoms, attached at position 5 of pyrimidine ring may undergo elimination or, in the presence of the excess of nucleophile, subsequent substitution. As a result, a mixture of 6- and 5-substituted derivatives was obtained, as shown in Scheme 1 [3,4].

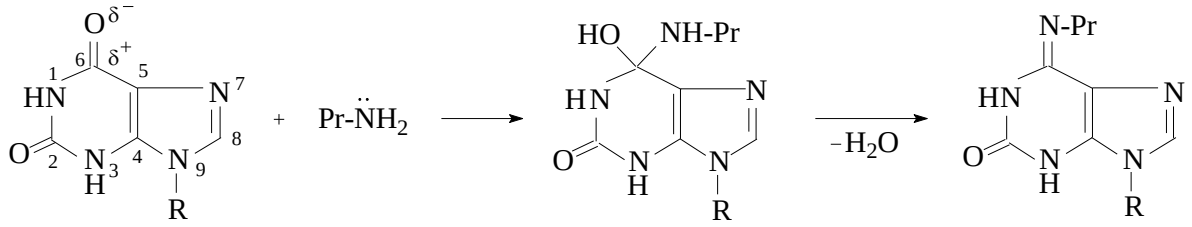




A_N-addition product

5-substituted derivative

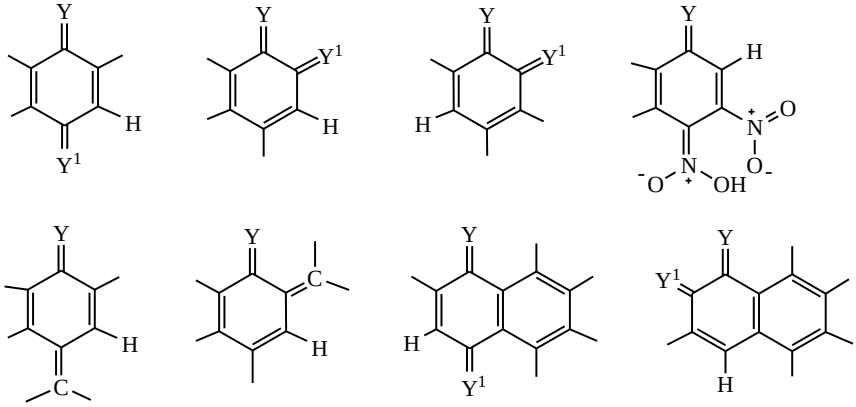
The carbon-oxygen double bonds at C-2 and/or C-6 positions in the purine derivatives and at C-2 and/or C-4 positions in the pyrimidines can also undergo nucleophilic addition reactions [4,5]. The initially formed product could be stable under appropriate reaction medium or may spontaneously undergo an elimination reaction, especially dehydration, leading to the Schiff base formation (Scheme 2).



A_N-product

Schiff-base derivative

Set of chemicals used for profile development	Pyrimidines and Purines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Yajima, N., K. Kondo, K. Morita, Reverse mutation tests in *Salmonella typhimurium* and chromosomal aberration tests in mammalian cells in culture on fluorinated pyrimidine derivatives. *Mutat Res.*, **1981**, 88(3), 241-254. 2. Ishidate, M. Jr., M.C. Harnois, T. Sofuni, A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 3. Boncel, S., A. Gondela, K. Walczak, Uracil as a target for nucleophilic and electrophilic reagents. *Curr. Org. Synth.*, **2008**, 5(4), 365-396. 4. Hermanson, G.T., Bioconjugate Techniques, 2nd ed., Elsevier Inc., **2008**, pp. 55-59. 5. Shabarova, Z.A., A.A. Bogdanov, Advanced Organic Chemistry of Nucleic Acids, 2nd ed., John Wiley & Sons, **2008**, pp. 44-47, 393-394.

Individual profile/alert	
Name	Quinoid compounds
Type of profile	Structural alert
Description/applicability domain	 **Classification:** ➢ *ortho*- and *para*-Quinones, quinoneimines and quinonedimines: Y and Y^1 = O, N ➢ 1,4- and 1,2-Naphthoquinones, naphthoquinoneimines and naphthoquinonedimines: Y and Y^1 = O, N ➢ *ortho*- and *para*-Quinone methides and quinoneimine methides: Y = O, N and Y^1 = Csp^2 {acy, scy} ➢ *para*-Quinoid oximes: Y = O, N and Y^1 = N-OH ➢ *ortho*- and *para*-Nitroquinones and nitroquinoneimines: Y = O, N
Mechanism	A_N2, Michael-type addition, quinoid structures Radical mechanism , ROS generation

<u>Direct formation of covalent adducts with chromosomal proteins</u>

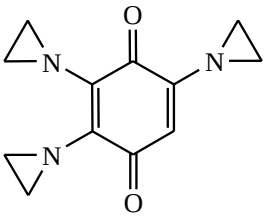
Quinones as the most representative sub-class of quinoid compounds constitute an important class of naturally occurring chemicals found in plants, fungi and bacteria [7]. Quinones are Michael acceptors, and modifications of cellular processes could occur through alkylation of crucial cellular proteins and/or DNA [3].

As “soft” electrophiles, quinones are particularly susceptible to conjugation via Michael addition reactions with cellular nucleophiles such as glutathione as well as cysteine and lysine residues in proteins [3,8]. These reactions can lead to loss or disruption of protein function [8] and induction of structural and numerical chromosomal aberration in mammalian cells [9].

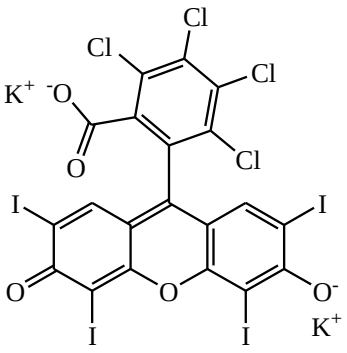
It was suggested that chromosomal aberrations in cells might affect chromosomal translocations by inhibition of topoisomerase II [10]. For instance, para-benzoquinone at micromolar concentrations causes concentration-dependent inhibition of topoisomerase II activity, probably by interaction with

the essential thiol groups of topoisomerase II [10,11].

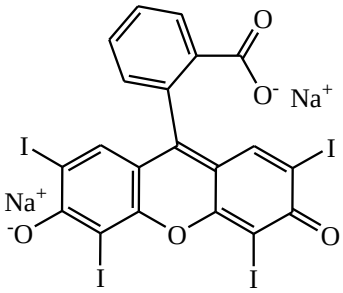
Some compounds with quinone or quinone methide fragment in their molecular structures such as as Trenimon, Rose Bengal and Erythrosine were reported to cause in vitro chromosomal aberrations in mammalian cells when tested without metabolic activation [12]:



Trenimon



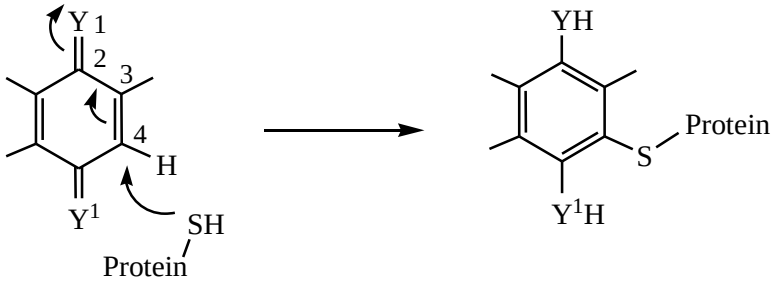
Bengal rose



Erythrosine

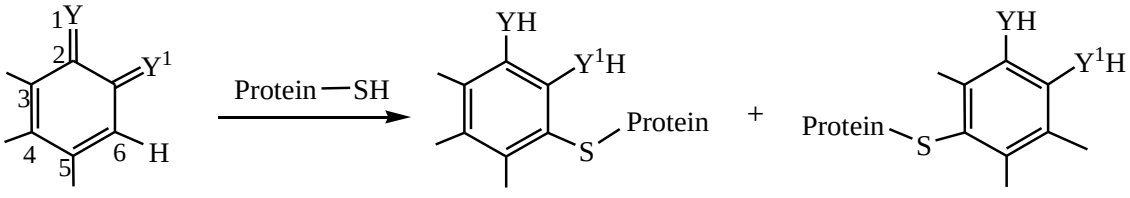
The proposed reaction schemes for formation of protein adducts between quinone-type compounds and cysteine and lysine residues in proteins are shown below:

- 1,4-Michael addition reaction and formation of covalent bonded protein adduct with *para*-quinone type compounds



para-Quinones Protein adduct

- 1,4- and 1,6-Michael addition reactions and formation of covalent bonded protein adducts with *ortho*-quinone type compounds

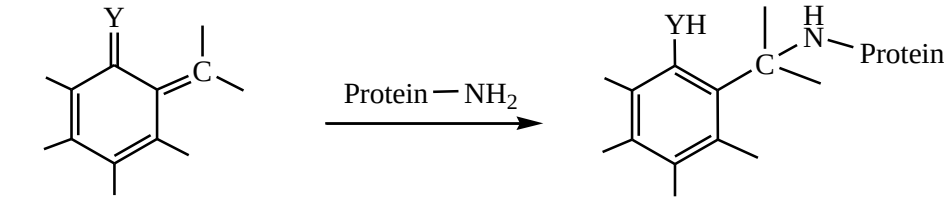


ortho-Quinones Major protein adduct Minor protein adduct

These reactions can lead to loss or disruption of protein function [8] and induction of structural and numerical chromosomal aberration in mammalian cells [9].

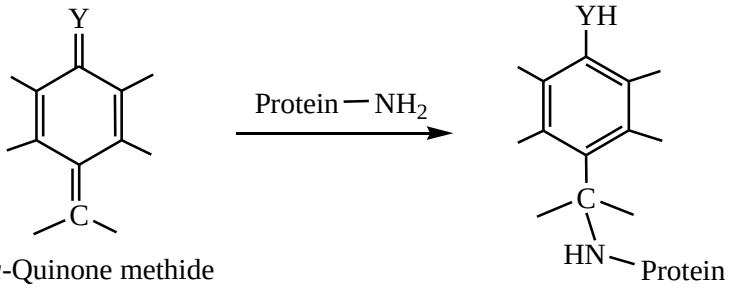
Moreover, *ortho*- and *para*- quinone methides interacting as Michael acceptors can form

benzylic-type adducts when binding thiol and amino groups in proteins to their exocyclic methylene fragments, according to the following schemes [13,14]:



ortho-Quinone methide

Protein adduct



para-Quinone methide

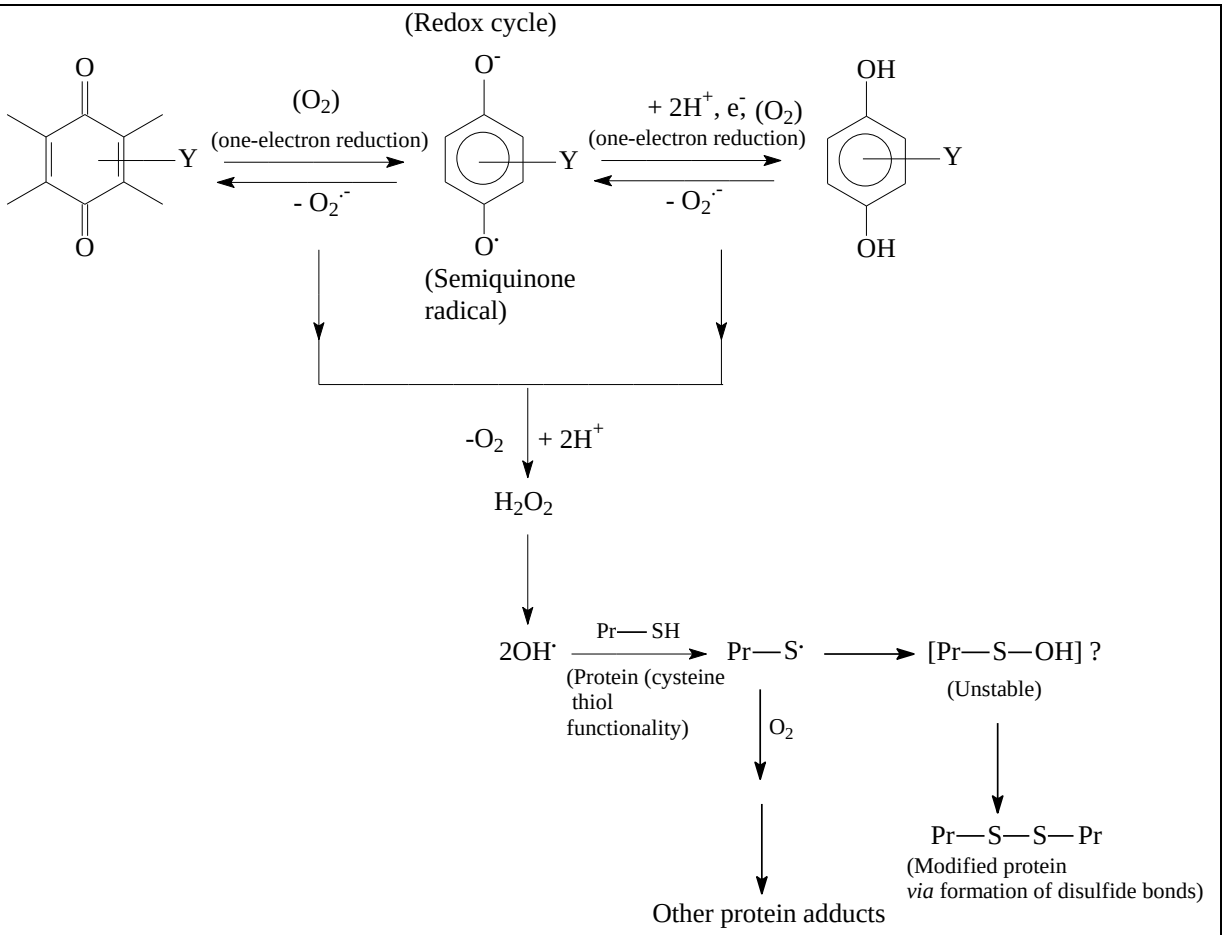
Protein adduct

The conversion of thiol groups to S-benzyl derivatives may alter the redox status of cells, but the susceptibility of these species to nucleophilic displacement reactions suggests that the relatively stable N-benzyl adducts may be of more toxicological importance [13].

Oxidative stress

Chemical interactions between thiol-containing compounds and reactive oxygen species (ROS) play a central role in the oxidation-reduction balance in the living cell. Experiments in aqueous solutions have indicated that the various thiol compounds (cysteine, cysteamine, glutathione, captopril, N-acetylcysteine, etc.) are very efficiently oxidized by hydroxyl radical (HO.) generated as ROS [15].

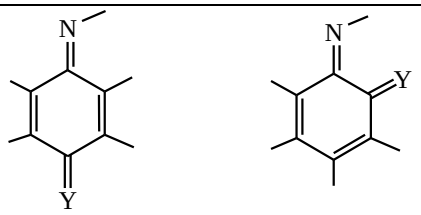
Not only cysteine residues in proteins are affected. Scheme I below provides an example of suggested mechanistic pathway of generation of ROS such as hydroxyl radical from quinones, with the subsequent modification of the cysteine thiol functionalities in proteins [3-5]:



Set of chemicals used for profile development	Quinoid compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis*, **1987**, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. *Chem. Res. Toxicol*., **2017**, 30(1), 376-387. 3. Bolton, J.L., Dunlap, T., Formation and biological targets of quinones: cytotoxic versus cytoprotective effects. *Chem. Res. Toxicol*., **2017**, 30(1), 13-37. 4. Yu, D., Berlin, J.A., Penning, T.M., Field, J., Reactive oxygen species generated by PAH *o*-quinones cause change-in-function mutations in *p*53. *Chem. Res. Toxicol*., **2002**, 15(6), 832-842. 5. Kovacic, P., Jacintho, J.D., Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. *Curr. Med. Chem*., **2001**, 8(7), 773-796. 6. do Céu Silva, M., Gaspar, J., Duarte Silva, I., Leão, D., Rueff, J., Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells. *Mutagenesis*, **2003**, 18(6), 491-496.

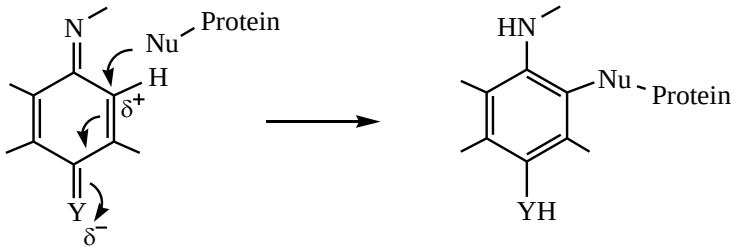
	7. Monks, T.J., Jones, D.C., The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. *Curr. Drug Metab.*, **2002**, 3(4), 425-438. 8. Penning, T.M., Genotoxicity of *ortho*-quinones: reactive oxygen species *versus* covalent modification. *Toxicol. Res.*, **2017**, 6(6), 740-754. 9. Turchi, G., Glatt, H.R., Seidel, A., Puliti A, Sbrana I. Structure-activity relationship in the induction of chromosomal aberrations and spindle disturbances in Chinese hamster epithelial liver cells by regioisomeric phenanthrene quinones. *Cell Biol. Toxicol.*, **1997**, 13(3), 155-165. 10. Hutt, A.M., Kalf, G.F., Inhibition of human DNA topoisomerase II by hydroquinone and *p*-benzoquinone, reactive metabolites of benzene. *Environ. Health Perspect.*, **1996**, 104 Suppl. 6, 1265-1269. 11. Lindsey, R.H. Jr, Bromberg, K.D., Felix, C.A., Osheroff, N., 1,4-Benzoquinone is a topoisomerase II poison. *Biochemistry*, **2004**, 43(23), 7563-7574. 12. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 13. Di Valentin, C., Freccero, M., Zanaletti, R., Sarzi-Amadè, M., *o*-Quinone methide as alkylating agent of nitrogen, oxygen, and sulfur nucleophiles. The role of H-bonding and solvent effects on the reactivity through a DFT computational study. *J. Am. Chem. Soc.*, **2001**, 123(34), 8366-8377. 14. Bolton, J.L., Turnipseed, S.B., Thompson, J.A., Influence of quinone methide reactivity on the alkylation of thiol and amino groups in proteins: studies utilizing amino acid and peptide models. *Chem. Biol. Interact.*, **1997**, 107(3), 185-200. 15. Enescu, M., Gardey, B., Mechanism of cysteine oxidation by a hydroxyl radical: a theoretical study. *Chemphyschem.*, **2006**, 7(4), 912-919. 16. O'Brien, P.J., Molecular mechanisms of quinone cytotoxicity. *Chem. Biol. Interact.*, **1991**, 80(1), 1-41. 17. Chan, K., Jensen, N., O'Brien, P.J., Structure-activity relationships for thiol reactivity and rat or human hepatocyte toxicity induced by substituted *p*-benzoquinone compounds. *J. Appl. Toxicol.*, **2008**, 28(5), 608-620. 18. Mbiya, W., Chipinda, I., Siegel, P.D., Mhike, M., Simoyi, R.H., Substituent effects on the reactivity of benzoquinone derivatives with thiols. *Chem. Res. Toxicol.*, **2013**, 26(1), 112-123.

Individual profile/alert	
	Quinoneimines protein binding alert
Type of profile	Structural alert

Description/applicability domain	 *o*- and *p*-Quinoneimines and quinonediimines Y = O, NH
Mechanism	A_N2, Michael addition to the quinoid type structures

The proposed reaction pathways of protein adduct formation between quinone(di)imines and cysteine and lysine residues in proteins are shown below:

- Michael addition reaction and formation of covalent bonded protein adduct with para-quinone(di)imines [5]:

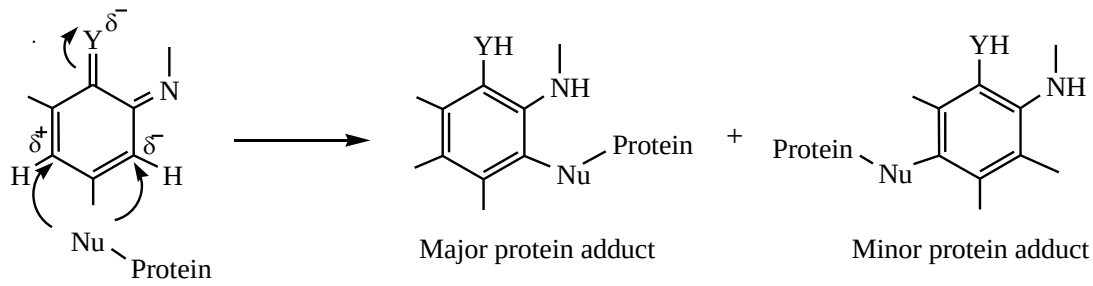


para-Quinone(di)imine

Nu = SH, NH_2; Y = O, NH;

Protein adduct

- Michael addition reaction and formation of covalent bonded protein adducts with ortho-quinone(di)imines [5] :



ortho-Quinone(di)imine

Nu = SH, NH_2; Y = O, NH;

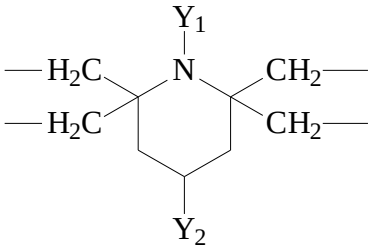
Major protein adduct

Minor protein adduct

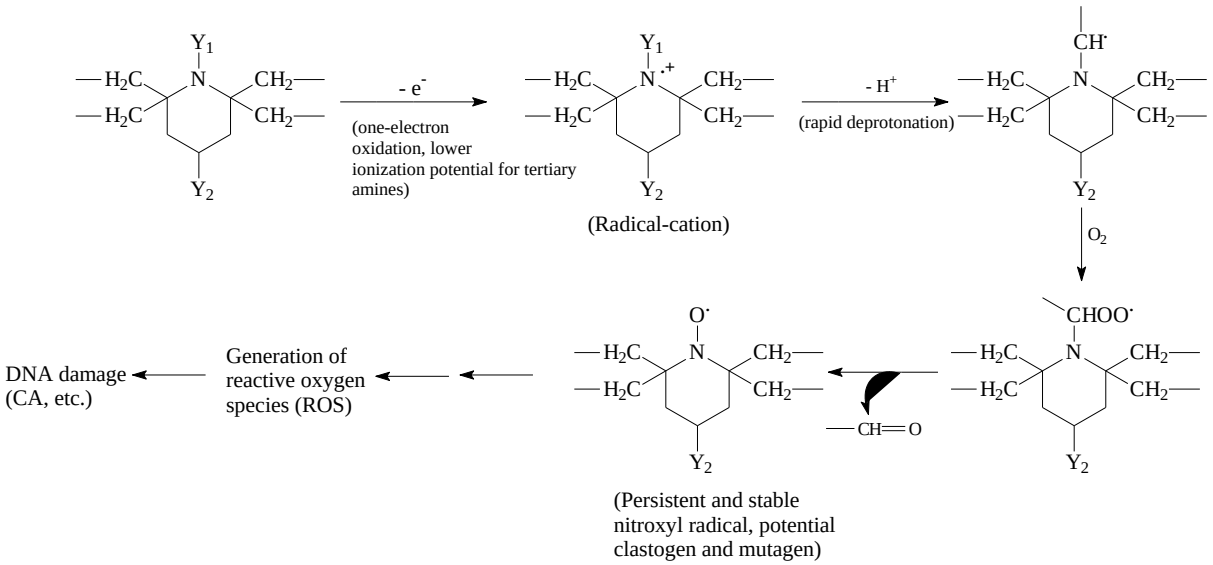
Set of chemicals used for profile development	Quinoneimines protein binding
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Klopčič, I., Dolenc, M.S., Chemicals and drugs forming reactive quinone and quinone imine metabolites. Chem. Res. Toxicol.,

	2019, 32(1), 1-34. 2. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 3. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 4. Powis, G., Hodnett, E.M., Santone, K.S., See, K.L., Melder, D.C., Role of metabolism and oxidation-reduction cycling in the cytotoxicity of antitumor quinoneimines and quinonediimines. Cancer Res., 1987, 47(9), 2363-2370. 5. Bolton, J.L., Dunlap, T., Formation and biological targets of quinones: cytotoxic versus cytoprotective effects. Chem. Res. Toxicol., 2017, 30(1), 13-37. 6. Kovacic, P., Jacintho, J.D., Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. Curr. Med. Chem., 2001, 8(7), 773-796. 7. Monks, T.J., Jones, D.C., The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. Curr. Drug Metab., 2002, 3(4), 425-438. 8. Penning, T.M., Genotoxicity of ortho-quinones: reactive oxygen species versus covalent modification. Toxicol. Res., 2017, 6(6), 740-754. 9. Turchi, G., Glatt, H.R., Seidel, A., Puliti A, Sbrana I. Structure-activity relationship in the induction of chromosomal aberrations and spindle disturbances in Chinese hamster epithelial liver cells by regioisomeric phenanthrene quinones. Cell Biol. Toxicol., 1997, 13(3), 155-165. 10. Enescu, M., Gardey, B., Mechanism of cysteine oxidation by a hydroxyl radical: a theoretical study. ChemPhysChem., 2006, 7(4), 912-919. 11. Chung, K.T., Murdock, C.A., Stevens, S.E. Jr, Li, Y.S., Wei, C.I., Huang, T.S., Chou, M.W., Mutagenicity and toxicity studies of p-phenylenediamine and its derivatives. Toxicol. Lett., 1995, 81(1), 23-32.

Individual profile/alert	
Name	Sterically Hindered Piperidine Derivatives
Type of profile	Structural alert

Description/applicability domain	 (Y_1 is -H or short-chain radicals such as $-CH_3$, $-CH_2CH_3$ or $-CH_2CH_2OH$ Y_2 is -H, -OH or -O-C{sp3})
Mechanism	Radical mechanism, ROS generation

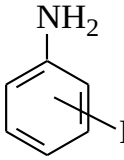
Tertiary sterically hindered amines such as 2,2,6,6-tetramethyl-substituted piperidines are easily oxidized by electron transfer to cation-radicals. These cation-radicals have been directly observed in non-polar medium by optical spectroscopy. In the presence of oxygen, the amine-derived radicals are oxidized to nitroxyl radicals. The probable mechanistic scheme of generation of active radical intermediate(s) for the sterically hindered piperidine derivatives, which is also associated with generation of reactive oxygen species (ROS) and in vitro chromosomal aberrations can be outlined as follows [6]:



It is known that 2,2,6,6-Tetramethylpiperidine-1-oxyl (Tempol) is stable nitroxyl-type free radical. Tempol is mutagenic in the in vitro mouse lymphoma assay (MLA) and induces micronuclei in TK6 cells. Oxidative stress may account for part of genotoxicity induced by Tempol in both cell lines and resulting in large genetic alterations, including chromosomal breakage [7]. Stable, membrane-permeating nitroxyl radicals possess antioxidant activity in various experimental systems, however, in parallel, they are considered as possible harmful oxidants. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is simultaneously anti-genotoxic agent but, also, cytotoxic and clastogenic chemical, depending on the concentration applied. Nitroxyl-type radicals are mutagenic in Salmonella typhimurium at concentrations > 50 mM and can aggravate the mutagenic effects of H2O2 in Salmonella typhimurium strain TA104, which is sensitive to oxidative DNA damage. Thus the mutagenic action of nitroxides is assumed to be due to their pro-oxidant properties, and to be

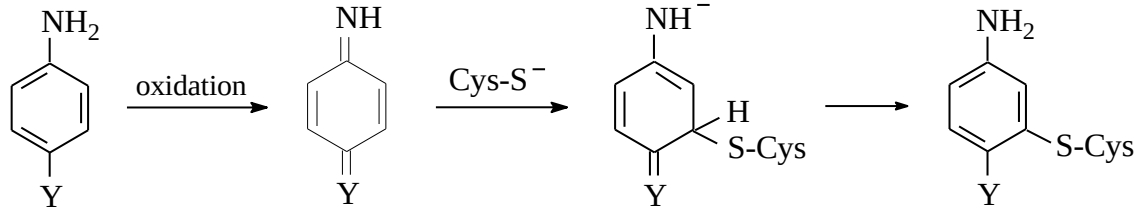
mediated by compounds formed upon oxidation of glutathione by nitroxides. The oxidative shift in the GSH/GSSG ratio induced by nitroxides, which affects the redox homeostasis in the cell may be also a factor favoring mutagenesis [8].	
Set of chemicals used for profile development	Sterically Hindered Piperidine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Allen, N. S., J. F. McKellar, D. Wislon, Photo-Stabilisation of Commercial Polypropylene by Piperidine Compounds: The Role of Stable Free Radicals, Polym. Degrad. Stability 1(3) (1979), 205 – 215. 2. CAS number: 52722-86-8, Registration dossier, ECHA, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-ethanol. 3. CAS number: 2403-88-5, Registration dossier, ECHA, 2,2,6,6-Tetramethylpiperidine-1-Ethanol. 4. VP Sanduvor PR-31, Full Public Report, National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (September 1997). 5. 2,2',6,6'-Tetramethylpiperidin-4-ol CAS No. 2403-88-5, SIDS Initial Assessment Report for SIAM 14, OECD SIDS, (26 – 28th March 2002). 6. Brede, O., D. Beckert, C. Windolph, H. A. Gottinger, One-Electron Oxidation of Sterically Hindrede Amines to Nitroxyl Radicals: Intermediate Amine Radical Cations, Aminyl, α-Aminoalkyl, and Aminylperoxyl Radicals, J. Phys. Chem. A 102 (1998), 1457 – 1464. 7. Guo, X., R. A. Mittelstaedt, L. Guo, J. G. Shaddock, R. H. Heflich, A. H. Bigger, M. M. Moore, N. Mei, Nitroxide TEMPO: A Genotoxic and Oxidative Stress Inducer in Cultured Cells, Toxicol. In Vitro 27 (2013), 1496 – 1502. 8. Lewinska, A., M. Wnuk, E. Slota, Gr. Bartosz, The Nitroxide Antioxidant Tempol Affects Metal-Induced Cyto- and genotoxicity in Human Lymphocytes in Vitro, Mutat. Res. 649 (2008), 7 – 14.

Individual profile/alert	
Name	Substituted Anilines
Type of profile	Structural alert

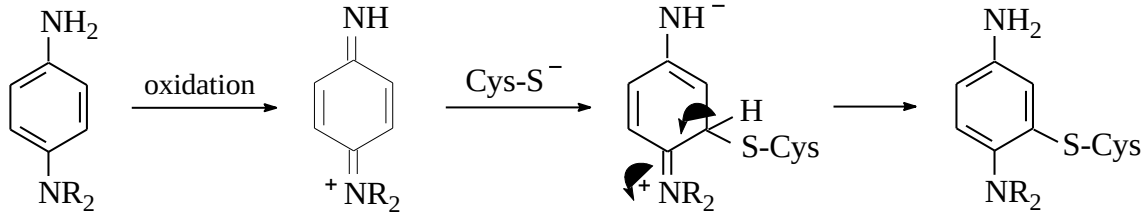
Description/applicability domain	 (R can be located in *o*-, *m*- or *p*-positions) R = Csp^3 (acy), OH, $OCsp^3$ (acy), Csp^3-Csp^2 (aryl), NH_2, $N(Csp^3)_2$, C(=O)- Csp^3, etc.
Mechanism	A_N2, Michael addition to the quinoid type structures such as quinone-imines, quinone-diimines, etc.

I. Abiotic and biotic oxidation of para- and ortho-substituted anilines

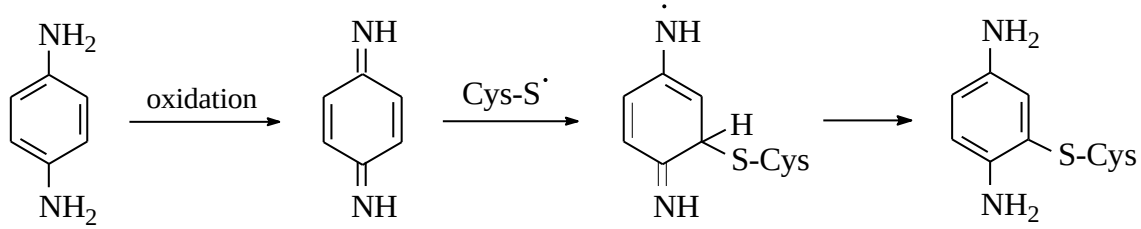
Substituted para- and ortho-aminophenols, phenylenediamines and alkylanilines are susceptible toward oxidation in the presence of air oxygen or peroxidases in the cellular systems [4-7]. The rate of their oxidation were shown to increase when solution pH increased from 6 to 8 [4]. The formation of the corresponding quinoid structures suggests the interaction mainly with protein thiolates [5-7]. The overall mechanism of this reaction can be summarized as shown in Scheme 1.



If one of the amino groups in benzene ring is tertiary, the corresponding diamine can be oxidized to a charged analogue (Scheme 2). This derivative would be more reactive as a Michael acceptor than the uncharged oxidized products [6].

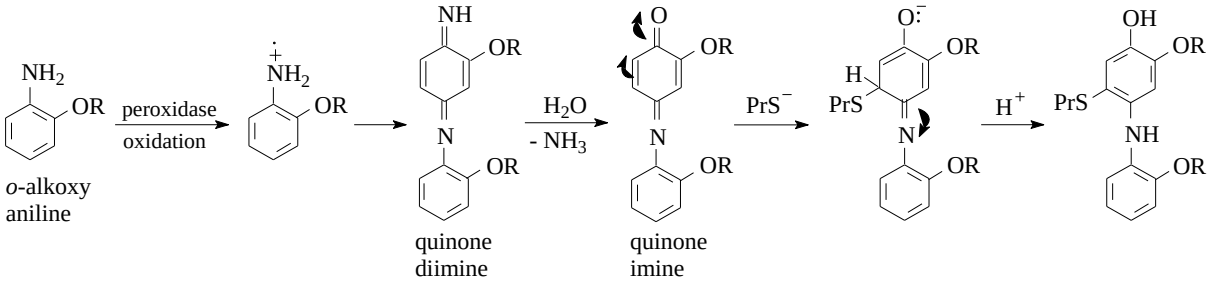


The quinone-imines and quinone-diimines can also react with protein-associated sulfhydryl radicals at a ring carbon atom in a radical analogue of the Michael addition (Scheme 3) [8].



The clastogenicity of *para*- and *ortho*-substituted alkoxyanilines (i.e. anizidines, phenetidines)

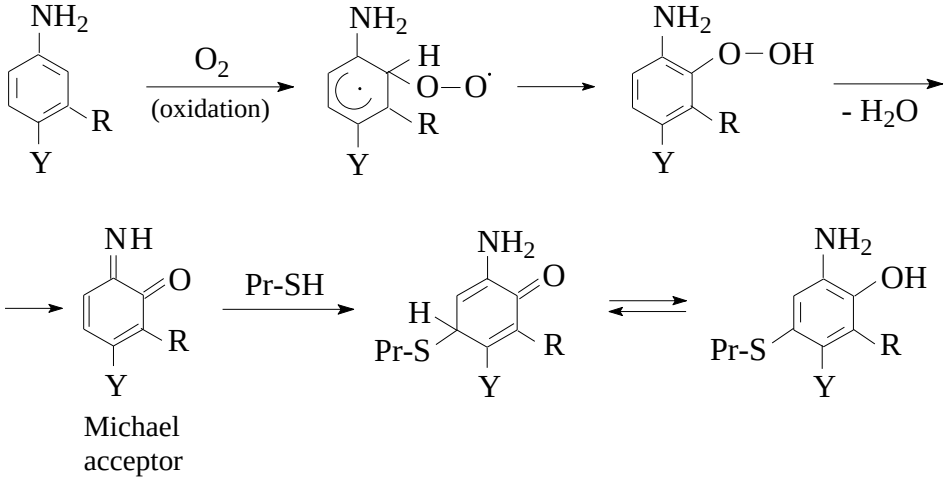
can be explained by the possibility to undergo one-electron oxidation in the presence of cellular peroxidases. Aromatic amines generally form nitrogen-centered cation-radicals when oxidized by peroxidases [9]. It was suggested that the protein reactive species could be *o*- and *p*-anisidine or phenetidine quinone-imine and quinone-diimine dimers formed as a result of peroxidative oxidation of the initial alkoxyanilines [8,9]. The putative mechanism of the oxidation and protein binding of *ortho*-isomers is shown in Scheme 4.



The reactivity of the p- and o-di-substituted aromatic compounds towards oxidative formation of quinoid structures at physiological pH can be arranged in the following manner: benzenediols > aminophenols > phenylenediamines [10]. The introduction of additional (1 to 3 in number) electron-donating substituents of pronounced positive inductive and/or resonance effects (such as alkyl, alkoxy, hydroxy, and amino groups) into the aromatic structure results in an enhanced reactivity towards oxidation, as expected. However, the rate of the next step of Michael-type cysteine (Cys-SH) conjugation can be decreased, due to the combination of such electronic factors. On the other hand, the presence of additional electron-withdrawing substituents such as -Cl, -Br, -NO2, -COOH, etc. in the aromatic structures can reduce the reactivity towards oxidation but increase the rate of the next nucleophilic addition of Cys-SH.

II. Perhydroxylation of meta-substituted anilines

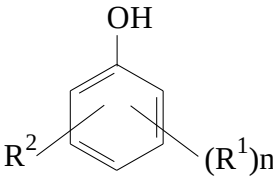
Aromatic amines can function as reducing cofactors for peroxidases, being oxidized in the process to free radicals [9]. The perhydroxylation of meta-substituted anilines is able to occur mainly in ortho- or para-positions towards amino group due to the strong stabilizing effect of a nitrogen atom in the formation of free radicals [8,11]. The ortho- and para-quinones or quinine-imines formed behave as electrophilic intermediates and may undergo Michael-type addition reaction in the presence of cellular nucleophiles (proteins, DNA). The process is shown as an abiotic perhydroxylation reaction sequence resulting from the attack of molecular oxygen in 2-position [8,11]. The protein binding of meta-substituted anilines is presented in Scheme 5.



R = OH, NH_2, Csp^3 (acy), $OCsp^3$ (acy); Y = H, Cl, $OCsp^3$ (acy), etc.

Set of chemicals used for profile development	Substituted Anilines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances, vol. 73, WHO, Lyon, **1999**, pp. 49, 52–55. 2. S.M. Galloway, M.J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A.D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B.H. Margolin, M.A. Resnick, B. Anderson, E. Zeiger, Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.*, **1987**, 10 (Suppl. 10), 1-175. 3. T. Sofuni, A. Matsuoka, M. Sawada, M. Ishidate Jr, E. Zeiger, M.D. Shelby, A comparison of chromosome aberration induction by 25 compounds tested by two Chinese hamster cell (CHL and CHO) systems in culture. *Mutat. Res.*, **1990**, 241(2), 175-213. 4. M. Uchimiya, A.T. Stone, Reversible redox chemistry of quinones: Impact on biogeochemical cycles. *Chemosphere*, **2009**, 77(4), 451-458. 5. A.O. Aptula, G. Patlewicz, D.W. Roberts, Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol.*, **2005**, 18(9), 1420-1426. 6. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, Mechanistic applicability domain classification of a Local Lymph Node Assay dataset for skin sensitization. *Chem. Res. Toxicol.*, **2007**, 20(7), 1019-1030.

	7. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.D. Cronin, A review of the elecrtophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol*., **2011**, 41(9), 783-802. 8. A.O. Aptula, S.J Enoch, D.W. Roberts, Chemical mechanisms for skin sensitization by aromatic compounds with hydroxyl and amino groups. *Chem. Res. Toxicol*., **2009**, 22(9), 1541-1547. 9. D.C. Thompson, T.E. Eling, Reactive intermediates formed during the peroxidative oxidation of anisidine isomers. *Chem. Res. Toxicol.*, **1991**, 4(4), 474-481. 10. A. Brunmark, E. Cadenas, Redox and addition chemistry of quinoid compounds and its biological implications, *Free Radic. Biol. Med.*, **1989, 7**(4), 435-477. 11. D.W. Roberts, A.O. Aptula, G. Patlewicz, Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol.*, **2007**, 20(1), 44-60.

Individual profile/alert	
Name	Substituted Phenols
Type of profile	Structural alert
Description/applicability domain	 where: (R1)n = (Csp3(acy))n alkyl groups at n = 1–4 and with linear or branched chains, containing between one and four carbons, but without tert-butyl groups in ortho-position toward OH group; (R1)n = Csp3(acy)-Csp2(vinyl or aryl) at n = 1 and located preferably in the ortho or para positions toward OH group; (R1)n = Hal (F, Cl, Br) atoms at n = 1–4; (R1)n can also represents CH=CH-Y at Y = C(O)-Csp3(acy) or N3+; (R1)n = Csp2 (aryl), mainly phenyl group; n = 1; (R1)n = para-NH-C(=O) group at n = 1 or ortho-C(=O)Y groups, where Y = H, OH, NH2 at n = 1; R2 should be at least one hydrogen atom located in appropriate position(s) of the corresponding compound.

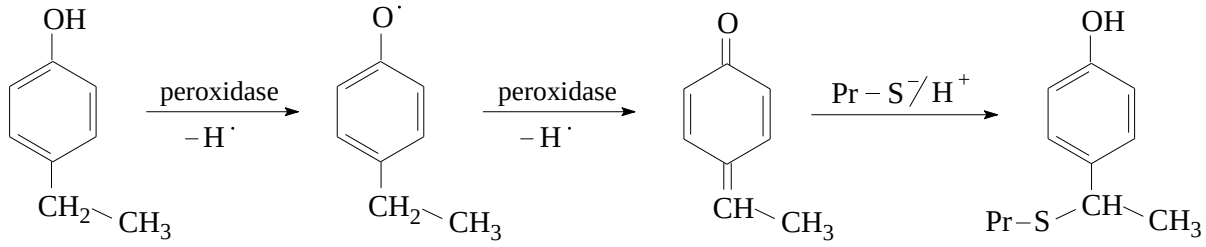
Mechanism	AN2, Michael-type addition to quinoid structures

I. Alkylated and alkenylated phenols

1. ortho- and para-Alkylated phenols containing primary and secondary alkyl groups

The quinone methides are able to be formed if alkyl groups possess at least one hydrogen atom in alpha-position relative to the arene ring and are also located in ortho- or para-positions toward hydroxyl group [7]. The observed in the in vitro clastogenicity in CHO and CHL cells without S9 mix is probably due to abiotic oxidation of alkylated phenols in an aqueous medium and in the presence of peroxidases and air oxygen [3].

For example, the one- and two-electron oxidative pathways of some ortho- and para-alkyl and arylalkyl phenols (2- and 4-ethylphenols, 2-sec-butylphenol, 2,3,6-trimethylphenol, bisphenol F, etc.) can lead to quinone methide formation (Scheme 1). The quinone methides are class of reactive electrophilic compounds which are capable of alkylating cellular proteins such as sulfur nucleophiles and also other nucleophilic sites in proteins [3,4] via an Michael-type addition reaction (Scheme 1).



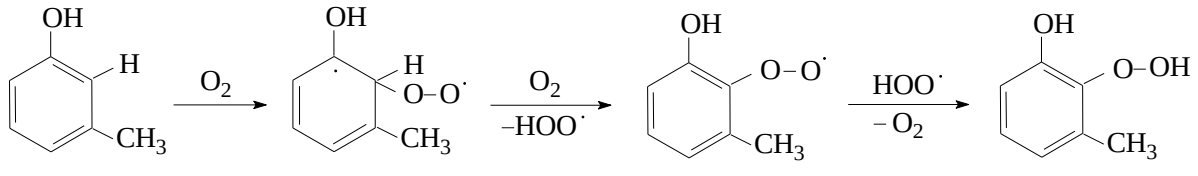
4-ethylphenol | 4-ethylphenoxy radical | 4-ethylidene-2,5-cyclohexadien-1-one | protein adduct

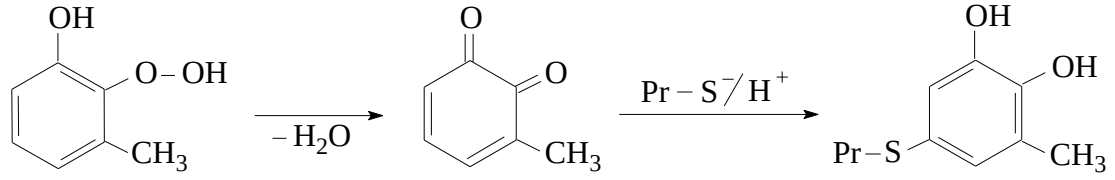
2. meta- and para-Alkylated phenols

Another group of substituted phenols are ***meta***- and ***para***-alkylated phenols such as *m*-cresol, *p*-*tert*-butyl phenol, bisphenol A, *p*-cumylphenol, etc., which can not be oxidized directly to quinone methides or quinones.

- *meta*-Alkylated phenols

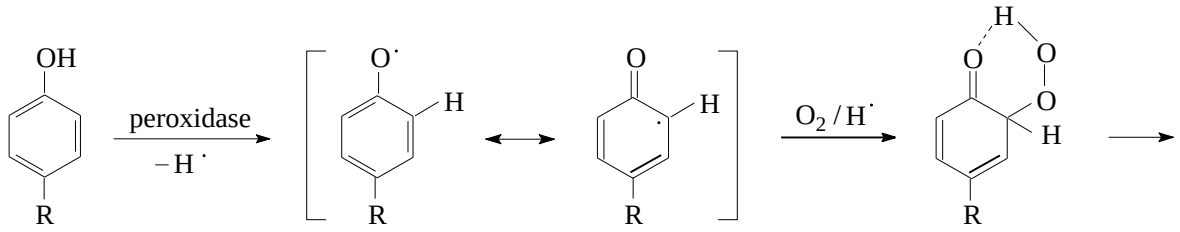
It may be suggested that the interaction of *m*-cresol with protein nucleophiles takes place similarly to that of 1,3-phenylenediamines, 3-aminophenols and 1,3-benzenediols. This process includes an abiotic perhydroxylation reaction sequence resulting from the attack of molecular oxygen in *ortho*- or *para*-positions and the formation of a free peroxyl radical which is converted into the corresponding perhydroxylated derivative [8,9]. The reactivity depends on the ability of the *meta* substituents to stabilize a free radical intermediate. The corresponding *ortho*- and *para*-benzoquinones are likely to be formed as a result of an intramolecular dehydration (Scheme 2).



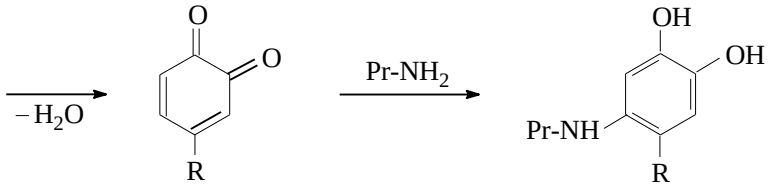


➢ *para*-Alkylated phenols

para-Alkylated phenols containing tertiary alkyl groups such as *p*-*tert*-butylphenol, bisphenol A, *p*-cumylphenol, etc. cannot be oxidized directly to quinones. The possible pathway of their activation in the absence of S9 fraction may be associated with an abiotic perhydroxylation under the influence of enzymes found in mammalian cells. The perhydroxylated phenol intermediate is easily dehydrated to the corresponding *ortho*-benzoquinone, which undergoes Michael type addition reaction (Scheme 3).



resonance-stabilized phenoxyl radical hydroperoxide formation

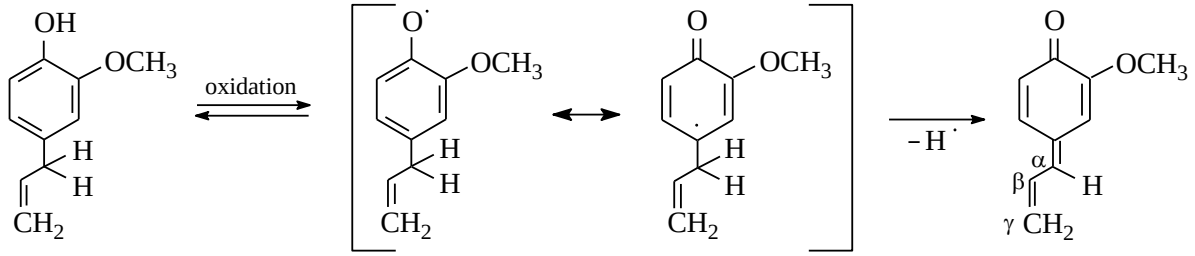


o-quinone-type fragment protein adduct

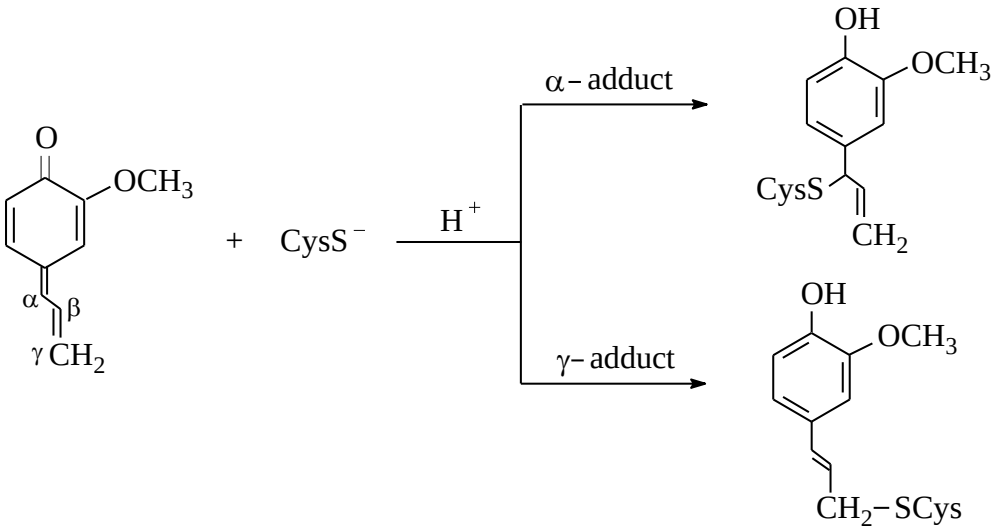
3. *Alkenyl-substituted phenols*

➢ Eugenol and eugenol-related compounds

Eugenol and eugenol-related compounds can also be oxidized in Chinese hamster cells. The reaction mechanism includes the formation of a phenoxyl radical via one-electron oxidation, which subsequently can change into a eugenol quinone methide that is able to bind covalently to thiol groups in proteins (Scheme 4) [11,12].



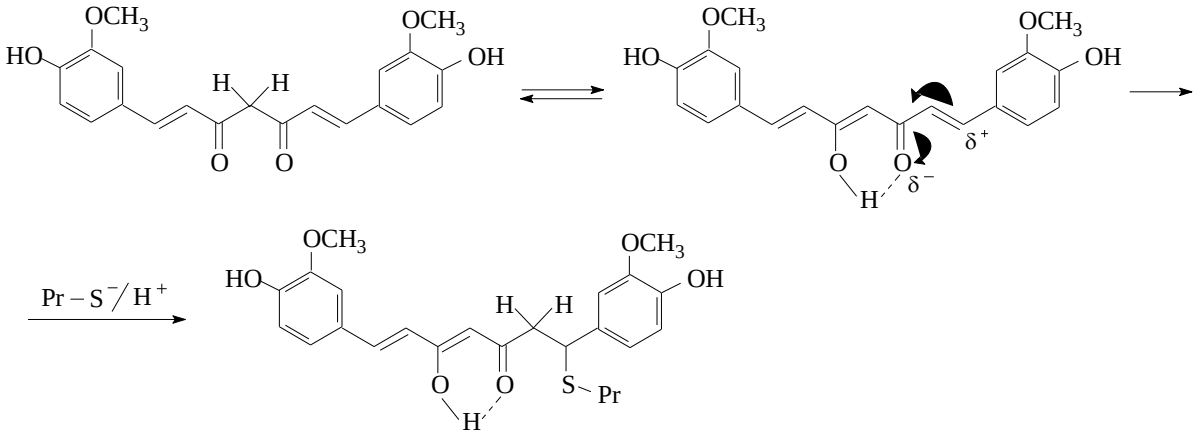
The so obtained conjugated quinone methide was found to give two types of adducts. The product resulting from the attack of a protein nucleophile at the alpha-position was produced under kinetic control, while the product of gamma-attack was formed under thermodynamic control (Scheme 5) [12].



- Curcumin reactivity

The equivocal results for chromosomal aberrations were found for alkenylated phenol curcumin in Chinese hamster ovary cells without metabolic activation [13,14]. The EFSA Panel considered that the indications provided by the positive results for curcumin in several in vitro and in vivo tests for genotoxicity, especially those detecting chromosomal aberrations and DNA adducts should not be disregarded, and that the available in vivo genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity.

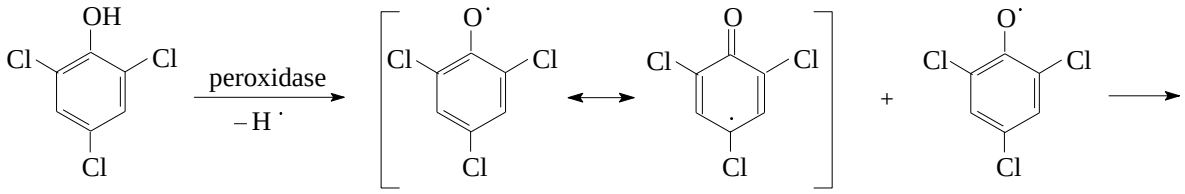
Curcumin can be regarded as a direct acting clastogen due to the presence of an α,β-unsaturated 1,3-dicarbonyl moiety in para-position against the hydroxyl group. Taking into account that it can be converted into a stable enol form, the mechanism of protein binding in the in vitro assay without metabolic activation can be presented as a Michael-type nucleophilic addition to the α,β-unsaturated carbonyls (Scheme 6).



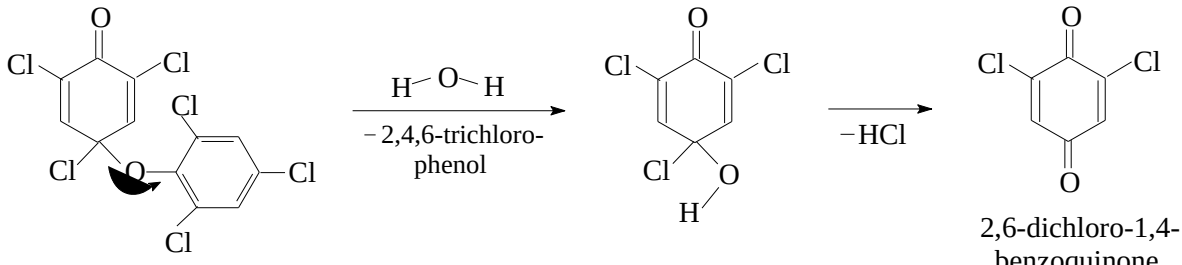
II. Halogenated phenols

The halogenated phenols are able to undergo one- or two-electron oxidation under the influence of

different peroxidases and/or dihydrogen peroxide [15,16]. Sturgeon et al. [15] established that the transient 2,4,6-trichlorophenoxyl radical intermediate can exist free in solution as a result of one-electron peroxidase oxidation (Scheme 7). Then, 2,4,6-trichlorophenoxyl radical intermediate undergoes enzyme-independent reactions, such as radical-radical (carbon-oxygen) coupling, leading to the formation of a two-electron oxidized 2,6-dichloro-1,4-benzoquinone product [15]. The latter can bind to protein nucleophiles via a Michael-type addition reaction (Scheme 7).



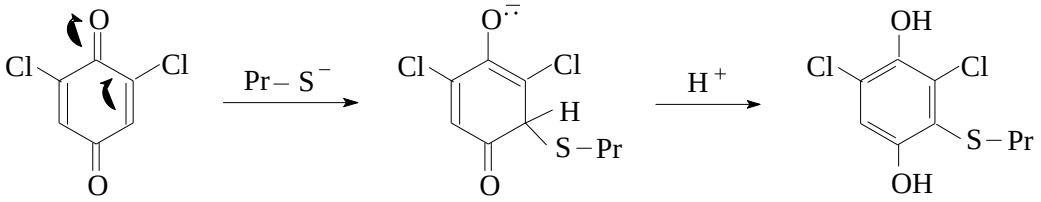
resonance-stabilized phenoxyl radical



dimer formation by radical-radical coupling

2,6-dichloro-1,4-benzoquinone

Michael-type addition reaction



protein-adduct formation

Depending on the substrate, peroxidases are thought to carry out both one- and two-electron oxidations [15]. The condensation products arising from coupling reactions of resonance-stabilized halophenol radicals could be formed in the non-irradiated systems. Different types of condensation products were found such as chlorinated hydroxylated biphenyls, hydroxylated diphenyl ethers, etc. [16]. However, the presence of halogens in positions 3, 4 and 5 against hydroxyl group will impede the access of the phenoxyl radical to the carbon-centered radical, limiting the formation of the intermediate dimer and the corresponding quinone (Scheme 7 above).

The mechanism of action of triclosan, a chlorinated aryloxyphenol, has been considered to be similar to the other halogenated phenols, targeting proteins and leading to their coagulation and precipitation. These effects clearly include the cell surface (cell wall and cell membrane) [17]. However, more recent studies have shown that triclosan specifically binds to an enzyme (enoyl reductase) and causes conformational changes in its protein structure [18,19].

III. Hydroxylated biphenyls

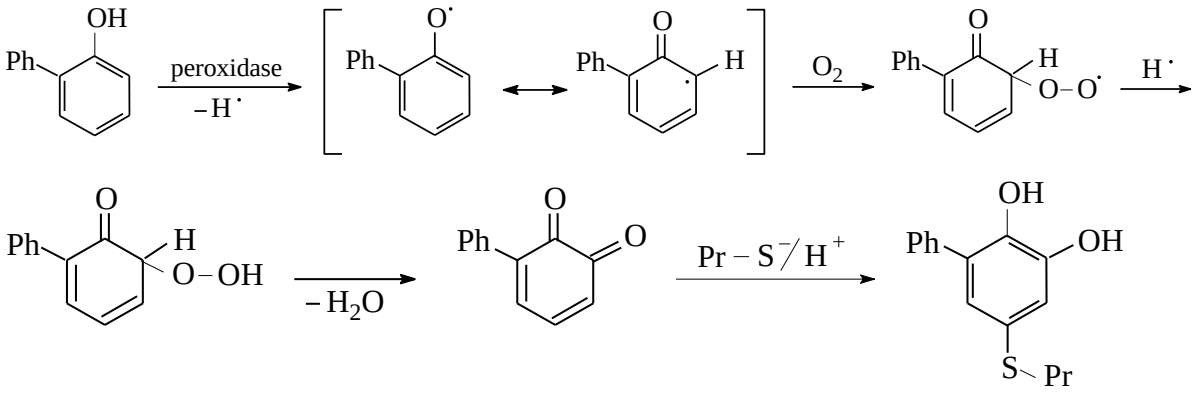
ortho-Phenylphenol (OPP, 2-hydroxybiphenyl) and its sodium salt (SOPP, sodium o-phenylphenate) are examples of biphenyl derivatives with a low degree of hydroxyl substitution. OPP and SOPP are broad spectrum antimicrobials with a variety of applications [20-24].

OPP and SOPP are believed to be oxidized by mixed function oxidases (CYP enzymes) to 2,5-dihydroxybiphenyl. Oxidation of dihydroxybiphenyl leads to 2-phenyl-1,4-benzoquinone via reactive semiquinone radicals. These semiquinone intermediates and/or the quinone obviously are responsible for the tumor initiating activity of OPP and SOPP observed [26].

The microsomal oxidative metabolism of OPP is an essential prerequisite for the covalent binding of this substance to proteins [25]. In addition, OPP was found to bind covalently to calf thymus DNA in the presence but not in the absence of microsomes, indicating that its conversion to an activated metabolite is required [21].

Generally, the investigators concluded that two different processes may have caused the clastogenicity of OPP: the direct effect of OPP in the absence of metabolic activation and electrophilic reaction of OPP metabolite(s) (e.g., phenylhydroquinone, phenylbenzoquinone) in the presence of metabolic activation [27]. According to another study of Tayama and Nakagawa [28], the involvement of reactive oxygen species (such as H2O2 and $O2^{\cdot -}$) in the presence of metabolic activation was minor. However, Li et al. [24] suggested that the disruption of lysosomal membrane integrity and the oxidative stress, leading to DNA fragmentation, may be the mechanism of DNA damage induced by OPP.

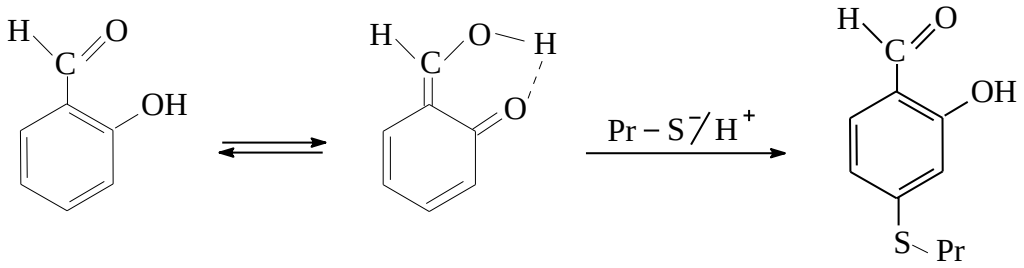
Then, it could be assumed that in the absence of metabolic activation oxygen-derived free radicals may be formed by normal cellular metabolism (peroxidase availability) and/or in aerobic conditions and by exogenous sources such as ionizing radiations, UV radiation, etc. The possible perhydroxylation of resonance stabilized ortho- and para-phenoxy radicals may consistently lead to the formation of the ortho- or para-quinone moieties that take part in Michael-type addition reaction with protein nucleophiles (Scheme 8).



IV. Salicylic acid derivatives

Among the salicylic acid derivatives (such as o-, m-, and p-hydroxybenzaldehydes, salicylic acid and salicylamide), positive results in Chinese hamster cell lines without metabolic activation have been found for salicylaldehyde (o-hydroxybenzaldehyde), salicylic acid and salicylamide.

It may be hypothesized that salicylaldehyde can become reactive via the formation of the corresponding tautomeric oxo-enol form in analogy with 2,6-dihydroxy-4-methylbenzaldehyde [29]. Regardless of the disturbed aromaticity of benzene ring, oxo-enol forms are expected to be partially stabilized by intramolecular hydrogen bond. The ortho-quinone methide structure of enol form may exhibit certain reactivity against protein nucleophiles according to Michael-type addition reaction, as shown for salicylaldehyde in Scheme 9.



keto form oxo-enol form protein-adduct formation

meta- and para-Hydroxybenzaldehydes cannot form an intramolecular hydrogen bond and are more susceptible to air oxidation being converted to the corresponding meta- and para-hydroxybenzenecarboxylic acids.

As with salicylic acid, Reszka et al. [30] were found that it may form phenoxyl radicals and subsequently a biphenol quinone dimmer in the presence of myeloperoxidase and lactoperoxidase systems together with dihydrogen peroxide. This process takes place effectively in slightly acidic to near neutral medium. The reactive intermediate biphenol quinone can form adducts with protein nucleophiles.

Set of chemicals used for profile development	Substituted Phenols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Brooke, D., Mitchell, R., Watts, C., Dungey, S., Indans, I., Environmental Risk Evaluation Report: *para*-C_{12}-Alkylphenols (Dodecylphenol and Tetrapropenylphenol). Science Report, Environmental Agency, UK, May **2007**. 2. Alkylphenols Category, Screening-Level Hazard Characterization. U.S. Environmental Protection Agency, September, **2009**. 3. Thompson, D.C., Thompson, J.A., Sugumaran, M., Moldeus, P., Biological and toxicological consequences of quinone methide formation. *Chem.-Biol. Interact*., **1992**, 86(2), 129-162. 4. Thompson, D.C., Perera, K., Krol, E.S., Bolton, J.L., *o*-Methoxy-4-alkylphenols that form quinone methides of intermediate reactivity are the most toxic in rat liver slices. *Chem. Res. Toxicol*., **1995**, 8(3), 323-327. 5. Okuda, K., Fukuuchi, T., Takigushi, M., Yoshihara, S., Novel pathway of metabolic activation of bisphenol A-related compounds for estrogenic activity. *Drug. Metab. Dispos*., **2011**, 39(9), 1696-1703. 6. Kolšek, K., Mavri, J., Dolenc, M.S., Reactivity of bisphenol A-3,4-quinone with DNA. A quantum chemical study. *Toxicol. In Vitro*, **2012**, 26(1), 102-106. 7. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.D. Cronin, A review of the elecrtophilic reaction chemistry involved in covalent protein binding relevant to toxicity, *Crit. Rev.*

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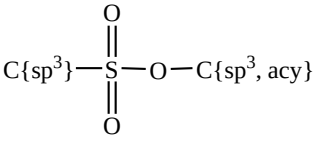
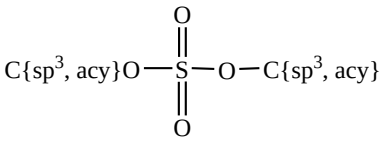
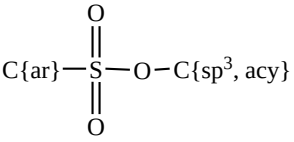
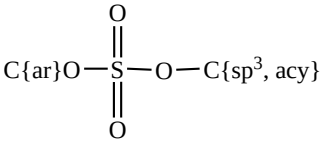
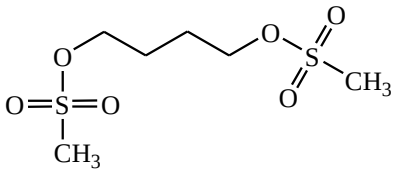
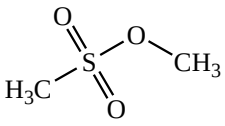
Individual profile/alert

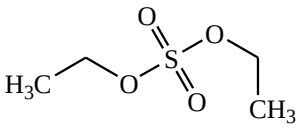
Name	Sulfonates and sulfates
Type of profile	Structural alert
Description/applicability domain	 Alkyl alkanesulphonates  Dialkyl sulfates  Alkyl arenesulfonates  Alkyl aryl sulfates
Mechanism	S_N2, Nucleophilic substitution at sp3-carbon atom (alkylation)

Mechanism of protein binding by sulfonates

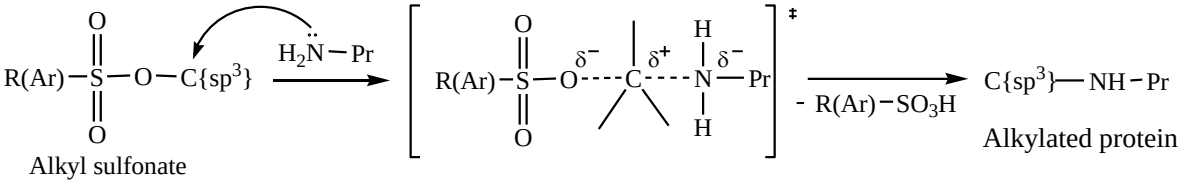
The sulfonate group in alkyl alkane- and arenesulfonates and sulfate group in dialkyl and alkyl aryl sulfates are excellent leaving groups therefore compounds with active structural fragments as well as shown above are alkylating agents via bi-molecular nucleophilic substitution (SN2) mechanism. Busulfan (myleran), methyl methanesulfonate, and diethyl sulfate were found to be active in test for chromosomal aberration in mammalian cells without metabolic activation [4,5].


Busulfan

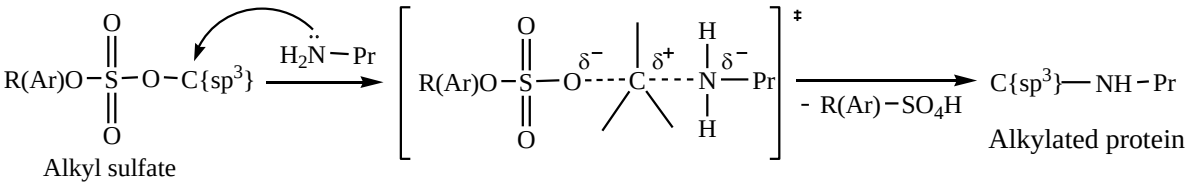

Methyl methanesulfonate


Diethyl sulfate

The activity of these compounds in chromosomal aberration assay is assumed to be result of alkylation of nuclear proteins associated with DNA. The formation of protein adducts via SN2 reaction of sulfonates and sulfates [6] with nucleophilic residues in proteins are shown below:

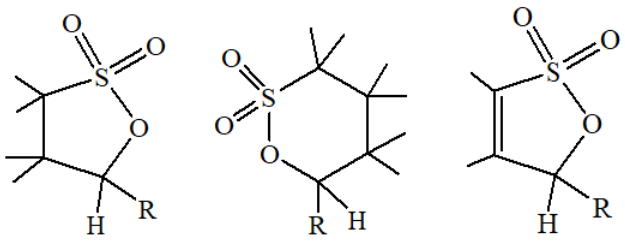

Alkyl sulfonate

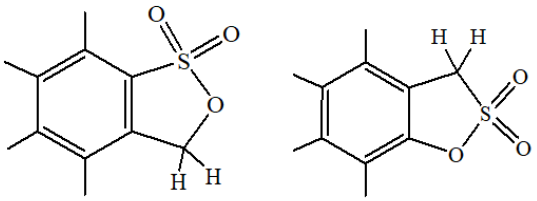
Alkylated protein


Alkyl sulfate

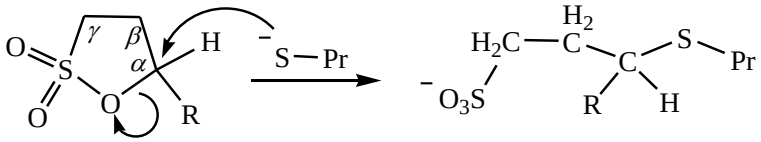
Alkylated protein

Set of chemicals used for profile development	Sulfonates and Sulfates protein binding
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Mekenyan, O., Todorov, M., Serafimova, R., Stoeva, S., Aptula, A., Finking, R., Jacob, E., Identifying the structural requirements for chromosomal aberration by incorporating molecular flexibility and metabolic activation of chemicals. *Chem. Res. Toxicol.*, **2007**, 20(12), 1927-1941. 2. Puyo, S., Montaudon, D., Pourquier, P., From old alkylating agents to new minor groove binders. *Crit. Rev. Oncol. Hematol.*, **2014**, 89(1), 43-61. 3. Estrada, E., Molina, E., Automatic extraction of structural alerts for predicting chromosome aberrations of organic compounds. *J. Mol. Graph. Model.*, **2006**, 25(3), 275-288. 4. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 5. CCRIS: Diethyl Sulfate, CAS No 64-67-5. https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=64-67-5. Last visited: July, 2021. 6. Enoch, S.J., Ellison, C.M., Schultz, T.W., Cronin, M.T., A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol.*, **2011**, 41(9), 783-802.

Individual profile/alert	
	Sultones protein binding
Type of profile	Structural alert
Description/applicability domain	

	 R = H, C{sp3, acy}
Mechanism	SN2, Ring opening SN2 reaction

The correlations between the biological action pattern and chemical reactivity of sultones as alkylating agents and rate constants for the reactions of 1,3-propane sultone and 1,4-butane sultone with a number of nucleophiles at physiological temperature have been determined [2]. The comparison degrees of alkylation of alkane sultones and some sulfonate and sulfate open-chain esters (for example methyl methanesulfonate and dimethyl sulfate) shown similarity in their reactivity as alkylating agents [2]. The activity of these compounds in chromosomal aberration assay is expertly assumed to be result of alkylation of nuclear proteins associated with DNA. The formation of a protein adduct of alkane 1,3-sultones by ring opening SN2 mechanism with nucleophilic residues in proteins [3,4] is shown below:



Sultone

R = H, alkyl chain

Alkylated protein

The presence of the alkyl group (R) in the □-position of alkane sultones leads to marked reduction in their electrophilicity [3].

Set of chemicals used for profile development	Sultones protein binding
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Ishidate, M. Jr, Odashima, S., Chromosome tests with 134 compounds on Chinese hamster cells in vitro--a screening for chemical carcinogens. Mutat. Res., 1977, 48(3-4), 337-353. 2. Osterman-Golkar, S., Wachtmeister, C.A., On the reaction kinetics in water of 1,3-propane sultone and 1,4-butane sultone: a comparison of reaction rates and mutagenic activities of some alkylating agents. Chem. Biol. Interact., 1976, 14(1-2), 195-202. 3. Lepoittevin, J.-P., Basketter, D.A., Goosens, A., and Karlberg, A.-T., Eds. Allergic Contact Dermatitis The Molecular Basis.

	Springer, Heidelberg. 1998, pp. 100-102. 4. Rüegg, U.T., Rudinger, J., Alkylation of cysteine thiols with 1,3-propane sultone. Int. J. Pept. Protein Res., 1974, 6(6), 447-456. 5. Roberts, D.W., Williams, D.L., Bethell, D., Electrophilic reactions of skin-sensitizing sultones. Chem. Res. Toxicol., 2007, 20(1), 61-71.