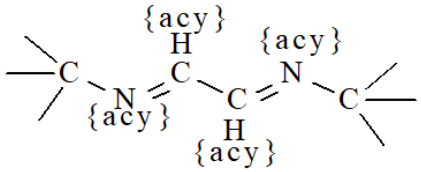
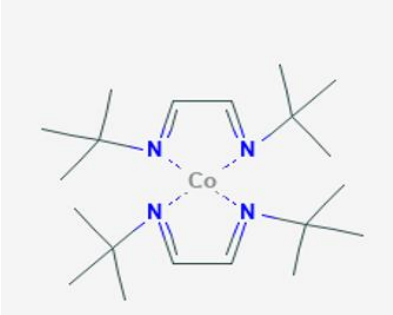
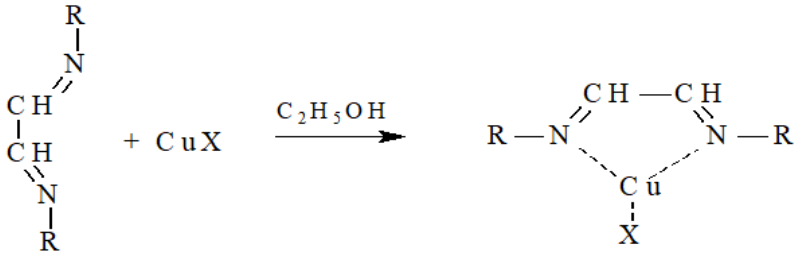
Individual profile/alert	
Name	1,4-Diazabutadiene Derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: Radical Mechanistic Alert: ROS generation

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]:

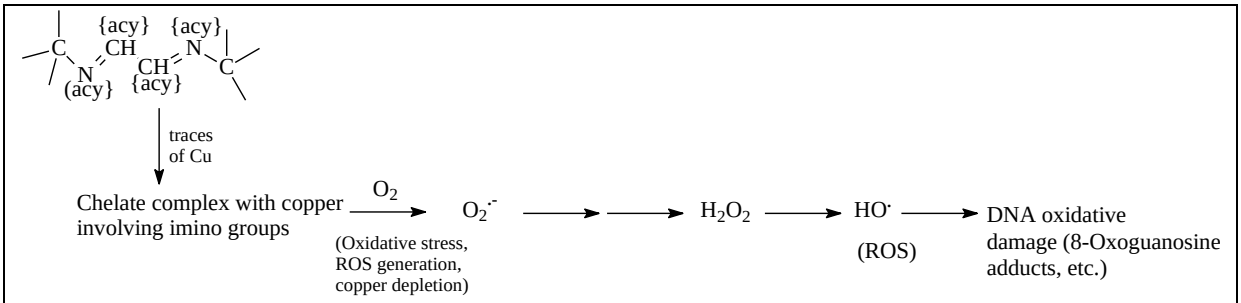


(X is Cl or I; R is C{ar}, C{sp3})

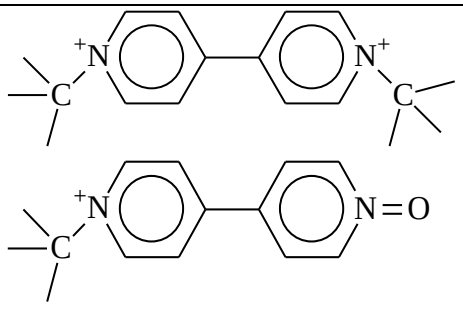
Also, novel tetrahedral copper(I) mixed-ligand complexes with other diimine-type ligands were synthesized, and genotoxicity effects of these complexes were suspected [3].

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].

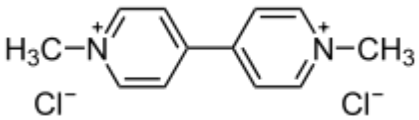
Based on the above speculations, and the presence of traces of transition metals such as coper in the incubation medium, the following, rather simplified mechanistic scheme can be expertly proposed:



Set of chemicals used for profile development	1,4-Diazabutadiene Derivatives.txt
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.

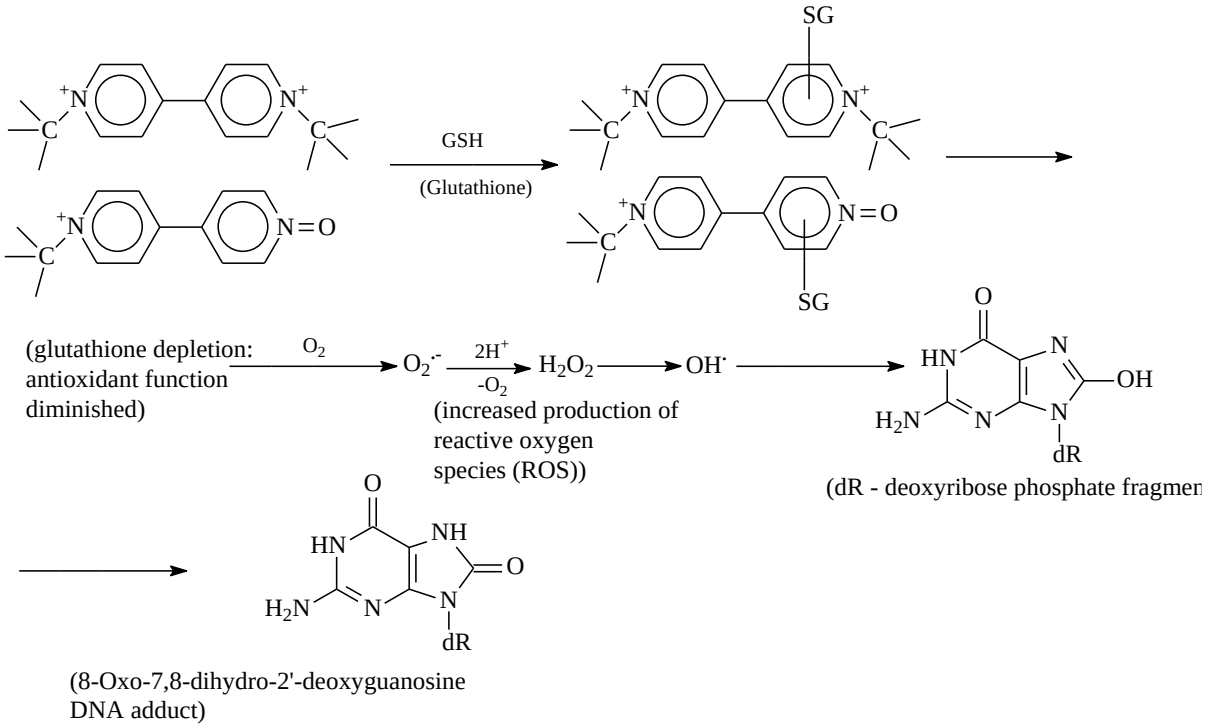
Individual profile/alert	
Name	4,4’-Bipyridinium Salts and N-Oxides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: Radical Mechanistic Alert: Radical mechanism via ROS formation

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



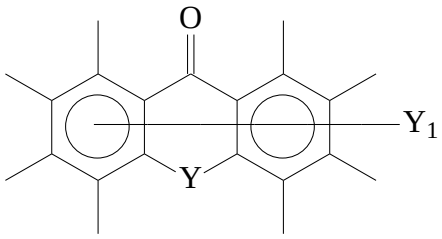
was used as an intracellular generator of oxygen free radicals, and was found to be highly mutagenic for Salmonella typhimurium. It caused both base-pair substitution and frameshift mutations. The mutagenicity of paraquat was dependent on the presence of a supply of both electrons and oxygen. The mutagenicity 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



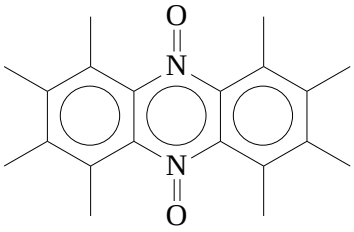
(Y is O, S{V_2}, N{V_3})

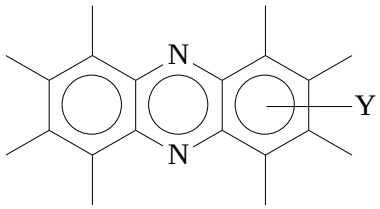
(Y_1 can be -OH, -O-CH_3, -NH{{sp3}{V3}, -CH_3, -CH_2OH, —C(=O)—NH

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



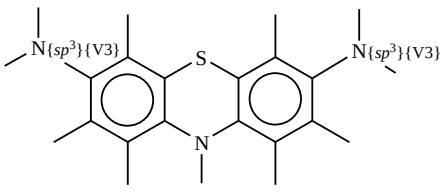


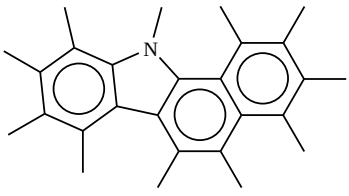
(Y can be combinations between -H and -NH_2 or -H, NH_2 and -OH or OCH_3)

(I) (II)

(IV): Phenothiazine derivatives derivatives

(V): Benzocarbazole



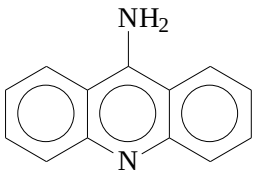


(IV) (V)

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].

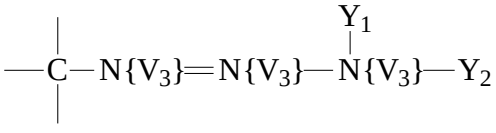
The bacterial mutagenicity of another class of chemicals, phenazine derivatives such as 2,3-diaminophenazine and 2-amino-3-hydroxyphenazine, both without and with S9 metabolic activation has been proved in the Salmonella typhimurium strain TA98. This suggests that, similarly to other major mutagenic 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].

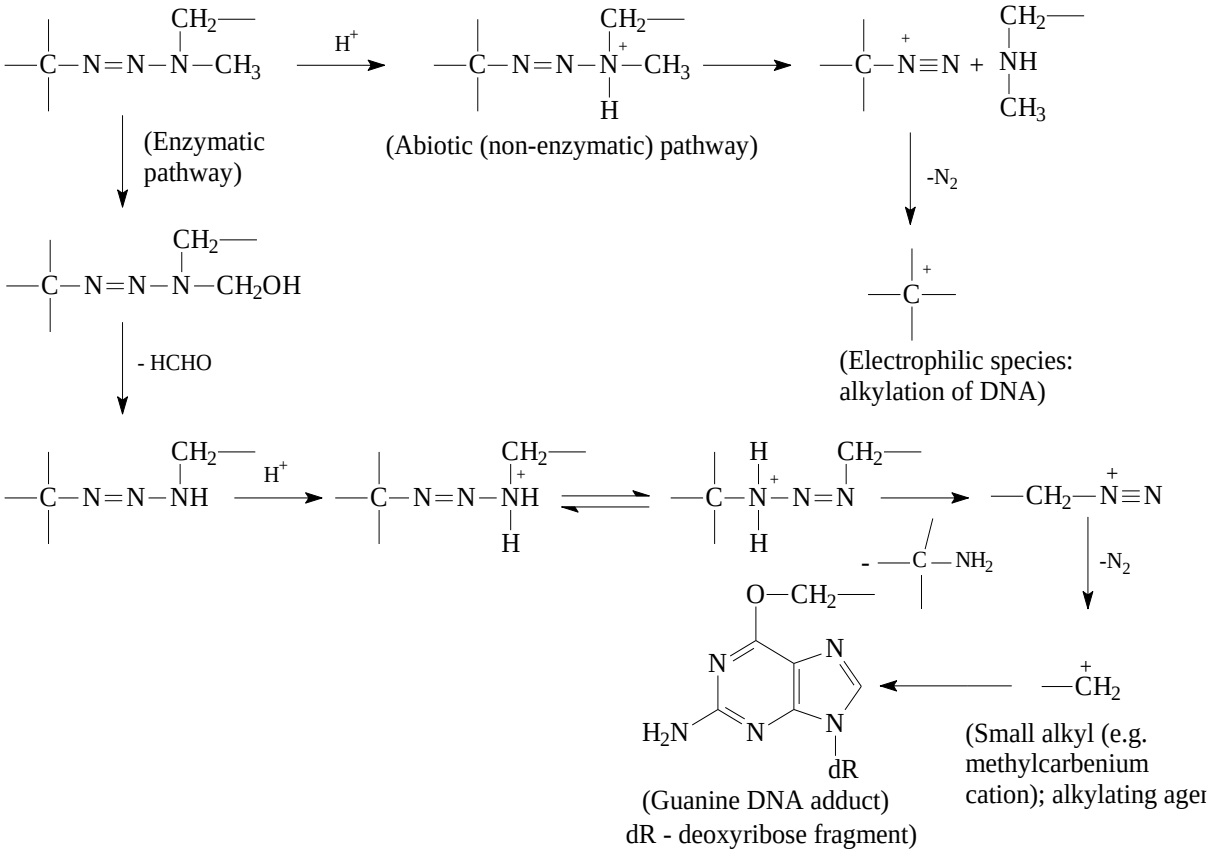
The planar structure of phenothiazine derivatives suggests DNA intercalation as a possible mechanistic explanation of their bacterial mutagenicity [10]. Such as assumption was also postulated for some benzocarbazole derivatives [11].

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.

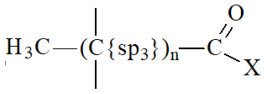
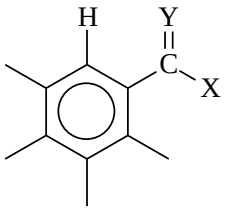
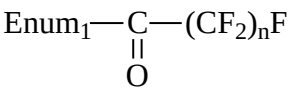
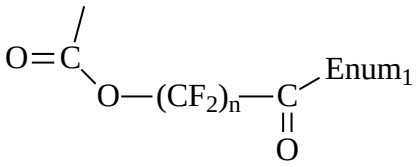
Individual profile/alert	
Name	Acyclic Triazenes
Type of profile	Structural alert
Description/applicability domain	 (Y_1, Y_2 are $-CH_3$, or $-H_2C-C_6H_5$ or $-CH_2CH_3$ or -H or $-CH(CH_3)_2$ (number of -H can be 0 or 1))
Mechanism	Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after 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:



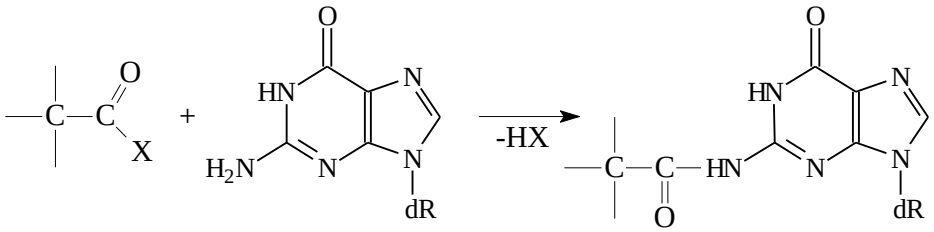
Set of chemicals used for profile development	Acyclic Triazenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

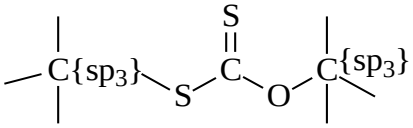
Such chemicals are believed to be direct-acting mutagens predominantly.
Mixed results for bacterial mutagenicity (Ames test) have been obtained for benzoyl chloride [1]. Generally, acyl halides are known to undergo nucleophilic substitution reactions and are mostly mutagenic, i.e., and capable of interacting 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.

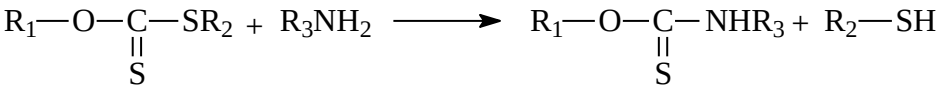
A mixture of methylglyoxal and hydrogen peroxide has been found to react with 2'-deoxyguanosine to form N2-acetyl-2'deoxyguanosine [4]. By analogy, direct DNA acylation mechanism by acyl halides such as acetyl chloride can be expertly suggested:



Deoxyguanosine fragment DNA acylated adduct

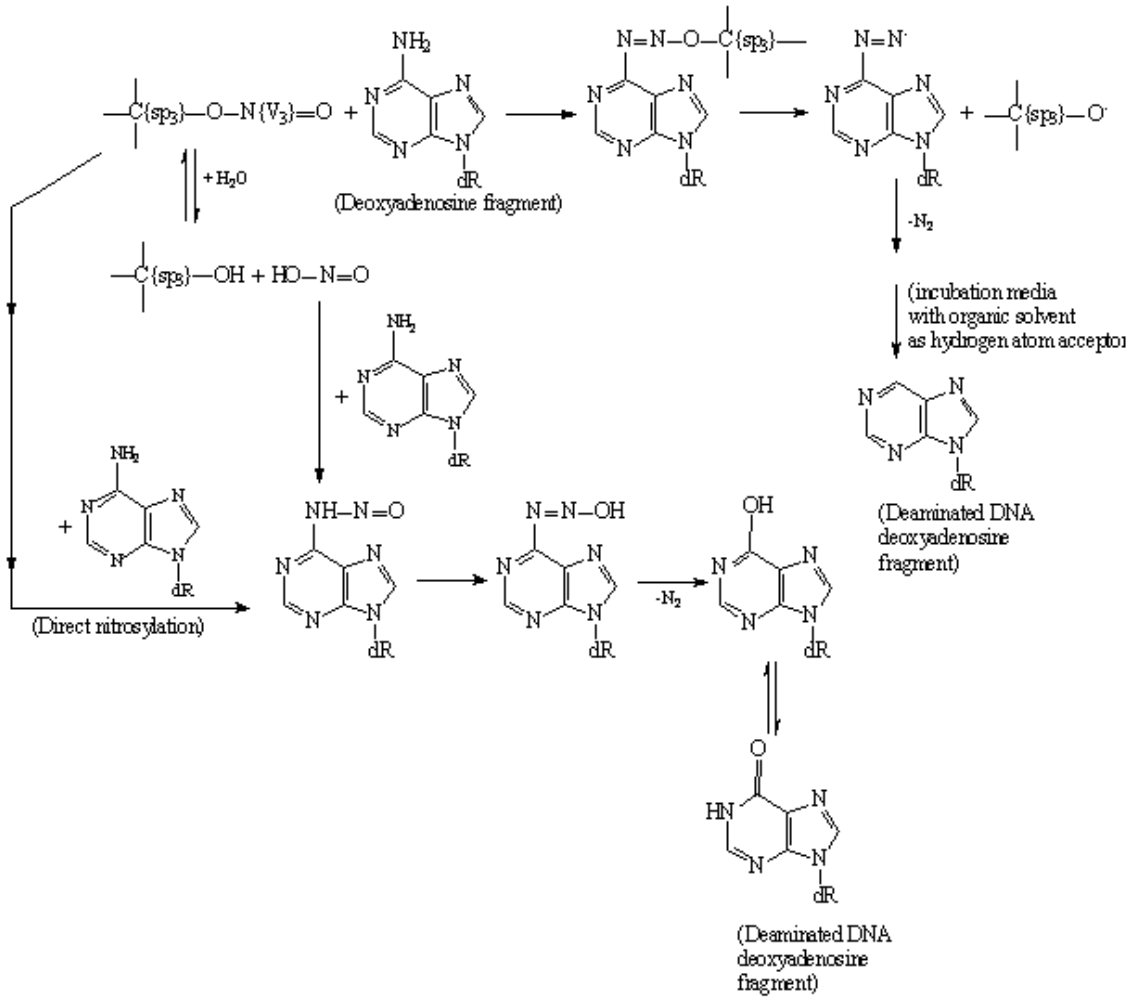
Set of chemicals used for profile development	Acyl Halides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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



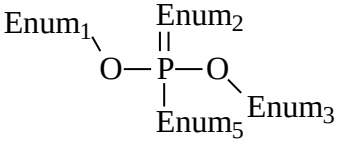
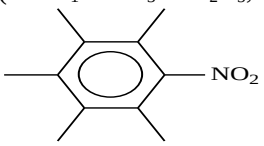
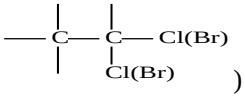
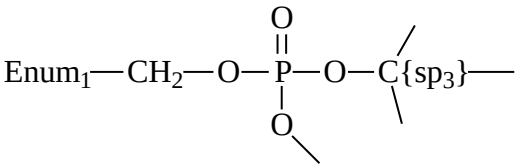
Set of chemicals used for profile development	Alkyl Xanthate Esters
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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	

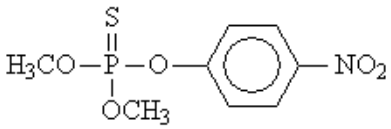


Set of chemicals used for profile development	Alkylnitrites
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

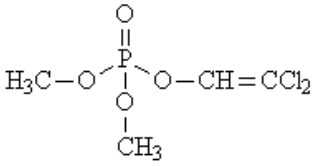
	Esters – A Genetical Hazard from Nitrogen Oxides (NO_x), Hereditas **92**(1) (1980), 127 – 130.

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:



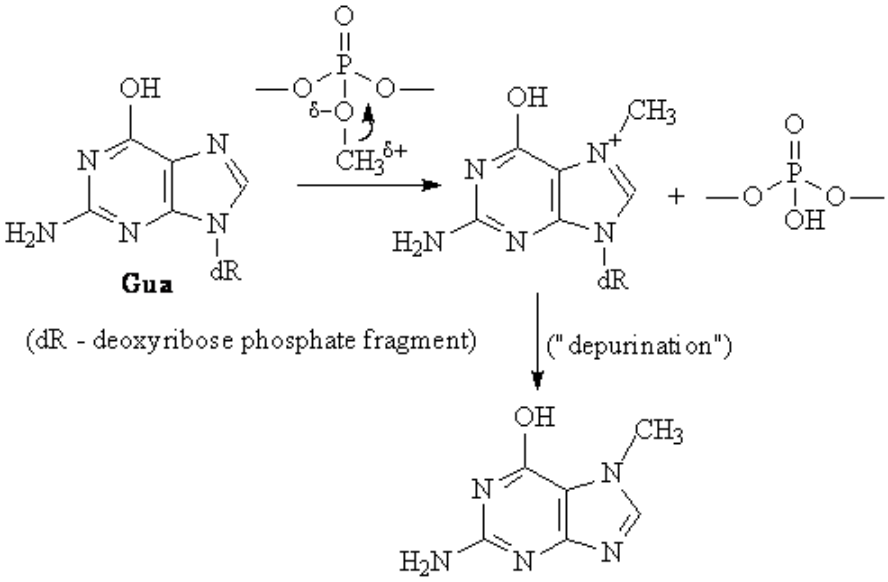
which belongs to the organothiophosphate group of insecticides also exhibits mutagenicity [2]: perhaps the aromatic nitro group strongly contributes to this effect. Alkylation of DNA has been proposed as the essential step for mutation interactions of the organophosphate insecticides dichlorovos and 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].

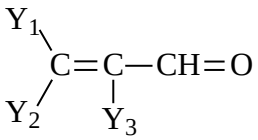
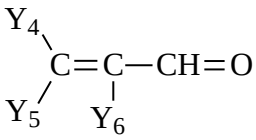
Therefore, the alkylation mechanism seems to be more plausible for this class of compounds, as expertly outlined below in Scheme 1

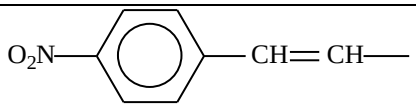


Scheme 1

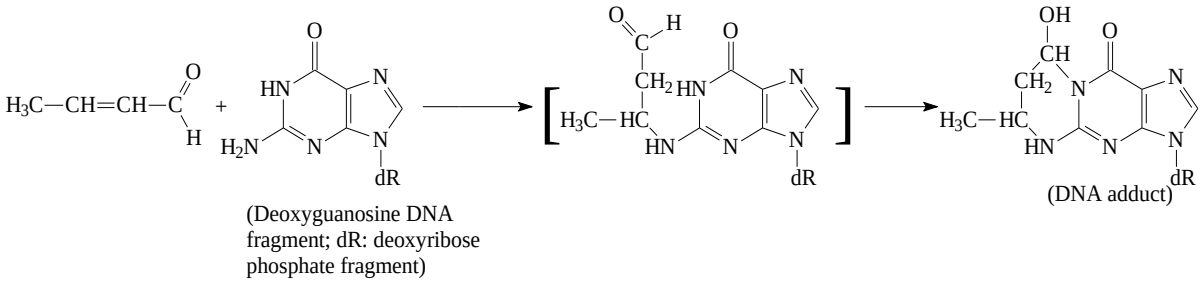
Set of chemicals used for profile development	Alkylphosphates, Alkylthiophosphates and Alkylphosphonates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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

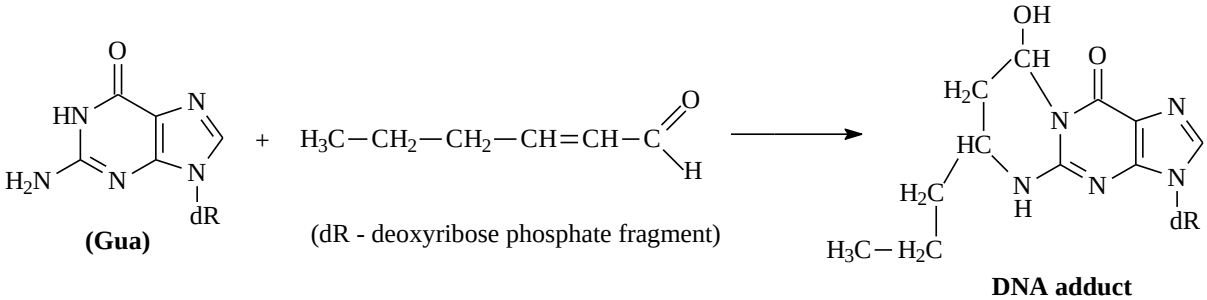
	 ; Y_6 is –H, X or CH_3
Mechanism	Mechanistic Domain: A_N2 Mechanistic Alert: Nucleophilic addition to α,β-unsaturated carbonyl compounds Mechanistic Domain: A_N2 Mechanistic Alert: Schiff base formation

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:



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]:

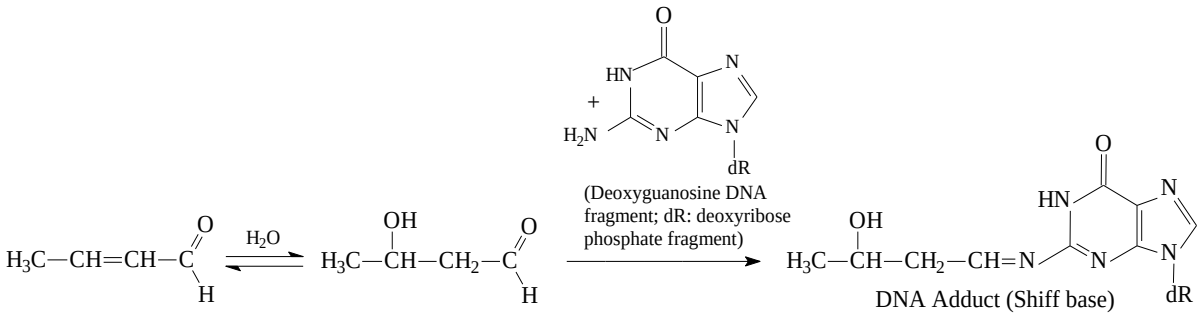


Scheme 2

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*

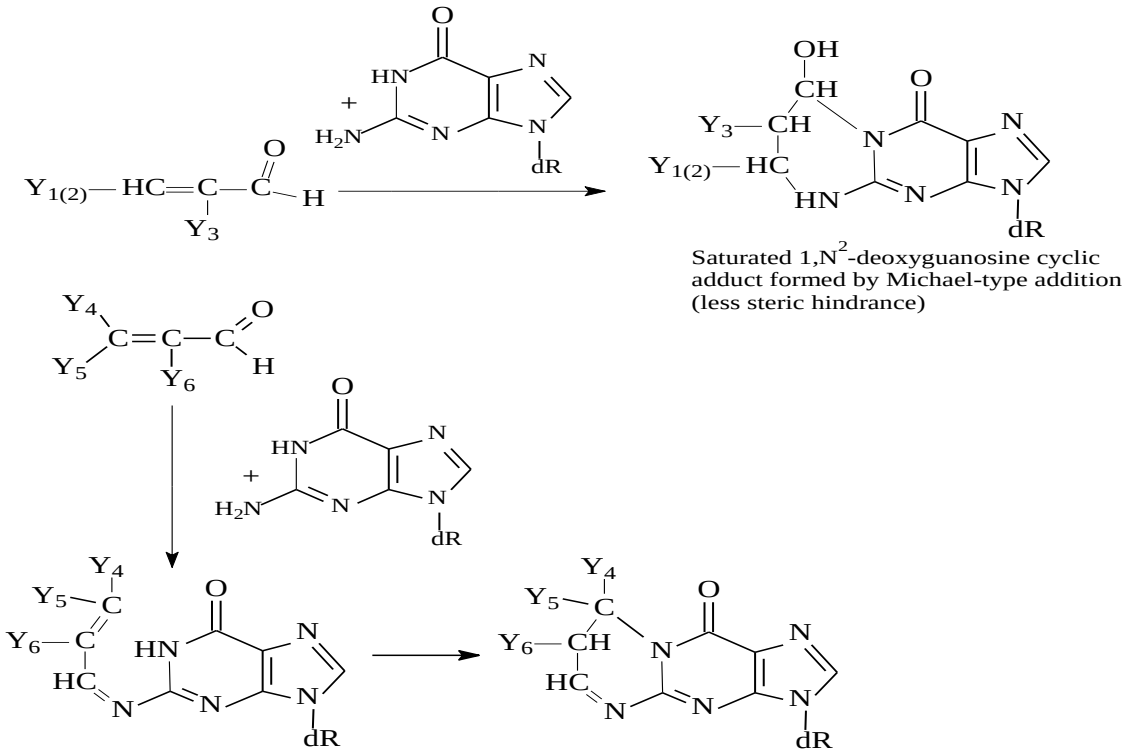
TA 100 without exogenous metabolic S9-activation system; however, the results were affected by the bacterial toxicity of alkylacroleins. This increasing toxicity was explained by the ability of compounds with longer alkyl chain to better penetrate into the bacterial cell, due to their higher hydrophobicity. 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:



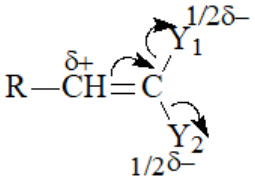
Scheme 4

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.

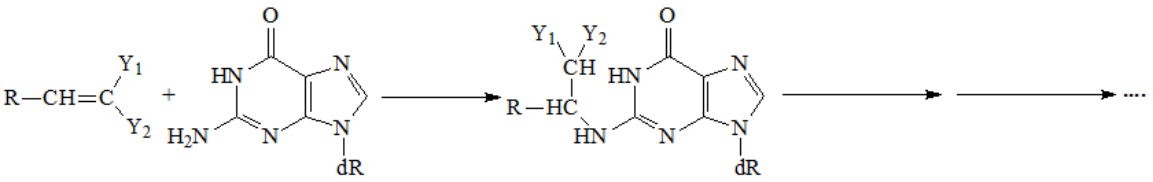
Individual profile/alert	
Name	Alpha-Beta Conjugated Alkene Derivatives with Geminal Electron-Withdrawing Groups
Type of profile	Structural alert
Description/applicability domain	 (R is C or H; Y_1, Y_2 are —C≡N or —NO_2 or —CH=O or —C(=O)OCH_3 or —C(=O)OH ; Y_1 and Y_2 belong to different-type functionalities))

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:



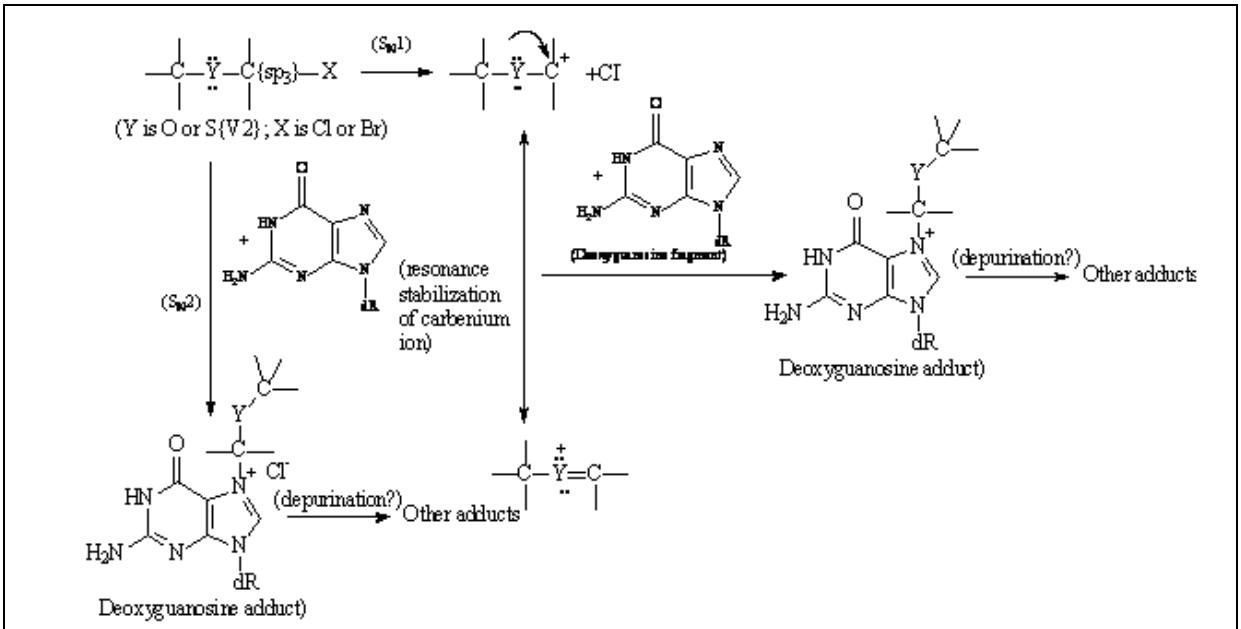
Thus some DNA alkylating capability becomes possible and it could materialize itself via mechanistic scheme, similar to Michael-type addition [4, 5], as follows:



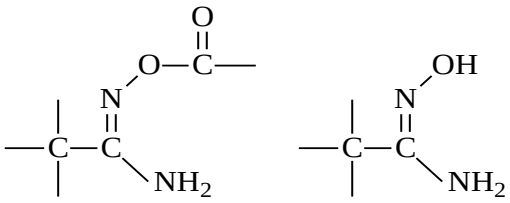
Set of chemicals used for profile development	Alpha-Beta Conjugated Alkene Derivatives with Geminal EWG
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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	—C—Y—C{sp_3}—X (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:

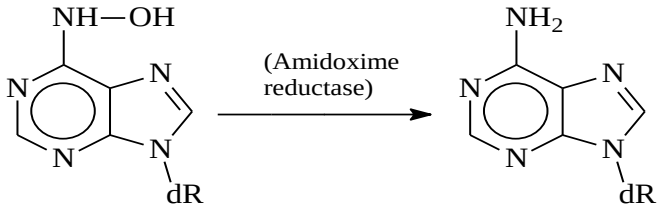


Set of chemicals used for profile development	Alpha-Haloethers
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

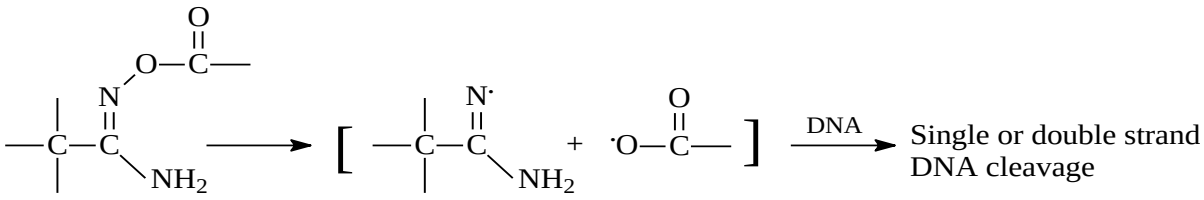
The metabolic reduction of some N-hydroxylated compounds such as amidoximes (N-hydroxylamines) as pro-drugs of amidines has been reported. Due their strong basicity, amidines, guanidines, and amidinohydrazones are protonated under physiological conditions, being very hydrophilic, and are usually not 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)

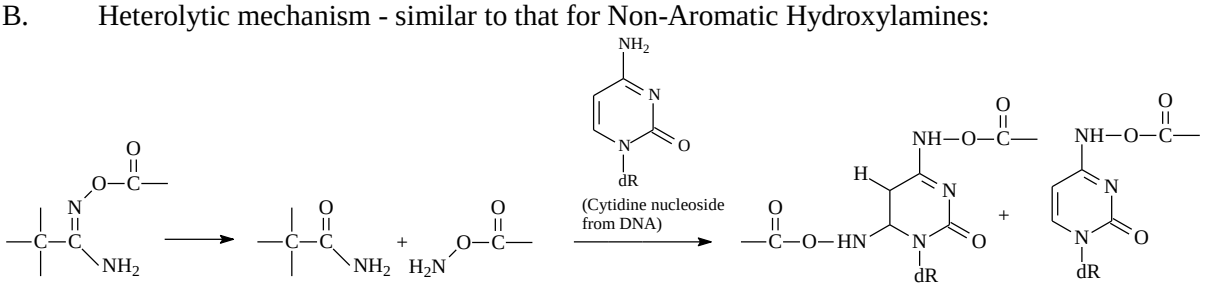
Some amidoxime ester derivatives have been subjected to UV irradiation, to undergo photo-cleavage of the N-O bond via radical mechanism. They act as metal-free DNA photo-cleavers, and the homolysis of the weak N-O bond of the oxime ester generates active aroyloxyl or heteroaroyloxyl radicals, able to 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)

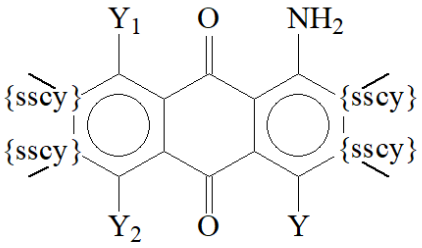
B. Heterolytic mechanism - similar to that for Non-Aromatic Hydroxylamines:

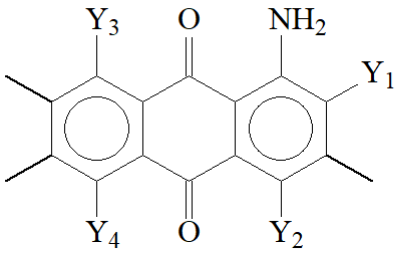


(Expertly assumed adducts)

Set of chemicals used for	Amidoxime Esters and Amidoximes

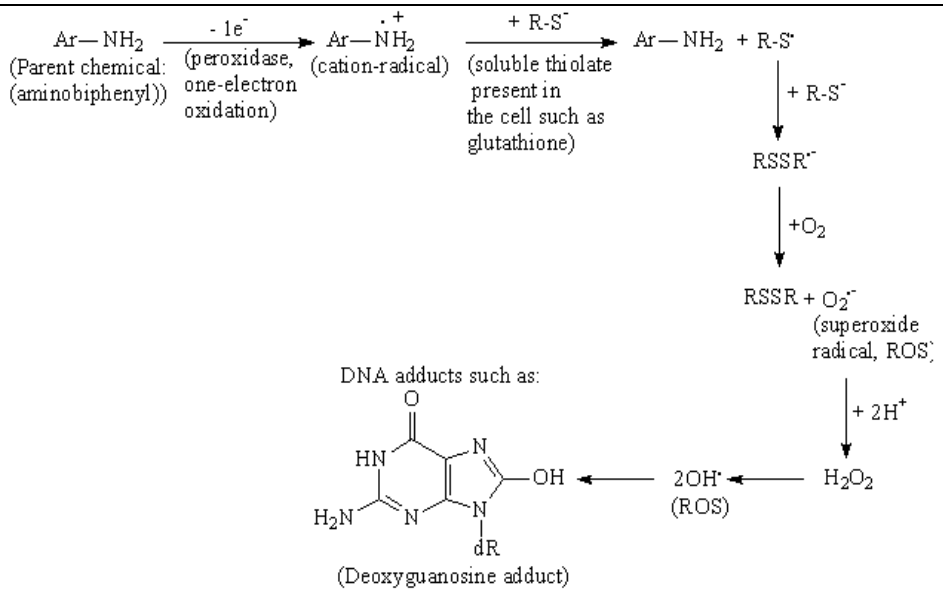
profile development	
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Clement, B., Reduction of N-Hydroxylated 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)

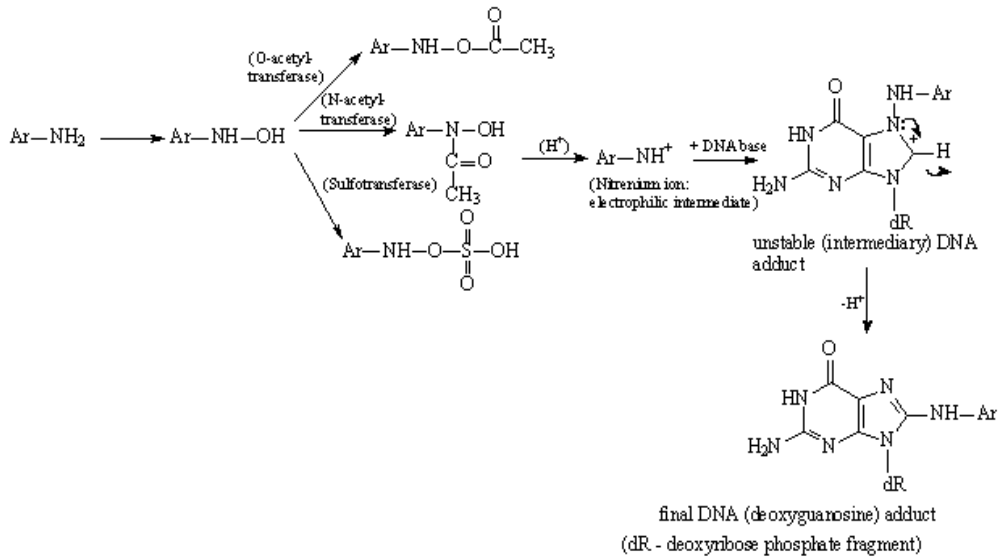
DNA intercalation: The presence of some electron-donating substituents with +M-effect can contribute to the direct mutagenicity of such chemicals, since the benzene rings become more electron-rich and this enhances the non-covalent interaction of the parent chemicals with DNA. Particularly important 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

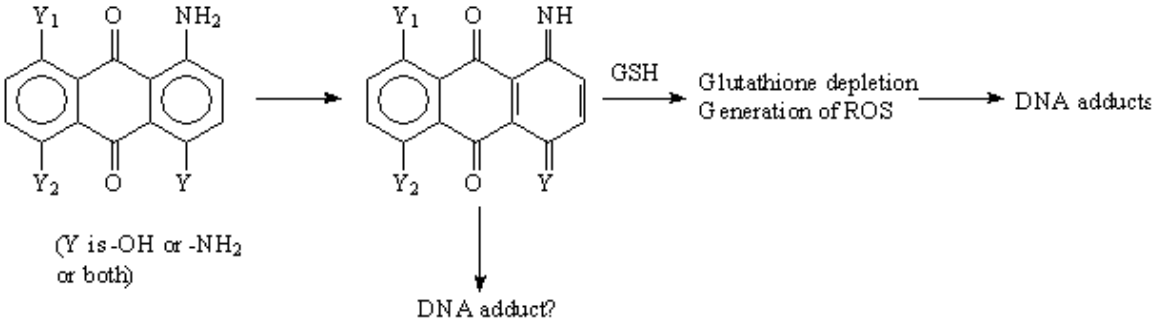
Mutagenicity after metabolic activation with S9 mix. There is strong evidence that aromatic amines, including aminoanthraquinone derivatives in many cases require metabolic activation with the external microsomal S9 system for eliciting mutagenicity and carcinogenicity. According to an excellent review 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

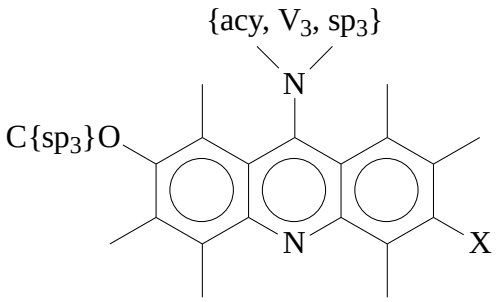
Not only is nitrenium ion chemistry implicated in the DNA damage. For some specific anthraquinone

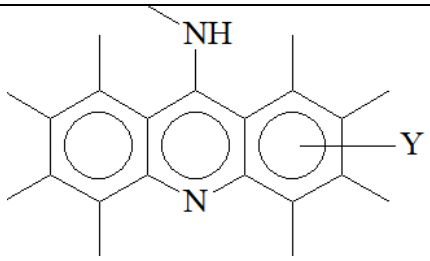
derivatives with electron-donating substituents mutually located at *p*- or *o*-positions, reactions associated with the formation of quinones, quinone imines or other quinoid structures could be involved in the elucidation of the overall mechanistic scheme of bioactivation shown below in Scheme 3 [12]:



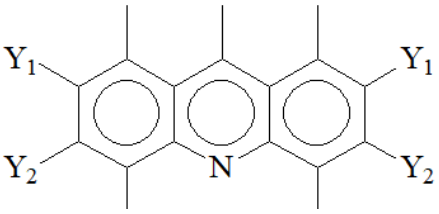
Scheme 3

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)

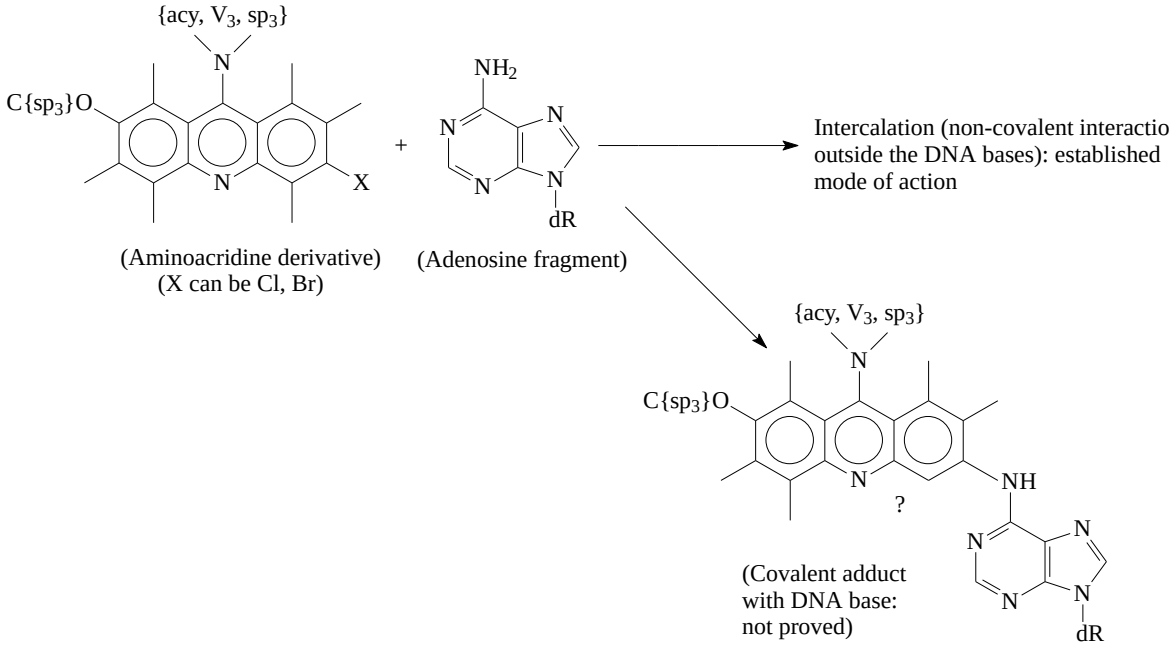


Y = -H, -NH_2

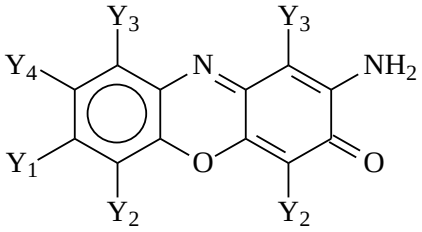


Y_1 = -H, -C{sp3}; Y_2 = -NH_2, -NHC{sp3}, -N(Csp3)$_2$

Mechanism	**Non-covalent interactions DNA intercalation**



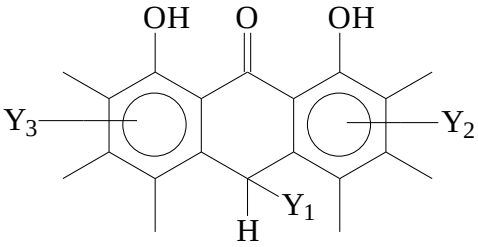
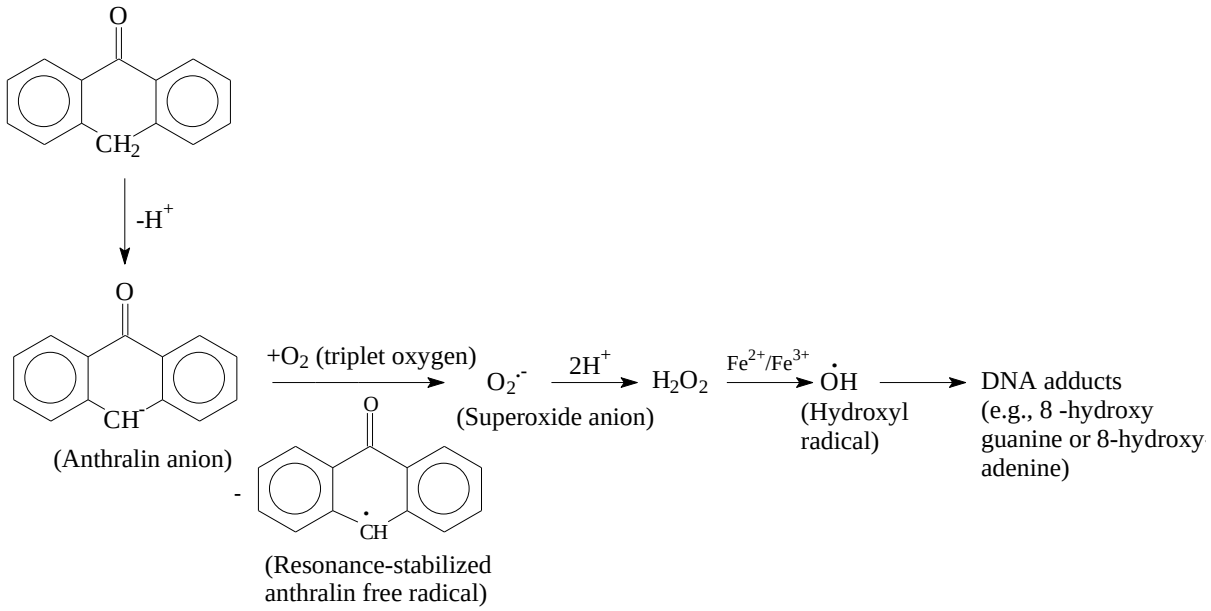
Set of chemicals used for profile development	Aminoacridine DNA Intercalators
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

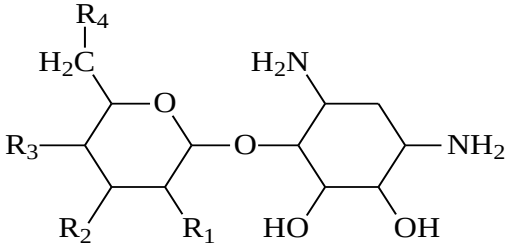
Individual profile/alert	
Name	Aminophenoxazinone Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y_1 is -H or -Cl or -Br; Y_2 is -H or -CH_3 Y_3 is -H or -C(O)NH-; Y_4 is -H or -OH)
Mechanism	**Non-covalent interactions DNA intercalation**

Scheme 1
contains aminophenoxazone principal structural fragment (see the bottom part of the above depiction). It is a potent anti-tumor antibiotic, that preferentially binds to DNA via the (G-C)·(G-C) steps on the duplex DNA [1]. The mutagenic activity of urine was evaluated in children receiving single and multiple anti-cancer agents chemotherapy to determine the duration of carcinogenic risk to health care personnel and family contacts. Urine samples from a group of children were evaluated before and daily for 5 days after chemotherapy administration. Mutagenic activity, a sensitive though not specific indicator of carcinogenic risk, was assayed using mutant strains of Salmonella typhimurium (the "Ames test"). None of the children tested demonstrated mutagenic activity before chemotherapy administration. Following single agent administration of several chemicals, among which, Dactinomycin, mutagenic activity was demonstrated for 2 days. Following multiple agent chemotherapy using two or three of the latter drugs on a single day, mutagenic activity was demonstrated for 4 or 5 days [2]. Therefore it could be assumed that the target chemical and its structurally similar derivatives possess positive in vitro bacterial mutagenicity.
Mechanistic considerations associated with positive in vitro mutagenicity and in vitro metabolism comments
The binding of the antibiotic Actinomycin D discussed above via hydrogen bonding between DNA C-G base pair and amino group in the molecule of phenoxazone derivative is the assumed intercalative mechanism of mutagenicity for the aminophenoxazone derivative in the Ames test [3]. In addition, such mode of binding to DNA fragments via intercalation is probably facilitated by the combination of the highly polar functionalities and conjugated double bonds, including the existing quinone imine-type fragment in the molecular structure of the target chemical.
No extended in vitro microsomal/S9 metabolism of 2-Amino-7-chloro-3H-phenoxazin-3-one as the target chemical, involving phase I transformations on the phenoxazinone ring can be expected. In addition, no other published data on the in vitro metabolism has been found. Phase II metabolites such as glucuronides were only detected [3].

Set of chemicals used for profile development	Aminophenoxazinone 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. Chen, H., Liu, X., Patel, D.J., J. Mol. Biol. 258(3) (1996), 457 - 479. doi: 10.1006/jmbi.1996.0262. 2. Maniar AC, Williams TW, Hammond GW, Johnson N, Kobrinsky NL. Excretion of mutagens following chemotherapy. Am. J. Pediatr Hematol. Oncol. 1991 Summer;13(2):160-3. doi: 10.1097/00043426-199122000-00010. PMID: 2069224; (Abstract:

	https://pubmed.ncbi.nlm.nih.gov/2069224/. Last visited: Martch, 2024. 3. Jensen B. M., Kh. B. Adhikari, H. J. Schnoor, N. Juel-Berg, I. S. Fomsgaard, L. K. Poulsen, Eur. J. Nutr. 2015; DOI 10.1007/s00394-015-1088-6; https://link.springer.com/article/10.1007/s00394-015-1088-6. Last visited: March, 2024.

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)
 (Anthralin anion) (Resonance-stabilized anthralin free radical) (Superoxide anion) (Hydroxyl radical) DNA adducts (e.g., 8 -hydroxy guanine or 8-hydroxy adenine)	
Set of chemicals used for profile development	Anthrones
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).

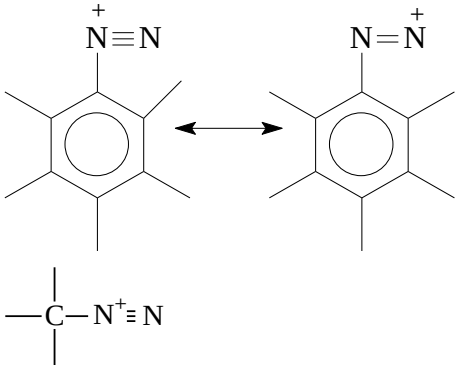
Individual profile/alert	
Name	Antibiotic Aminoglycoside Derivatives
Type of profile	Structural alert
Description/applicability domain	 (R_1 and R_4 are NH_2, R_2 and R_3 are OH; R_1 is NH_2, R_2 - R_4 are OH; R_1 is OH, R_2 is NH_2, R_3, R_4 are OH; R_1, R_2 are OH, R_3 is NH_2, R_4 is OH; R_1 – R_3 are OH, R_4 is NH_2 or CH=O)
Mechanism	Mechanistic Domain: Non-covalent interactions Mechanistic Alert: DNA intercalation

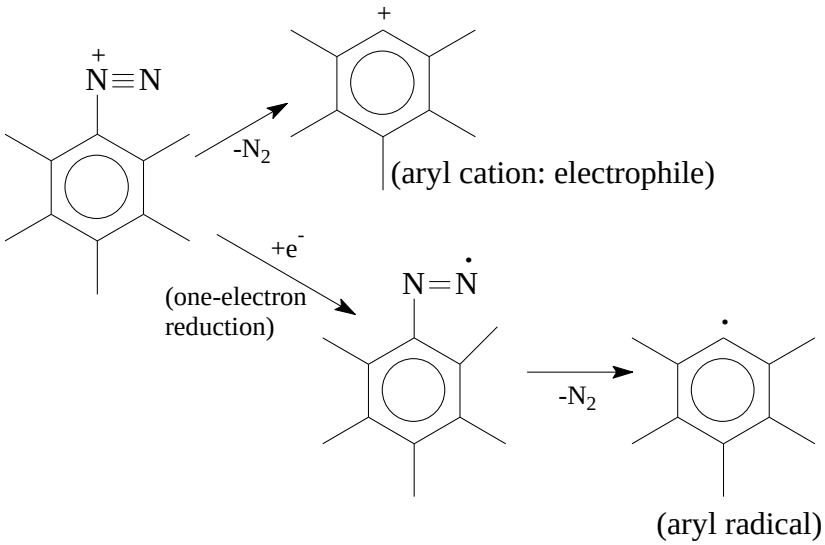
The structure/activity relationships of the pseudodisaccharide core found in aminoglycoside antibiotics were qualitatively studied with a series of synthetic analogues, in which the position of amino groups was varied around the glucopyranose ring. The naturally occurring compound Neamine was the most 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].

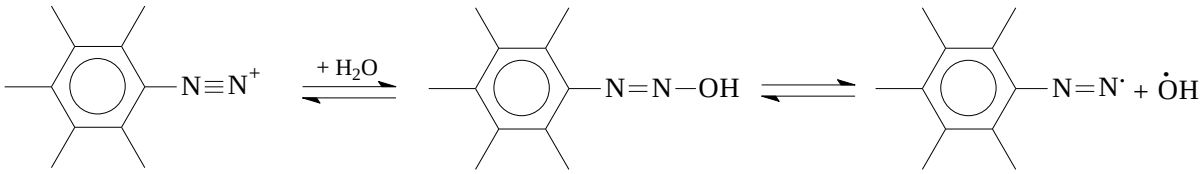
Set of chemicals used for profile development	Antibiotic Aminoglycoside Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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.

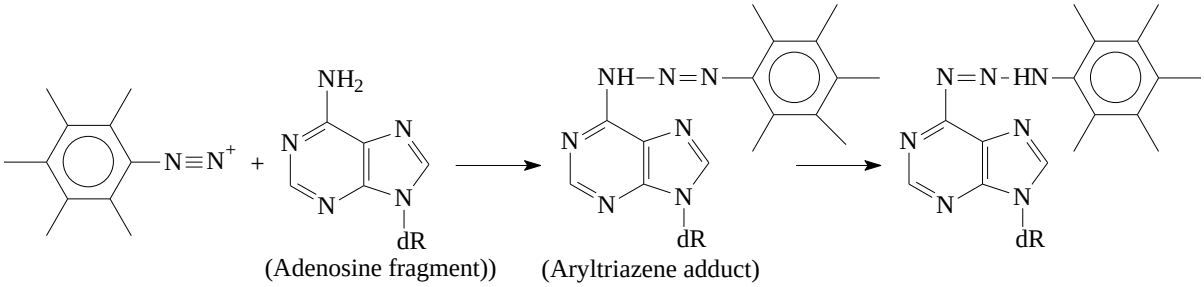
Individual profile/alert	
Name	Arenediazonium and Diazonium Salts
Type of profile	Structural alert
Description/applicability domain	 (C: any carbon atom) (Note: If C is C{ar}, then the two resonance forms should remain as active alerts, since in some (rare) cases, the 2D structure comes as the resonance 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:	

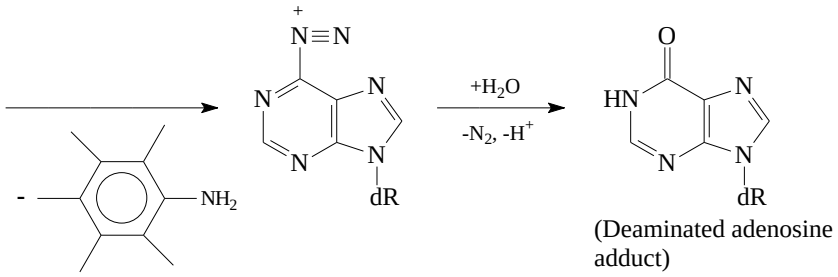


Also, hydroxyl radicals can be formed under these conditions:

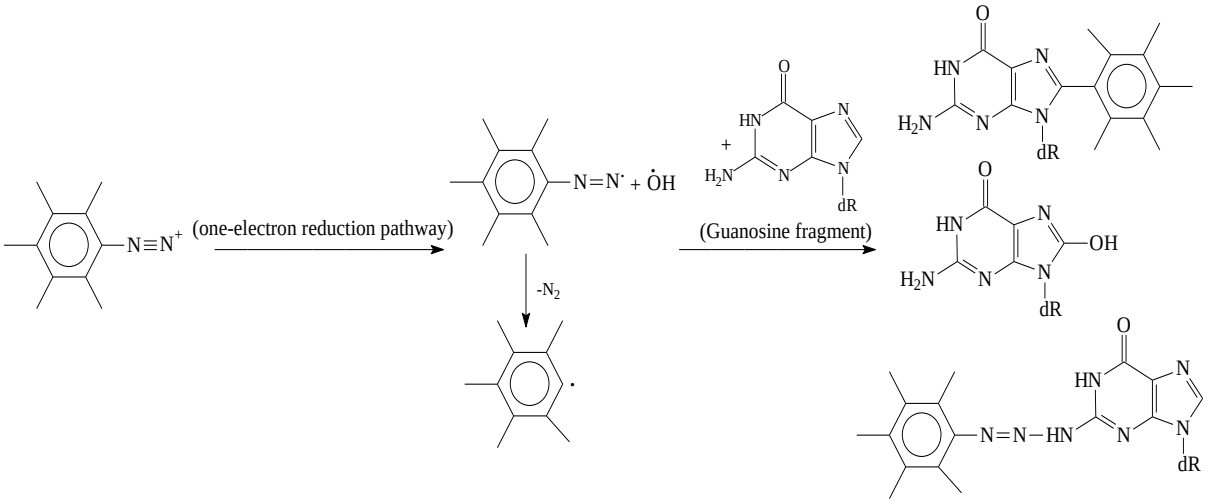


When the arenediazonium ion is the reactive intermediate, the following scheme for the formation of adduct seems to be operative:





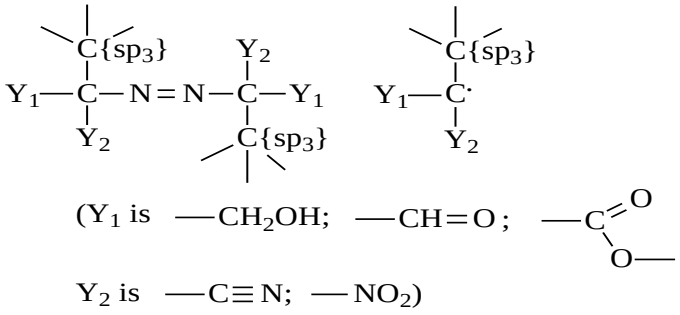
Alternatively, radical mechanism is also possible:



(Some possible adducts formed)

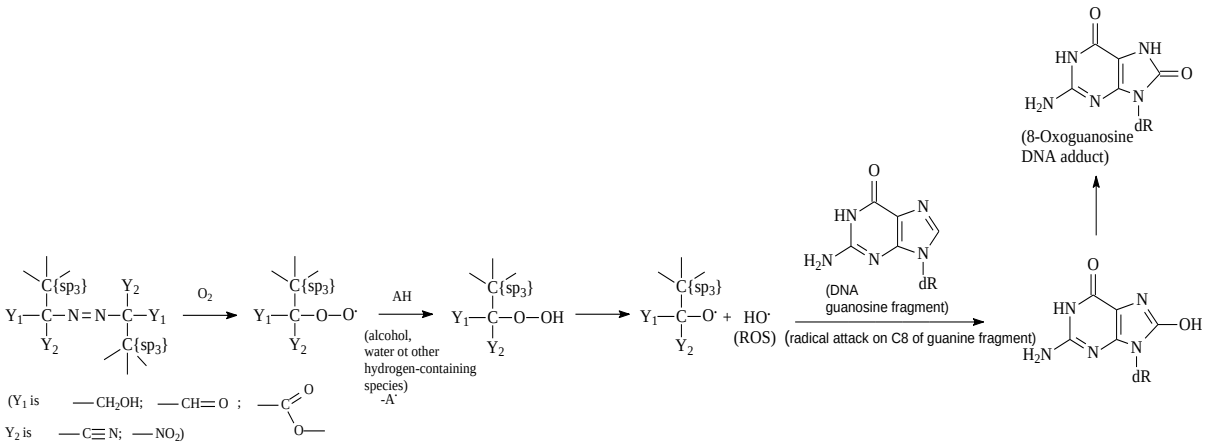
The C8 position of guanine is sufficiently nucleophilic to react directly with arenediazinium cations but it is more reactive to radicals, as shown in the scheme above. The nature of the reactive species depends mainly on the kind of substituent on the phenyl ring of the arenediazonium salt [1]. The 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 Elactron
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:

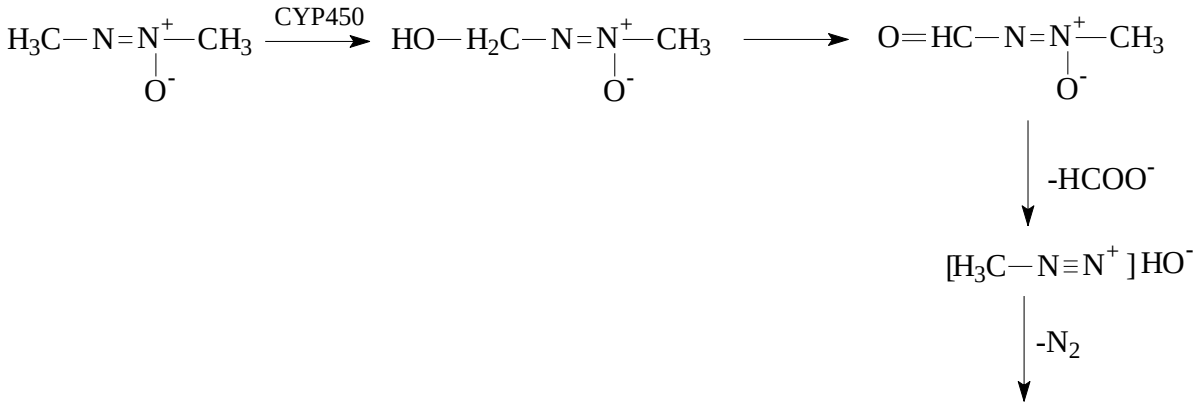


Set of chemicals used for profile development	Azoalkanes with Activating EWG
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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	—C{sp3}—N(=O)=N—C{sp3}— or —C{sp3}—N+(O-)=N—C{sp3}— —C{sp3}—N(=O)=N—CH2OH or —C{sp3}—N+(O-)=N—CH2OH
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]:



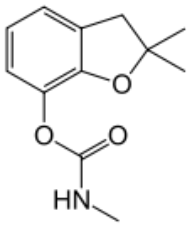
The mechanistic scheme is consistent with the assumption that the active end-products of chemicals such as 1,2-dimethylhydrazine and azoxymethane which cause mutagenic DNA lesions such as O6-methylated guanosine and O4-methylated thymidine [5].

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	Benzofuranyl Carbamate Derivatives
Type of profile	Structural alert

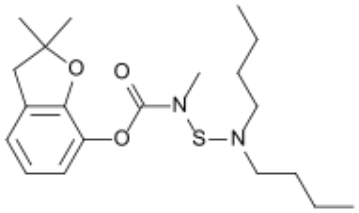
Description/applicability domain	
Mechanism	Non-covalent interactions

Positive bacterial mutagenicity results for the carbamate-type pesticide *Carbofuran* (2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate):



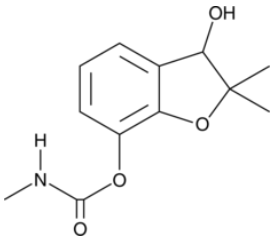
have been reported [1]. According to other published reviews, various carbofuran lots in the *Ames/Salmonella* test showed mixed results, including positive ones obtained with *Salmonella typhimrium* strains TA 1535 and TA 100 in the absence of an S9 rat microsomal activating system [2]. Positive *in vitro* genotoxicity of Carbofuran was also reported in other publications [3].

The positive *in vitro* mutagenicity for this sub-class of chemicals is also supported by the data from another structurally similar benzofuranyl carbamate derivative, the insecticide *Carbosulfan* (2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl [(dibutylamino)sulfanyl]methylcarbamate):

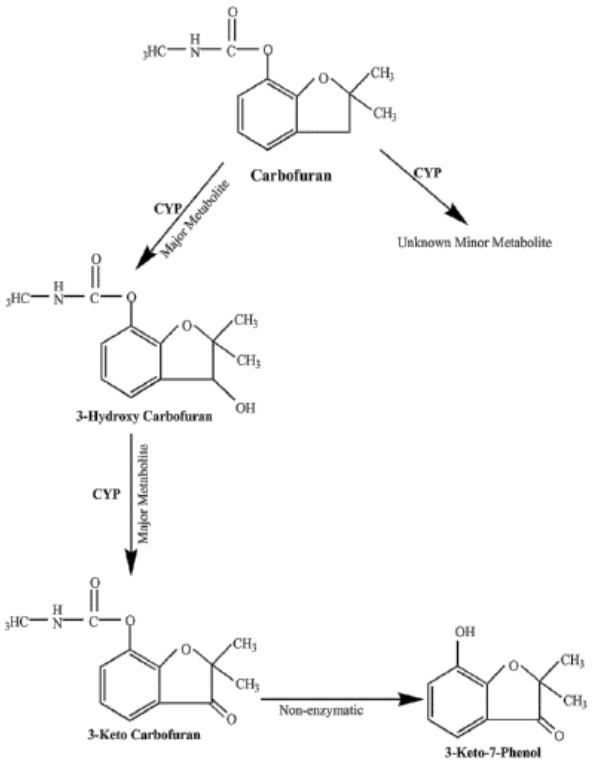


Carbosulfan was found to possess bacterial mutagenicity in both the TA98 and TA100 *Salmonella typhimurium* strains [4].

Another benzofuranyl carbamate derivative, *3-Hydroxycarbofuran* (*3-Hydroxy-2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate*):

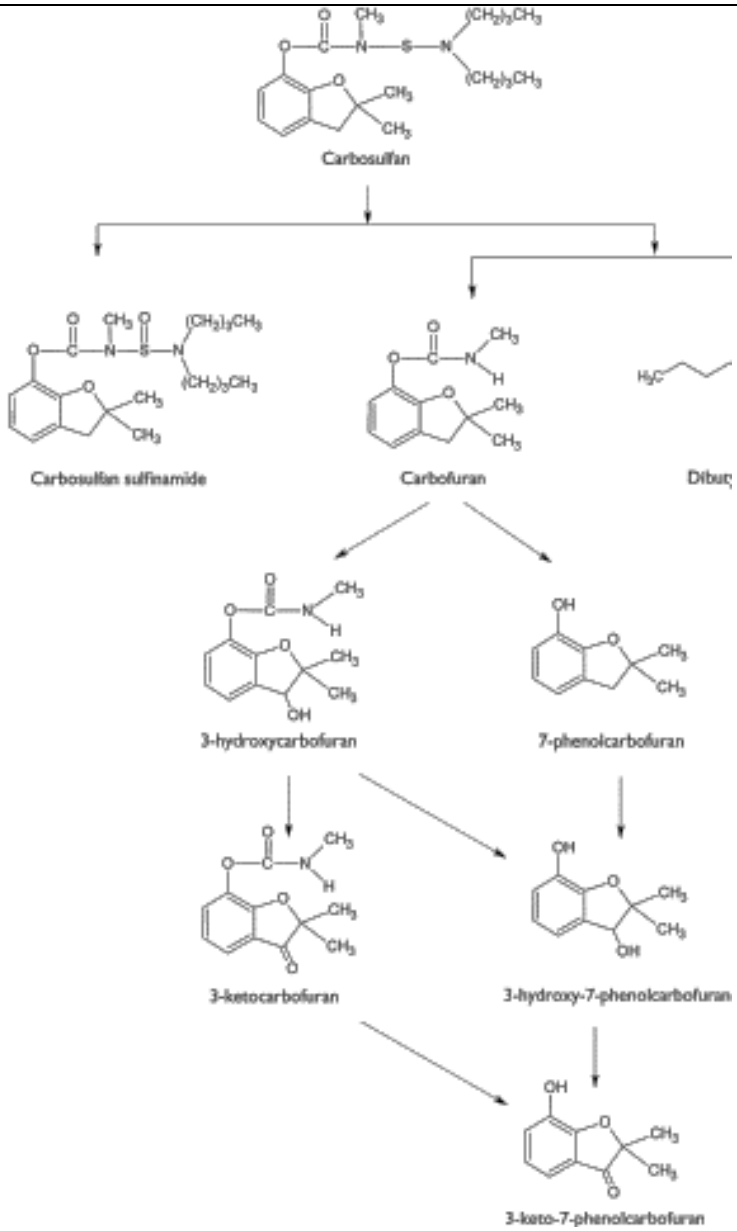


has very close structural similarity as metabolite of *Carbofuran* (Scheme 2) [6]:



Scheme 2

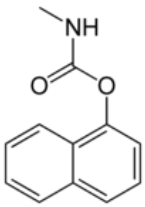
This suggests *positive* bacterial mutagenicity on incubation with microsomal/S9 fraction, as reported [5]. In addition, according to a publication on the *in vitro* metabolic pathway of the structurally close chemical, *Carbosulfan* (Scheme 3):



Scheme 3

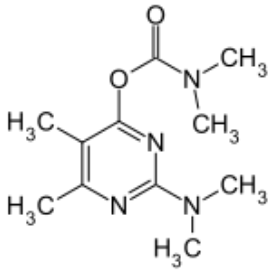
on incubation with liver microsomes from rat and mouse, some *toxic products* such as Carbofuran, *3-Hydroxycarbofuran, etc*. were prodced [7]. It could be therefore safely assumed that the *Ames*-active metabolite produced by *in vitro* metabolic activation of *3-Hydroxycarbofuran* tested as parent chemical is *3-Ketocarbofuran* (Scheme 3).

- *Intercalation* of *Carbofuran* molecule between DNA base pairs of *calf thymus*, to produce *DNA-carbofuran adducts* has been proposed [8].
- For another insecticide and structurally close cholinesterase inhibitor, *Carbaryl:*



it has been concluded that carbaryl molecules could *intercalate* into the base pairs of calf-thymus DNA helix [9].

- For still another carbamate-type insecticide, *Primicarb:*

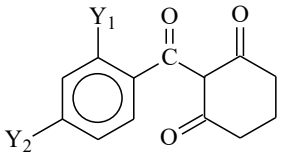
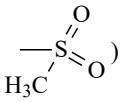
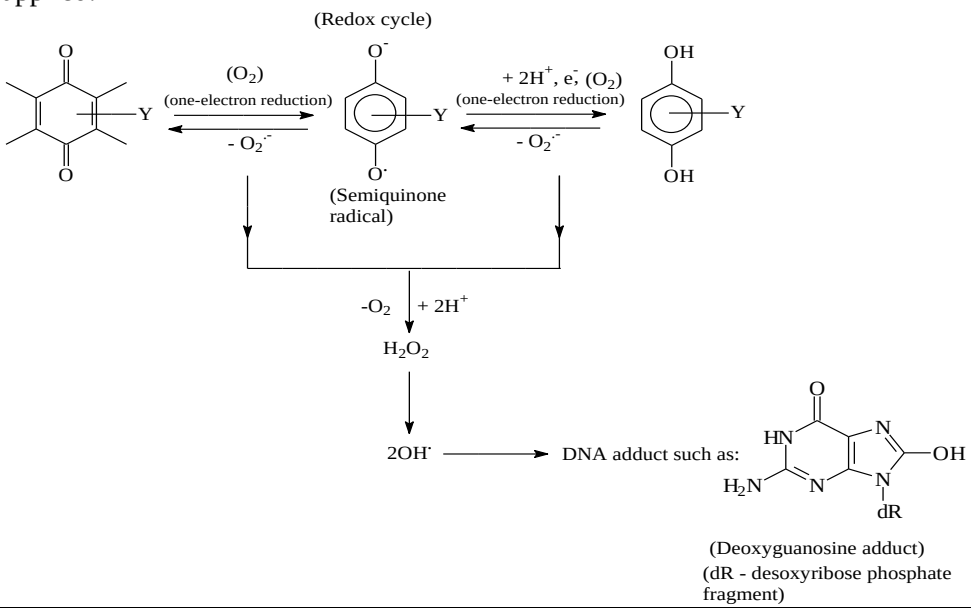


the analytical experimental results indicated that *Pirimicarb* can bind to DNA and the major binding mode is *intercalative binding* [10].

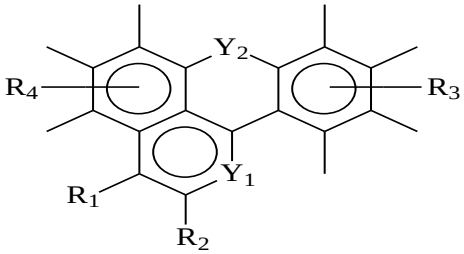
In conclusion, the intercalative binding of benzofuranyl carbamate derivatives to DNA (see Scheme 1 above) appears to be plausible mechanistic mode of action, determining the positive *in vitro* Ames mutagenicity of this sub-class of chemicals.

Set of chemicals used for profile development	Benzofuranyl Carbamate 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. Conclusion regarding the peer review of the pesticide risk assessment of the active substance carbofuran finalized: 28 July 2006 ; *EFSA Scientific Report* (2006) 90, 1-88, Conclusion on the peer review of carbofuran; https://doi.org/10.2903/j.efsa.2006.90r. Last visited: March`2024. 2. Carbofuran; https://inchem.org/documents/jmpr/jmpmono/v96pr03.htm. Last visited: March`2024. 3. Conclusion regarding the peer review of the pesticide risk assessment of the active substance Carbofuran (Question No EFSA-Q-2009-496), Issued on 16 June 2009, *EFSA Scientific Report* (2009) 310, 1 - 132; https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2009.310r. Last visited: March`2024. 4. Altinok, I., E. Capkin, H. Boran, Pest. Biochem. Physiolog. **102** (2012), 61 – 67. 5. Conclusion regarding the peer review of the pesticide risk assessment of the active substance carbofuran finalised: 28 July 2006 ; EFSA Scientific Report **90** (2006), 1 - 88; https://doi.org/10.2903/j.efsa.2006.90r). Last visited: March`2024. 6. Khawja A. Usmani, Ernest Hodgson, Randy L. Rose, Chem.-Biolog. Interact. **150** (2004) 221–232. 7. Abassa, Kh., P. Reponena, S. Mattilab, O. Pelkonena, Chem. Biol. Interact. **181** (2009) 210–219. 8. Li-Jin Zhang[1], Shun-Geng Min, Guo-Xue Li, Yan-Mei Xiong, Ying Sun, The mechanism of carbofuran interacts with calf thymus DNA, Guang Pu Xue, Yu Guang Pu Fen Xi, 25(5) (2005), 739 - 742 (Abstract, article in Chinese); https://pubmed.ncbi.nlm.nih.gov/16128077/. Last visited:

	March`2024. 9. Zhang, G., Xing Hu, Peng Fu, J. Photochem. Photobiol. B: Biology **108** (2012), 53 – 61. 10. G. Zhang, Xing Hu, Junhui Pan, Spectrochimica Acta Part A **78** (2011) 687 – 694.

Individual profile/alert	
Name	Benzoyl Cyclohexanedione Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y_1 is NO_2 or Cl or Br Y_2 is CF_3 or )
Mechanism	ROS generation
In the redox environment of biological systems, quinones and anthraquinones as cyclic dicabonyl componds may also cause genotoxicity through the formation of reactive oxygen species (ROS), which further may attack DNA by oxidative reactions. In such cases, the following combined scheme for bioactivation with predominance of the radical pathway as a part of the normal redox cycle can be applied:  (Redox cycle) (O_2) (one-electron reduction) / $- O_2^{\cdot-}$ $+ 2H^+, e^-, (O_2)$ (one-electron reduction) / $- O_2^{\cdot-}$ (Semiquinone radical) $-O_2$ $+ 2H^+$ H_2O_2 $2OH^{\cdot}$ → DNA adduct such as: (Deoxyguanosine adduct) (dR - desoxyribose phosphate fragment)	
Set of chemicals used for profile development	Benzoyl Cyclohexanedione 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. EFSA Scientific Report (2008) 150, 1 - 86, Conclusion on the peer review of sulcotrione; https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.150r. Last visited: April, 2024. 2. Boels, D., C. M. Ganiere, A. Turcant, M. Bretaudeau, P. Harry, Human and Experimental Toxicol. 32(7) (2013), 778 – 782; https://journals.sagepub.com/doi/pdf/10.1177/0960327112468908. Last visited: April, 2024. 3. Mesotrione 241–305 JMPR 2014; Pesticide residues in food 2014 REPORT Joint FAO/WHO Meeting on Pesticide Residues https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report2014/JMPR_2014_Full_Report.pdf. Last visited: April, 2024. 4. EFSA (2006) Public consultation on the active substance sulcotrione. https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-sulcotrione. Last visited: April, 2024. 5. EFSA (2015) Public consultation on the active substance mesotrione. https://www.efsa.europa.eu/en/consultations/call/150417. Last visited: April, 2024. 6. Genetic Toxicity Evaluation of Mesotrione in Salmonella/E.coli Mutagenicity Test or Ames Test, NTP Study Number G18020B (2021), NTP Study Number: G18020B Study Result: Positive; https://cebs.niehs.nih.gov/cebs/study/002-03383-0003-0000-2. Last visited: April, 2024. 7. Elsner, M., E. G. Convey, S. Lenzen, ANTIOXIDANTS & REDOX SIGNALING, 10(4) (2008), 691 - 699; DOI: 10.1089/ars.2007.1816. 8. Xiaoying Li, Lusheng Zhu∗, Zhongkun Du, Bing Li, Jun Wang, Jinhua Wang, Yanyan Zhu, Ecological Indicators 95 (2018) 436 – 443. 9. 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, Current Drug Metabol. 6 (2005), 161 – 225. 10. Yu, D., J. A. Berlin, Tr. M. Penning, J. Field, Chem. Res. Toxicol. 15 (2002), 832 – 842.

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].

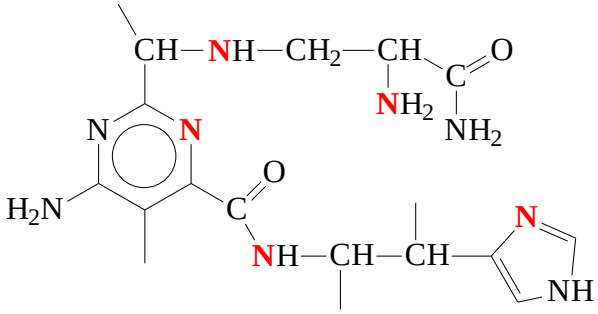
Therefore, it could be expertly assumed that a radical mechanism of generating reactive oxygen species (ROS) such as HO. and subsequent attack on DNA nucleoside bases may explain the genotoxicity of some benzanthrone derivatives. Generation of ROS is probably greatly facilitated by the abundance of phase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

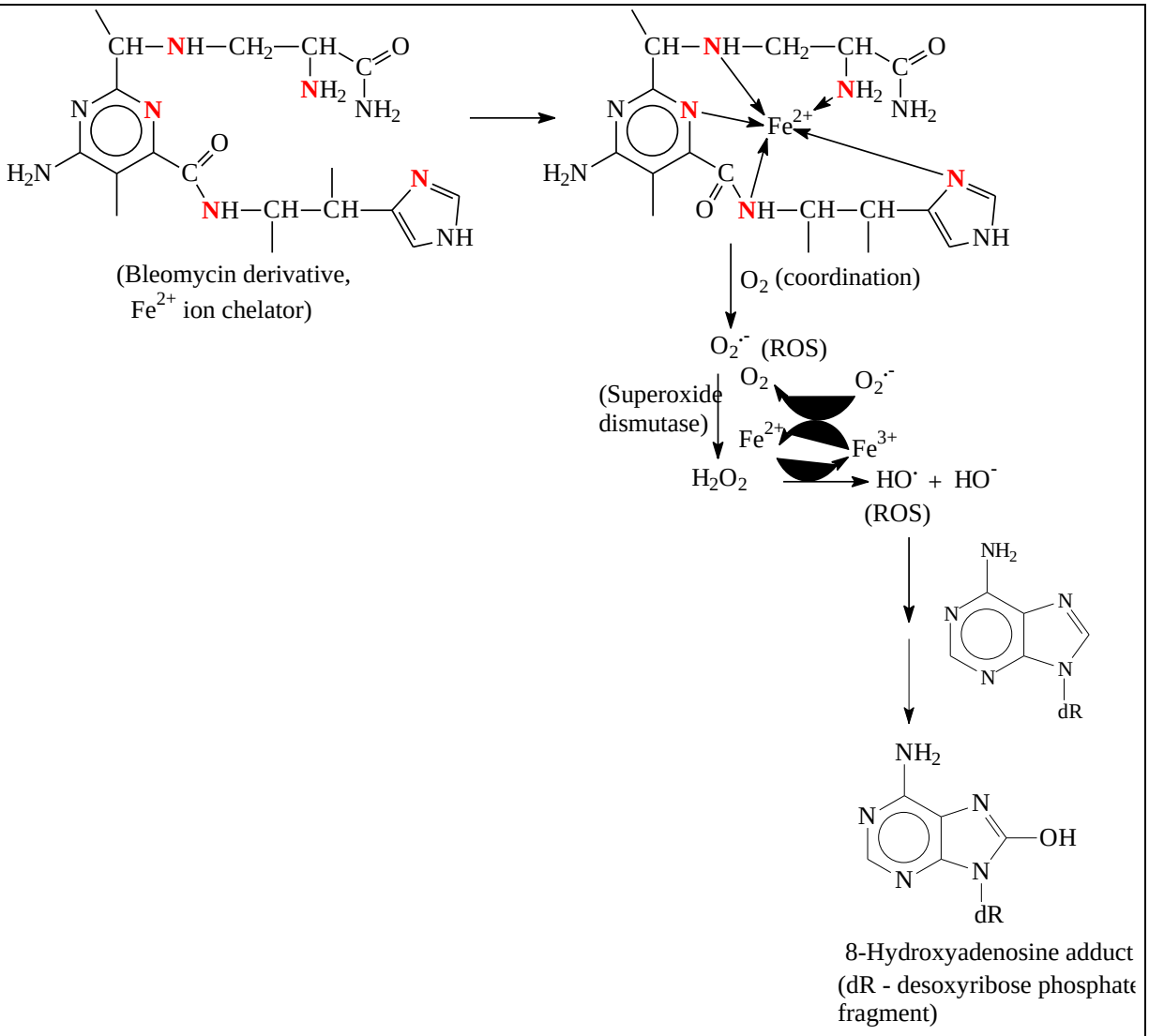
On the other hand, DNA intercalation mechanism cannot be excluded for the benzanthrone derivatives [4], similarly to a number of anthraquinones [5]. Based on the observed experimental data on mutagenicity (Table 1), some important points, regarding the structural boundaries of Ames-positive chemicals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

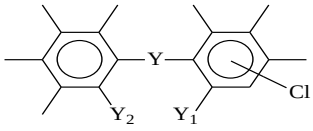
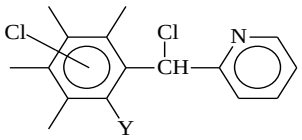
Set of chemicals used for profile development	Benzanthrone Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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.

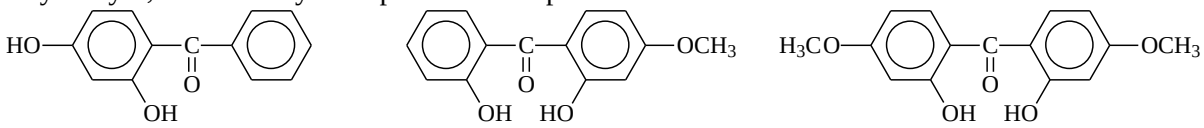
Individual profile/alert	
Name	Bleomycin and Structurally Related Compounds
Type of profile	Structural alert
Description/applicability domain	 (Nitrogen atoms, capable of coordination with Fe2+ are marked in bold and red)
Mechanism	Radical ROS generation & Non-covalent interactions DNA intercalation



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 (Cl) or C (O) ; 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].

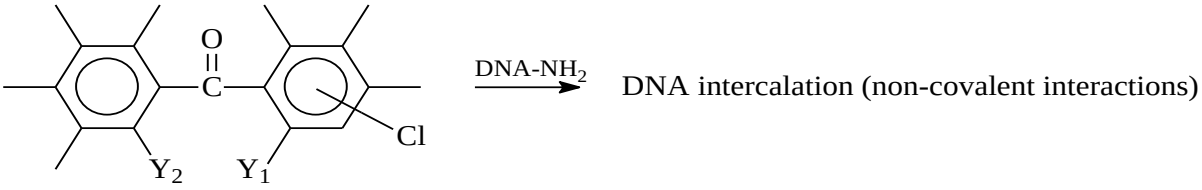
According to other publications, some hydroxylated benzophenone derivatives with structures shown above such as Benzophenone-1 (2,4-Dihydroxybenzophenone), Benzophenone-8 (2,2'-Dihydroxy-4-methoxybenzophenone), and particularly, their chlorination products have shown bacterial mutagenicity in some Salmonella 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

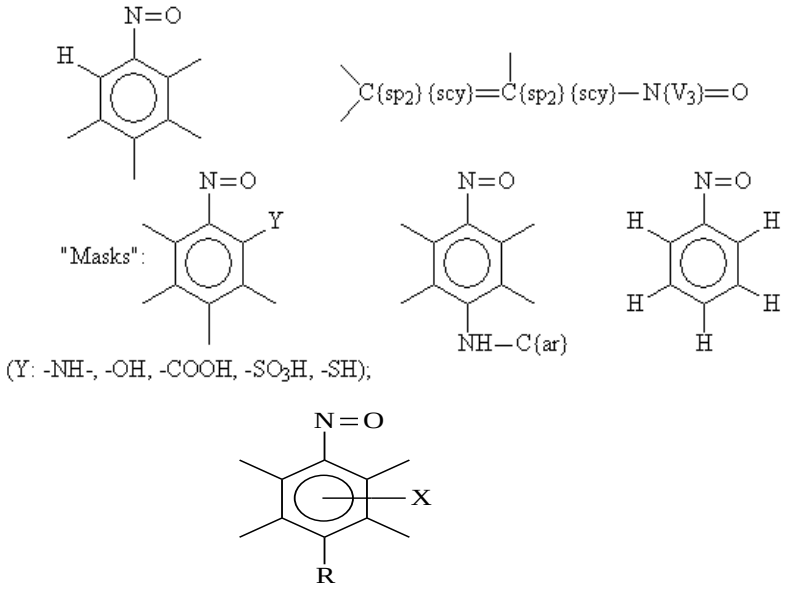
Scheme 1

(DNA adduct)

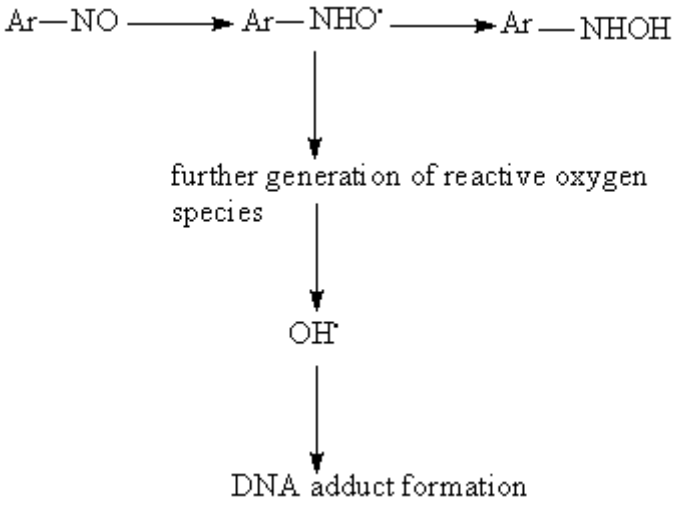
Scheme 2 (SN2 mechanism)

Set of chemicals used for profile development	Chlorinated Diphenylmethane and Benzophenone 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. 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.

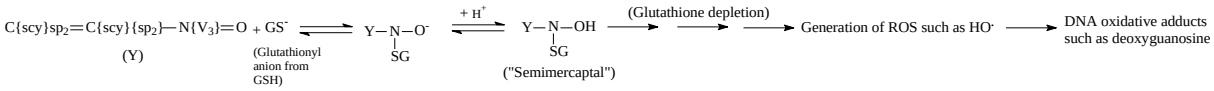
Individual profile/alert	
Name	C-Nitroso Compounds
Type of profile	Structural alert
Description/applicability domain	 X is Cl or Br (no more than two), no other substituents; R is H or OH or OCH_3 (only one of either OH or OCH_3); no other substituents
Mechanism	S_N1 Nucleophilic substitution after glutathione-induced 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]:



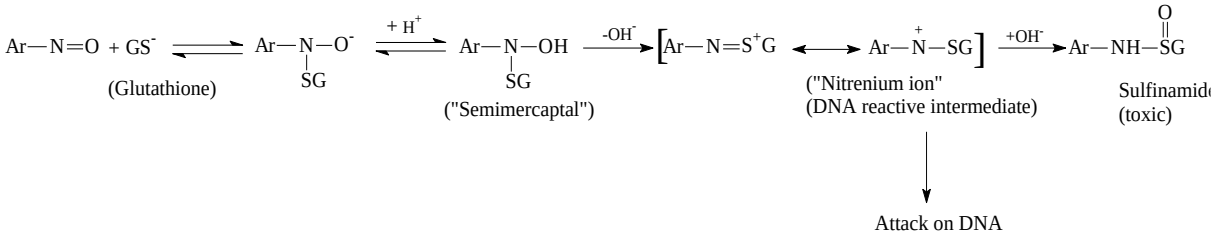
Scheme 1a

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

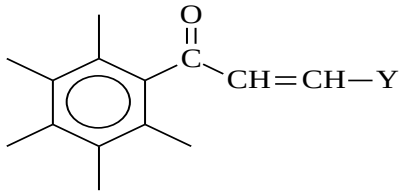
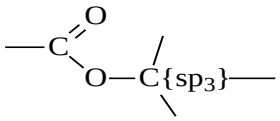
-Non-Radical Mechanism: pseudo-nitrenium ion formation with glutathione (or other thiols) Scheme 2 [4]:



Scheme 2

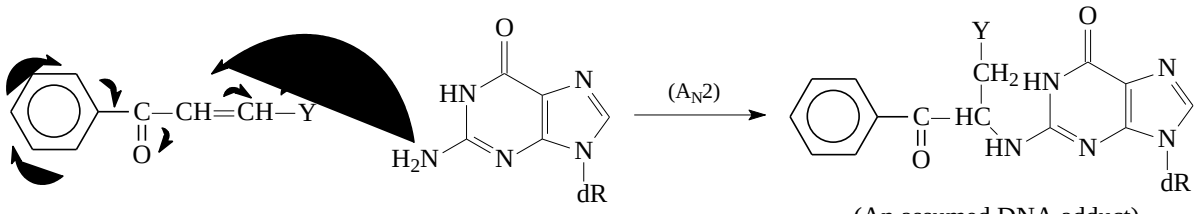
Set of chemicals used for profile development	C-Nitroso Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

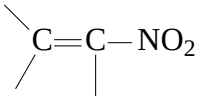
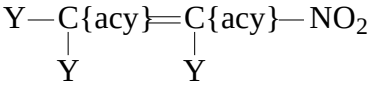
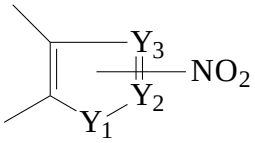
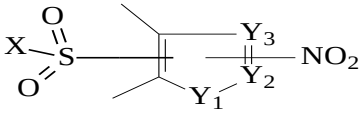
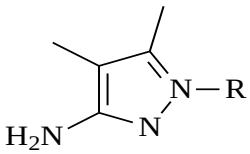


(Deoxyguanosine DNA fragment; dR: deoxyribose phosphate fragment)

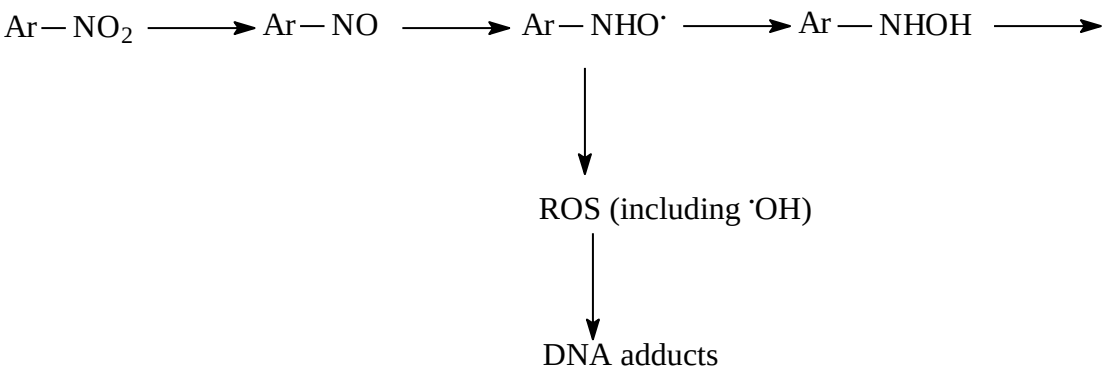
(An assumed DNA adduct)

Set of chemicals used for profile development	Conjugated Benzoylethene 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	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

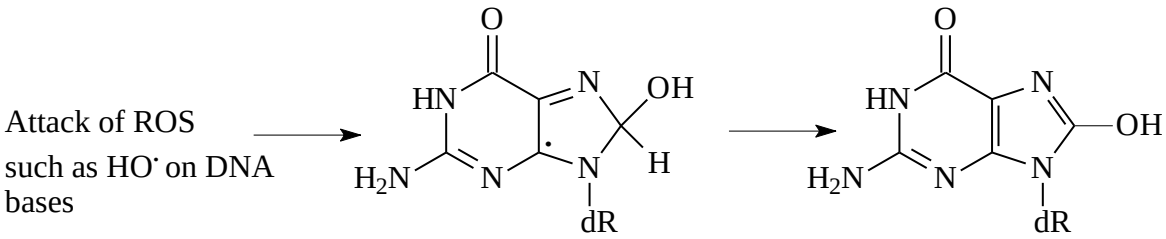
	 *Note*: The fragment:  where Y is acyclic C and/or H should be excluded (a "mask") B. Five membered aromatic nitroheterocycles:  Y_1 is N{V3}{sp3}or S{V2} or O Y_2 is N{V3}{sp2} or C{sp2}; Y_3 is N{V3}{sp2} or C{sp2} C. Five-membered aromatic nitroheterocyclic sylfonyl halides:  Y_1 is N{V3}{sp3}or S{V2} 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):



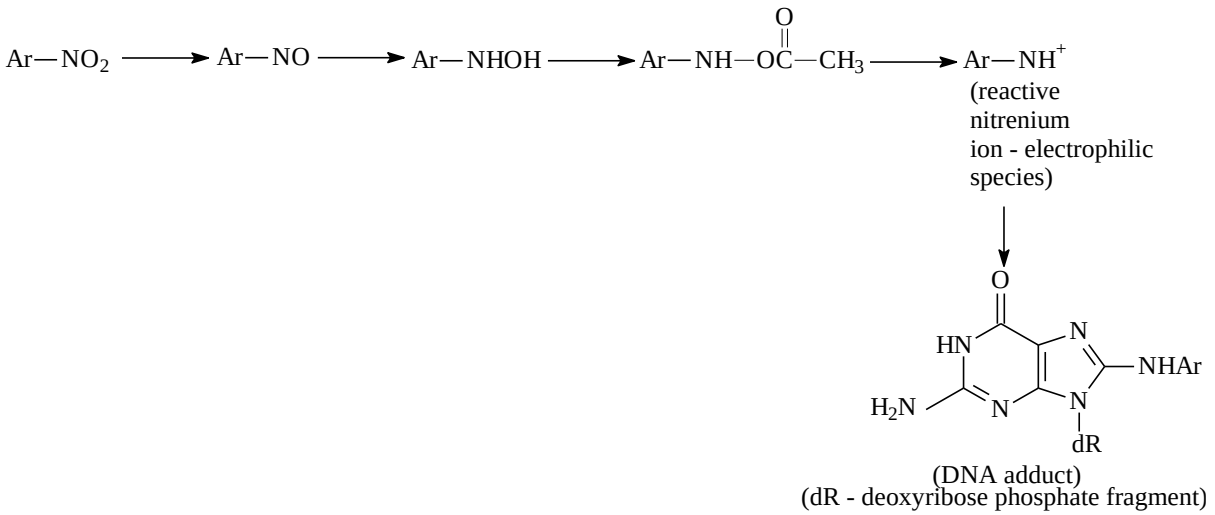
Scheme 1a

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]:

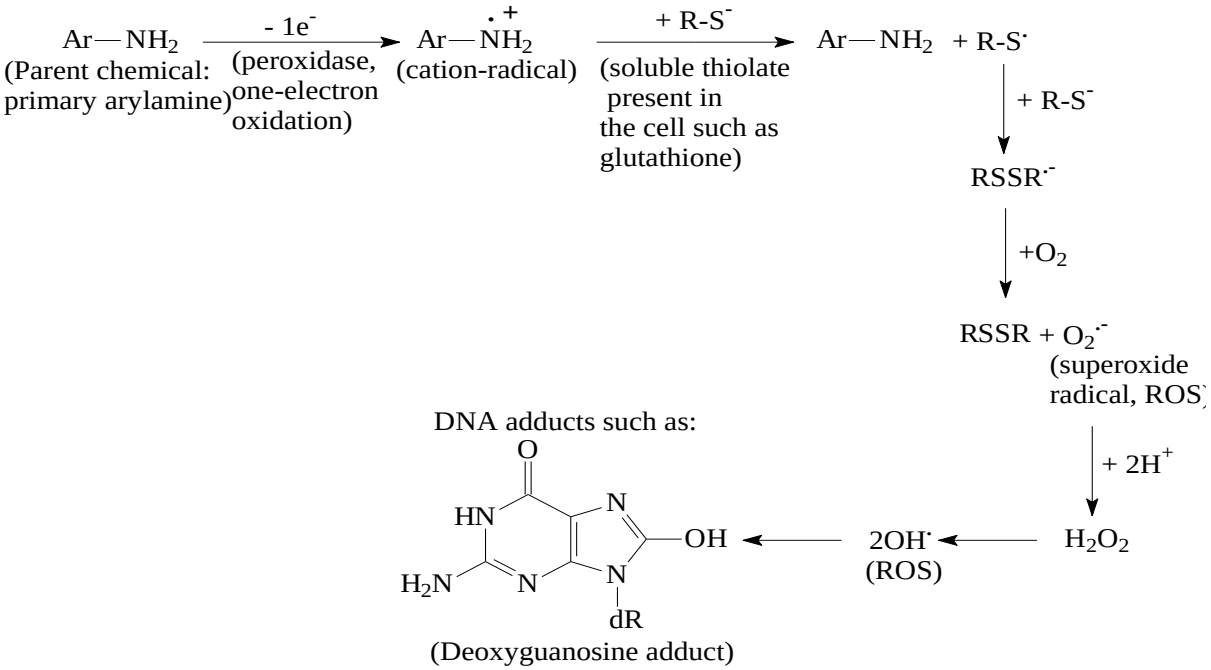


Scheme 1b

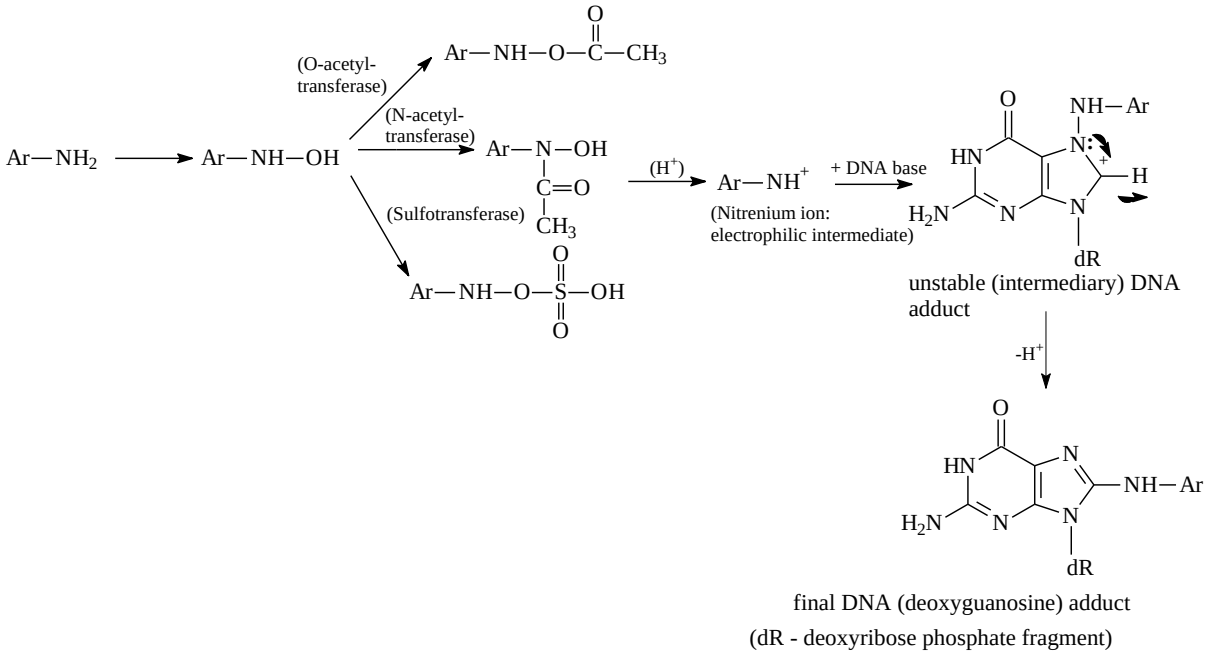
Heterolytic Mechanism. This is also an important mechanism, associated with the bacterial mutagenicity of nitroarenes and related compounds, and, more specifically, the sub-classes discussed here. It also operates in the presence of metabolic S9 activation system. The DNA damage, eliciting bacterial 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)



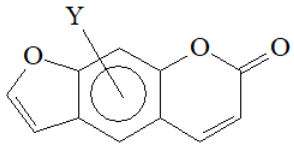
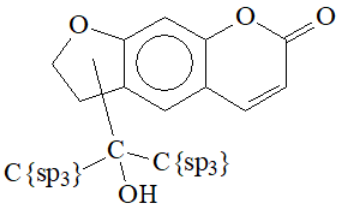
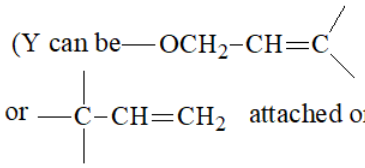
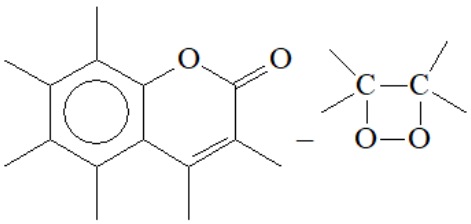
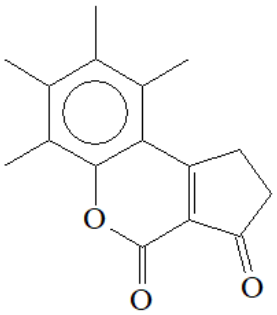
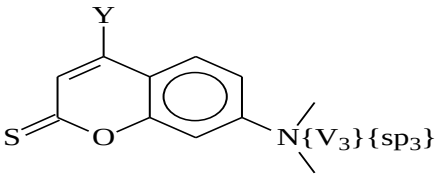
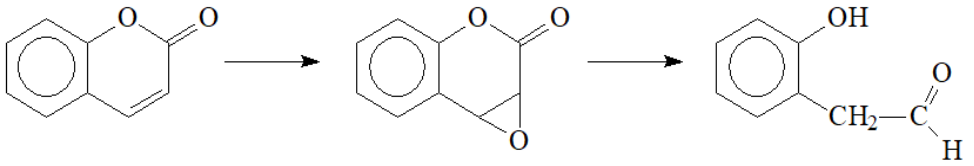
Scheme 3

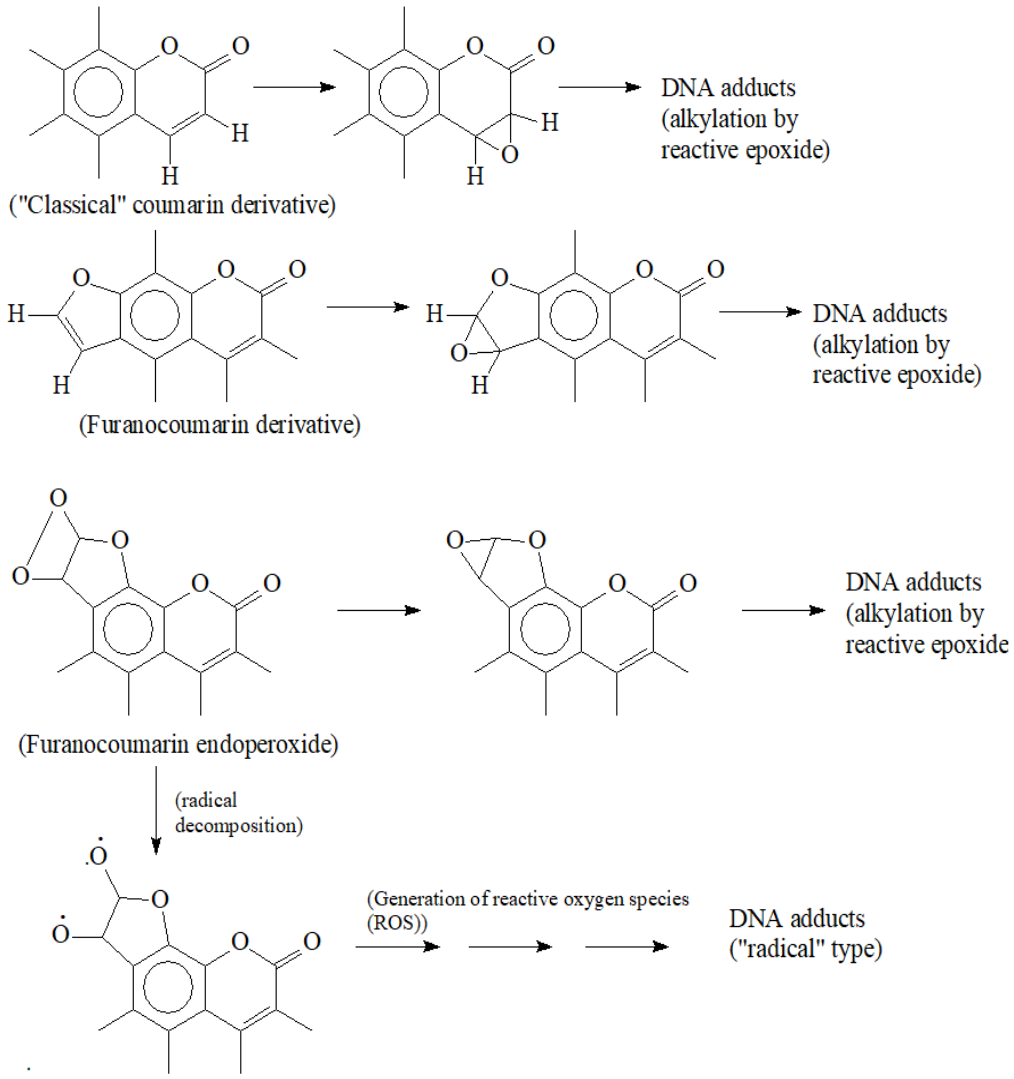


Scheme 4

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

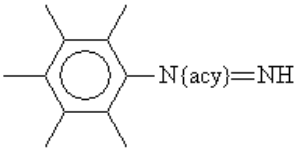
	Test for Bacterial Mutagens, Canc. Res. 39 (1979), 682 – 693.

Individual profile/alert	
Name	Coumarins and Thiocoumarins
Type of profile	Structural alert
Description/applicability domain	   (Y can be—OCH_2–CH=C or —C(–CH=CH_2) attached on the coumarin ring system *via* C {sp3} or O-atom) (The substituent is attached to the dihydrofuran ring)  (Two fragements within one molecular structure)   (Y is –CH3 or H)
Mechanism	S_N2 Direct acting epoxides formed after metabolic activation, Radical ROS generation, Non-covalent interactions DNA intercalation & S_N1 DNA alkylation
	



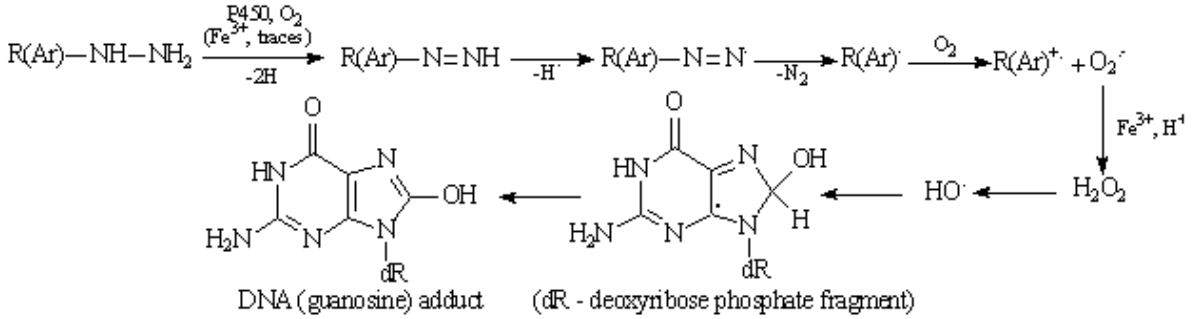
Set of chemicals used for profile development	Coumarins and Thiocoumarins
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

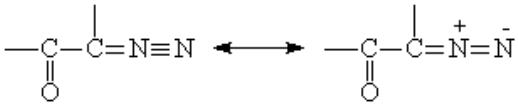
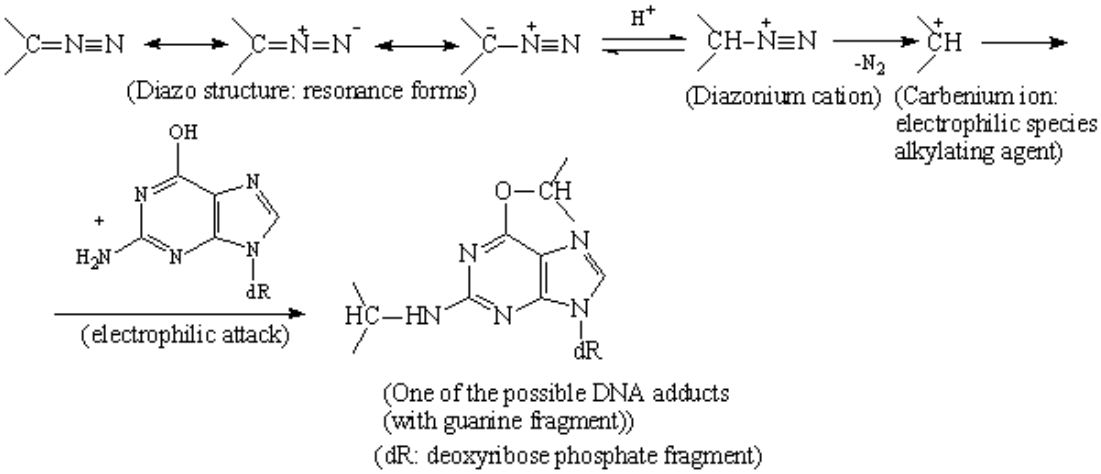


Scheme 1

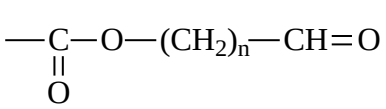
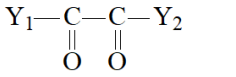
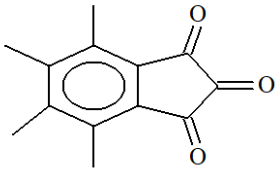
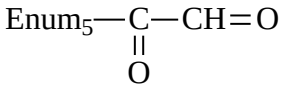
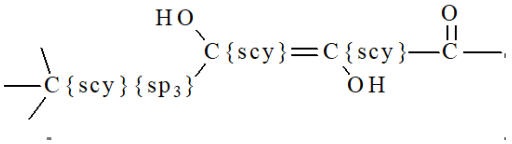
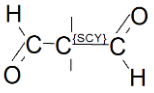
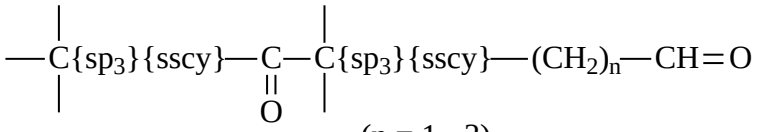
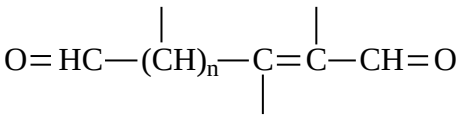
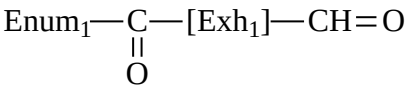
ROS can be also generated as a result from oxidation/reduction processes in bacteria without addition of exogenous S9 system. In such a case, the radical mechanism discussed above is likely to operate.

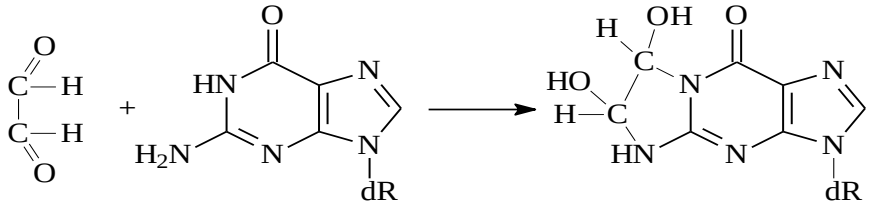
Set of chemicals used for profile development	Diazenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.

References	1. 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	Diazoalkanes
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.

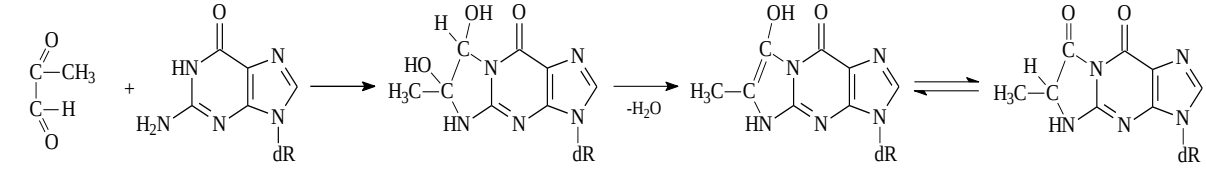
Individual profile/alert

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]:	



(Deoxyguanosine fragment) (Adduct)

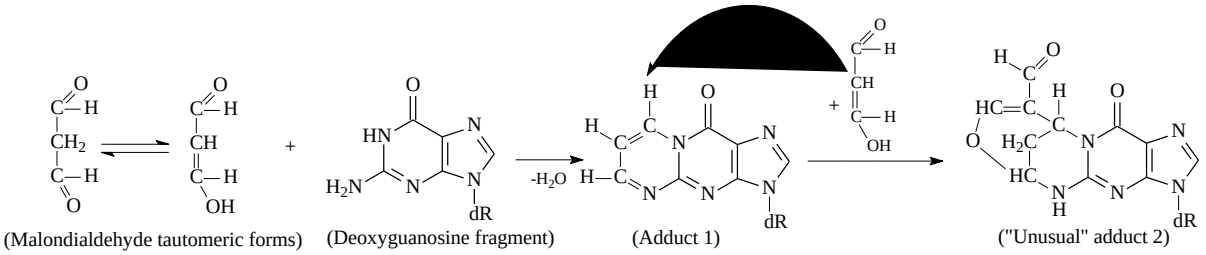
Methylglyoxal is a sugar degradation product, which is endogenously formed by fragmentation of triose phosphates during the metabolic glycolysis. It has shown bacterial mutagenicity in Salmonella typhimurium. The prolonged exposure of DNA to high concentrations of methylglyoxal under physiological conditions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



(Methylglyoxal) (Deoxyguanosine fragment) (Adducts)

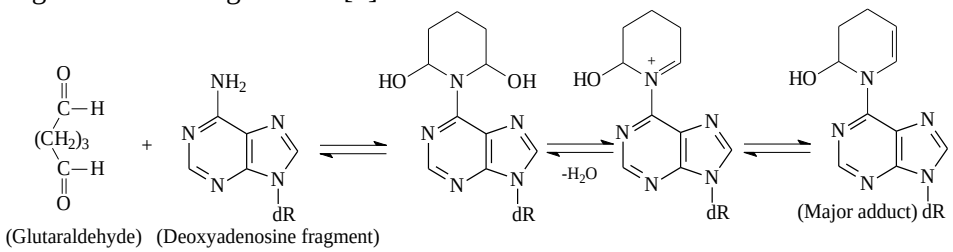
Reaction scheme, similar to those outlined above was suggested for the formation of DNA covalent adducts with the butter flavorant, diacetyl [6].

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]:



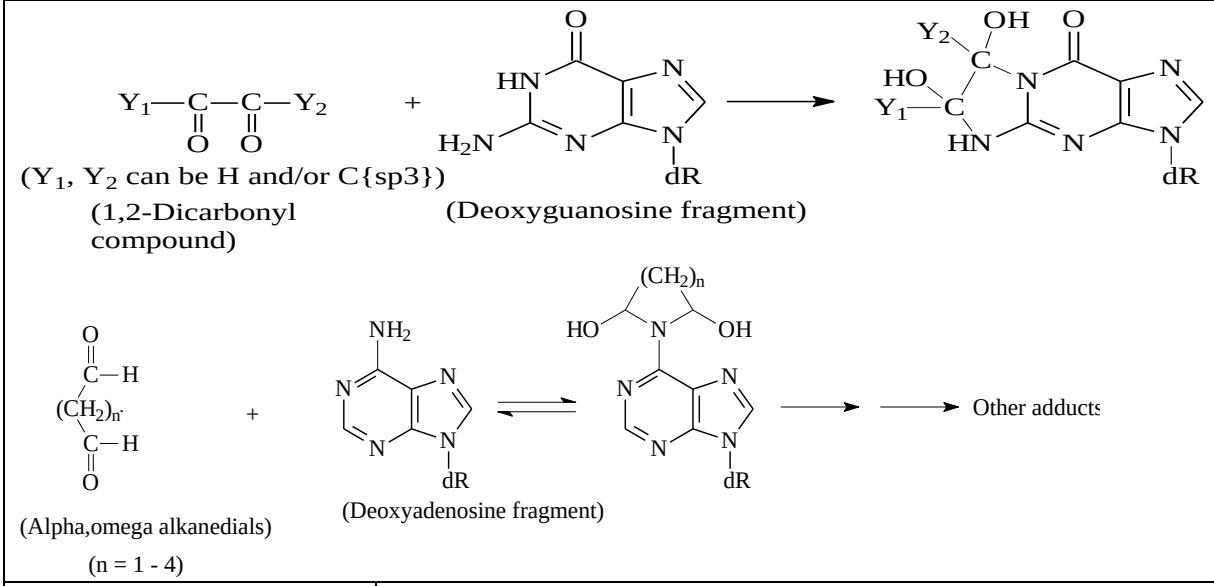
(Malondialdehyde tautomeric forms) (Deoxyguanosine fragment) (Adduct 1) ("Unusual" adduct 2)

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]:



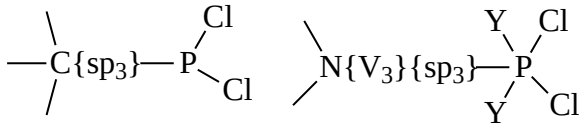
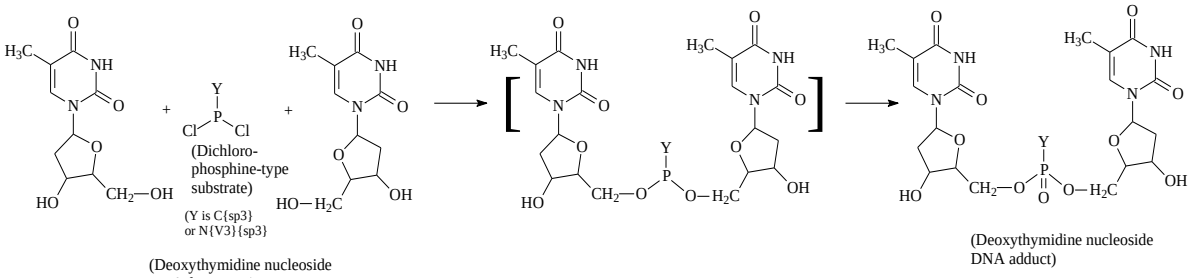
(Glutaraldehyde) (Deoxyadenosine fragment) (Major adduct)

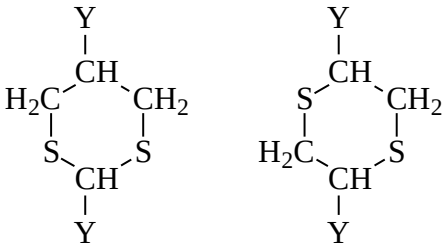
Based on the above considerations, the following generalized mechanistic schemes for this class of compounds can be outlined:



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):  (Deoxythymidine nucleoside DNA fragments) (Dichloro-phosphine-type substrate) (Y is C{sp3} or N{V3}{sp3} (Deoxythymidine nucleoside DNA adduct)	
Set of chemicals used for profile development	Dichlorophosphine and Dichlorophosphonium 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. 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)

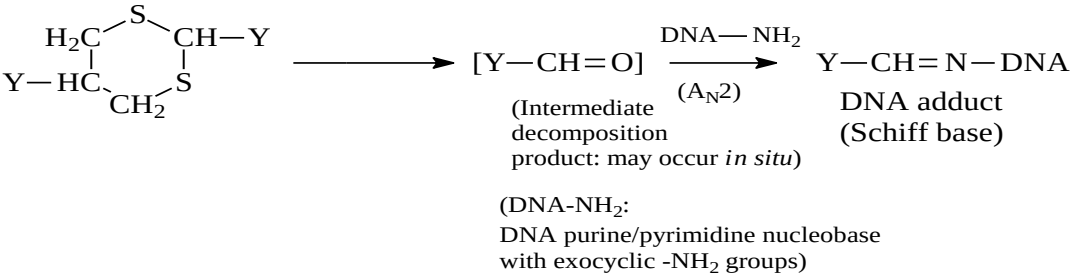
According to some authors, the decomposition of 1,3-dithiane may result in the release of low-molecular aldehyde derivative. The aldehyde can then undergo a Schiff-base reaction with DNA bases, causing mutagenicity [1].

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.

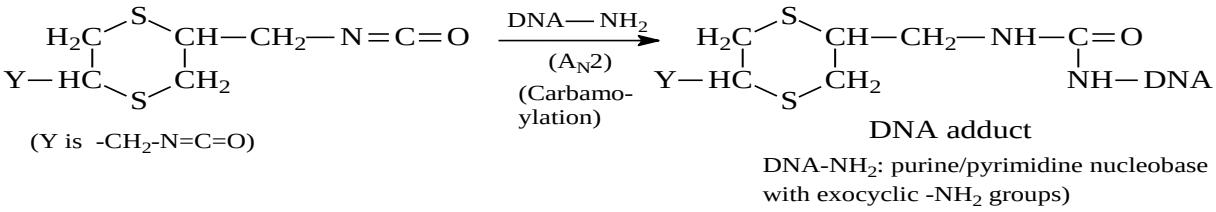
Based on the above information, the following simplified mechanistic schemes can be expertly proposed:

Mechanistic scheme A:

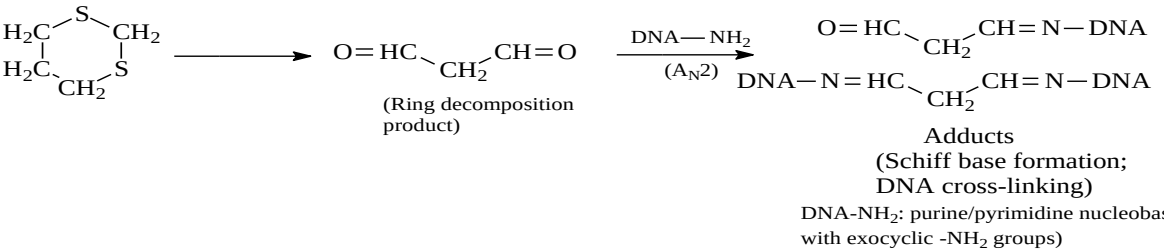


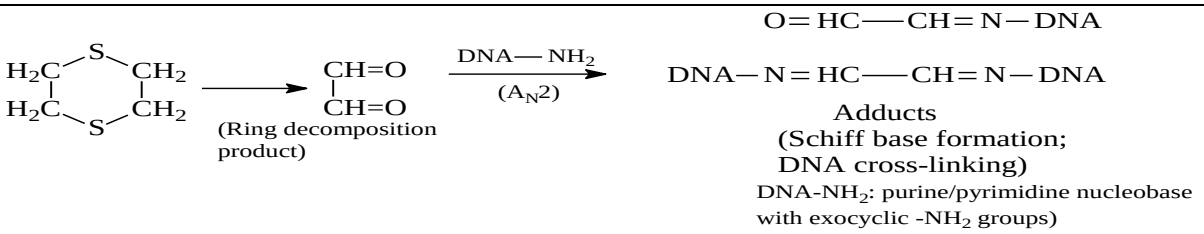
Note: If both Y are –CH=O DNA cross-linking may also occur.

Mechanistic scheme B:

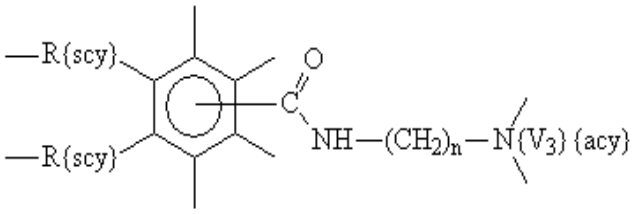
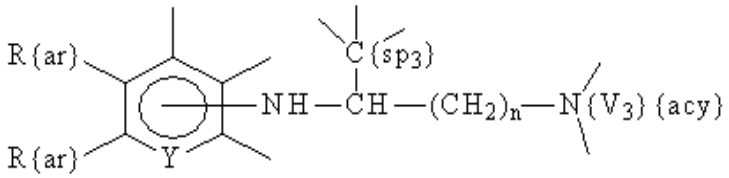


Mechanistic schemes C: Decomposition of 1,3- and 1,4-dithiane rings - more likely to be implemented if both Y are H:

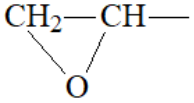


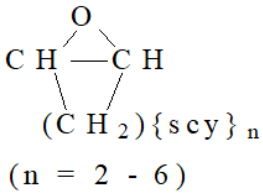


Set of chemicals used for profile development	Dithianes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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.

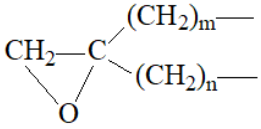
Individual profile/alert	
Name	DNA Intercalators with Carboxamide and Aminoalkylamine Side Chain
Type of profile	Structural alert
Description/applicability domain	 (n = 1 - 3; R{scy}: any atom in a cyclic (including aroma fragment condensed also with the aromatic ring)  (Y is N or C)
Mechanism	Non-covalent 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:

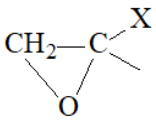


,1-Disubstituted epoxides and spiro-epoxides:



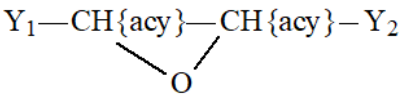
(m = 1 - 3; n = 1 - 3)

(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:

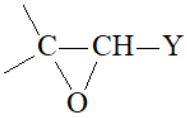
(a) $(-CH_2)_nH$ (n = 1 - 2)

(b) CH_2{scy} and -CH{scy}=CH{scy}-

(c) -CH{sp3}{scy} and O{scy} or -NH{scy}

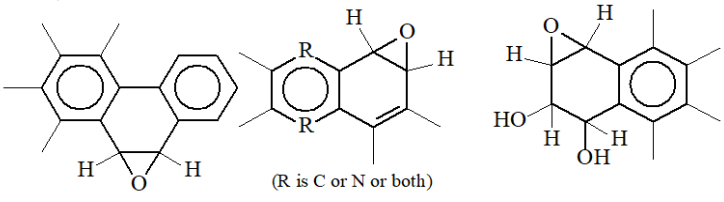
(d) Y_1 is Cl or Br; Y_2 is C

Other terminal polarized epoxides:

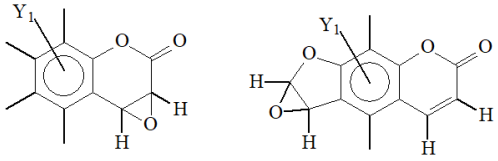


(Y can be Cl, Br or -CHO)

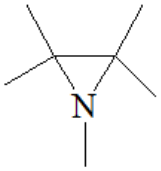
Metabolically derived epoxides from polycyclic aromatic hydrocarbons (PAH) and coumarin derivatives:

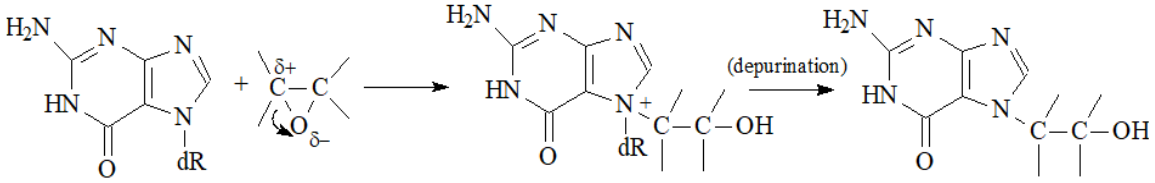


(R is C or N or both)

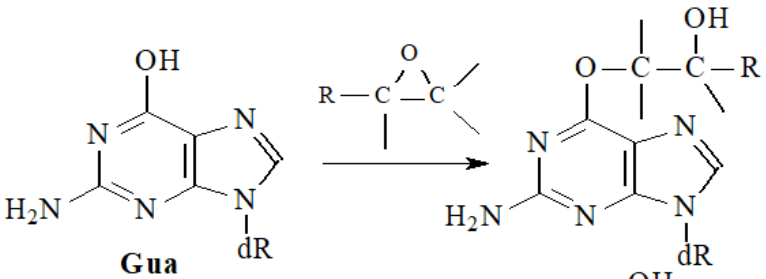


(Y_1 is –H (all) or combinations of H and $–OCH_3$, $-NH_2$, $-NO_2$, -NHOH, $-CH_3$, $-CH_2X$ (X is Cl, Br); no more than one substituent

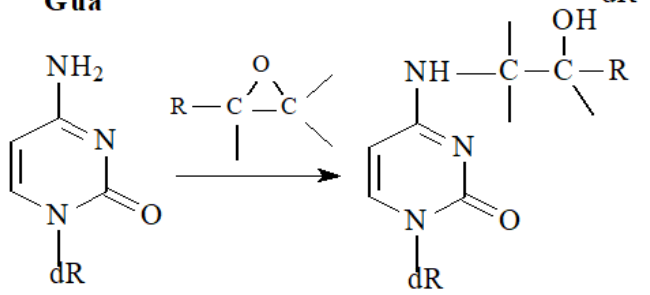
	Aziridines 
Mechanism	**S_N2 Alkylation, direct acting epoxides and related**



(DNA fragment)
(dR - deoxyribose phosphate fragment)

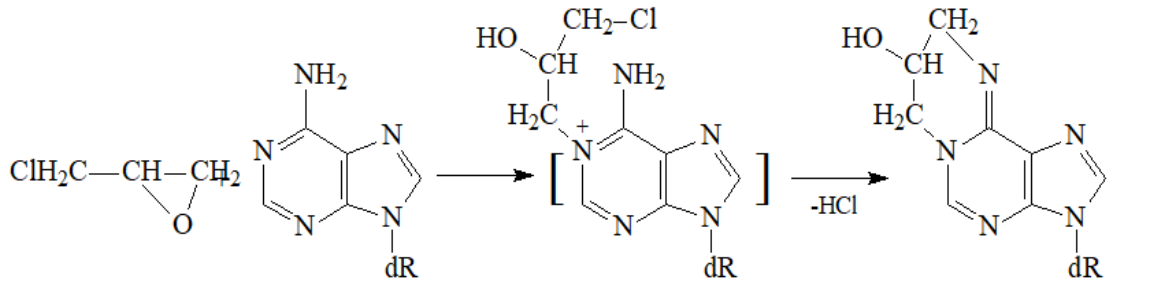


Gua

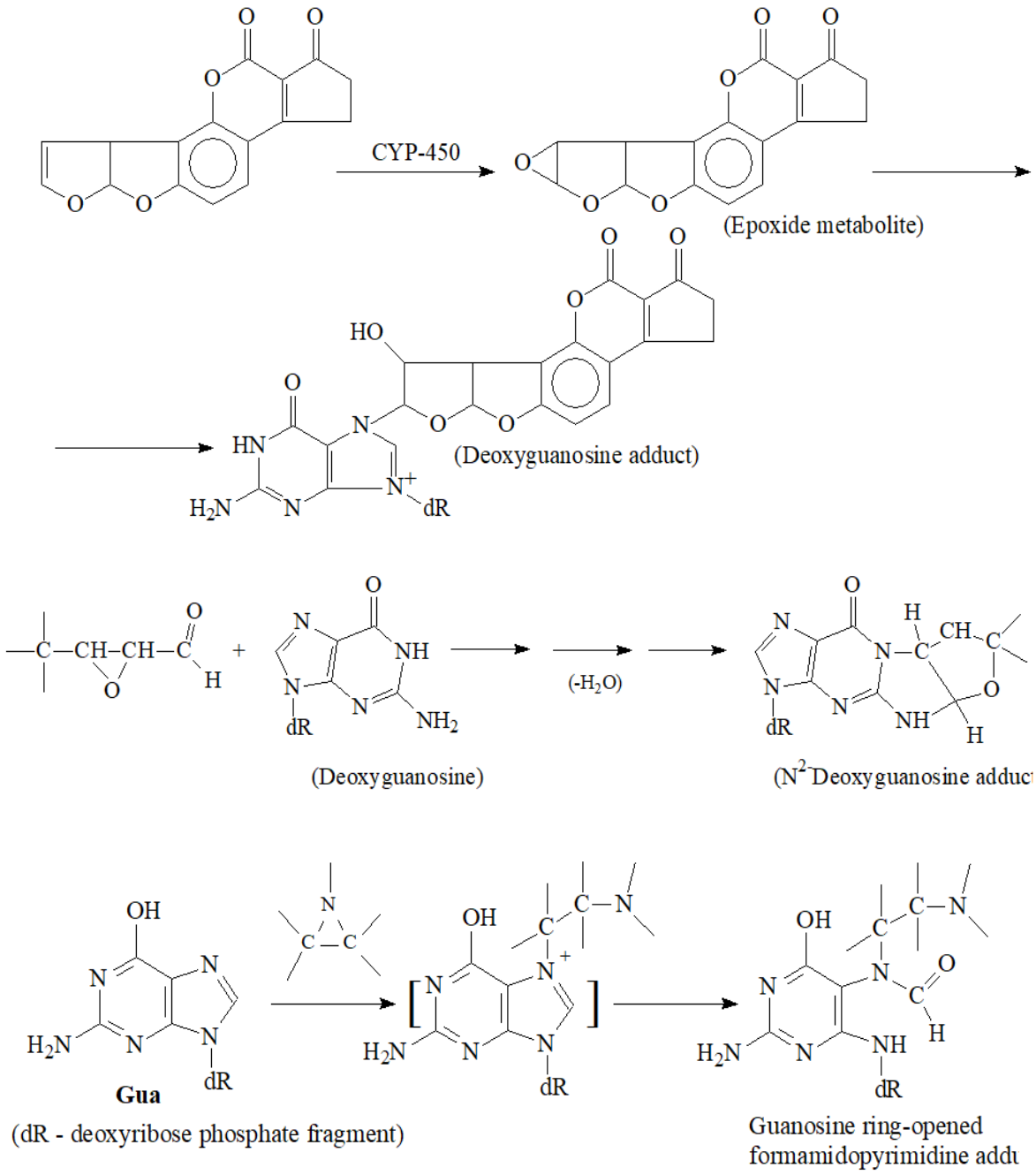


Cyt

(dR - deoxyribose phosphate fragment)

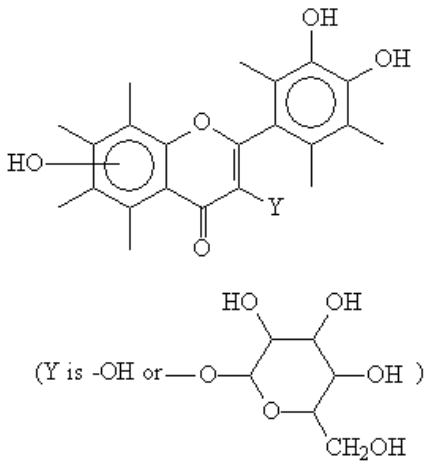


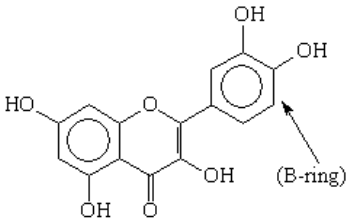
1,N6-2-hydroxypropanoadenine adduct



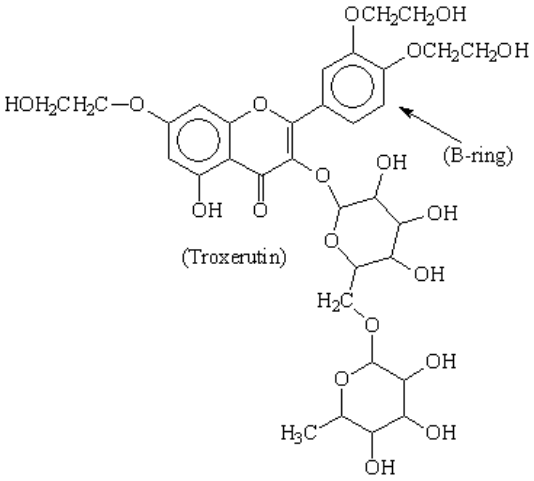
Set of chemicals used for profile development	Epoxides, Aziridines, Thiiranes and Oxetanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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]:	

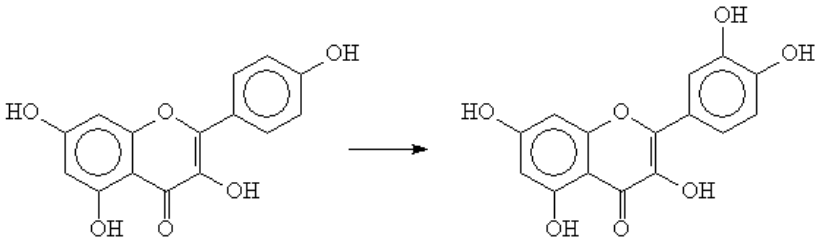


(Quercetin)



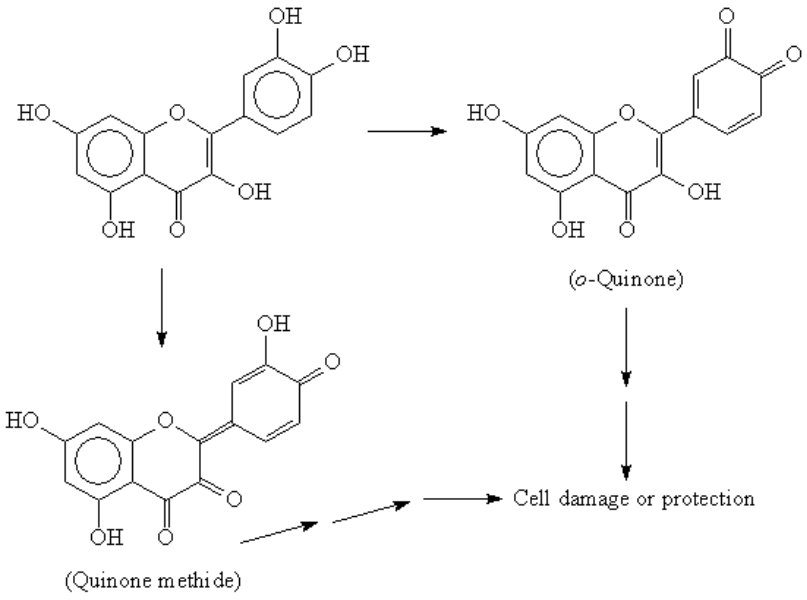
(Troxerutin)

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:



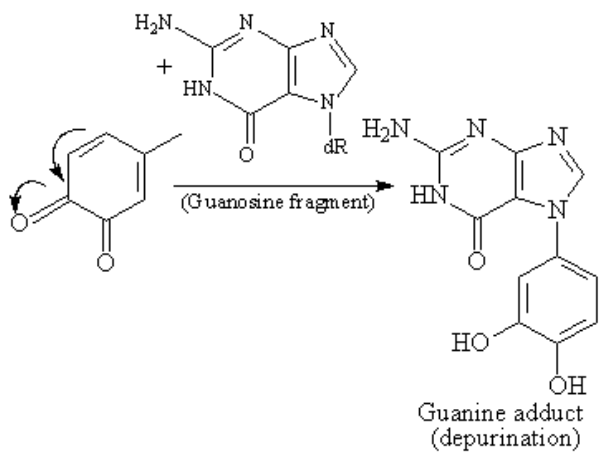
(Kaempferol)

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



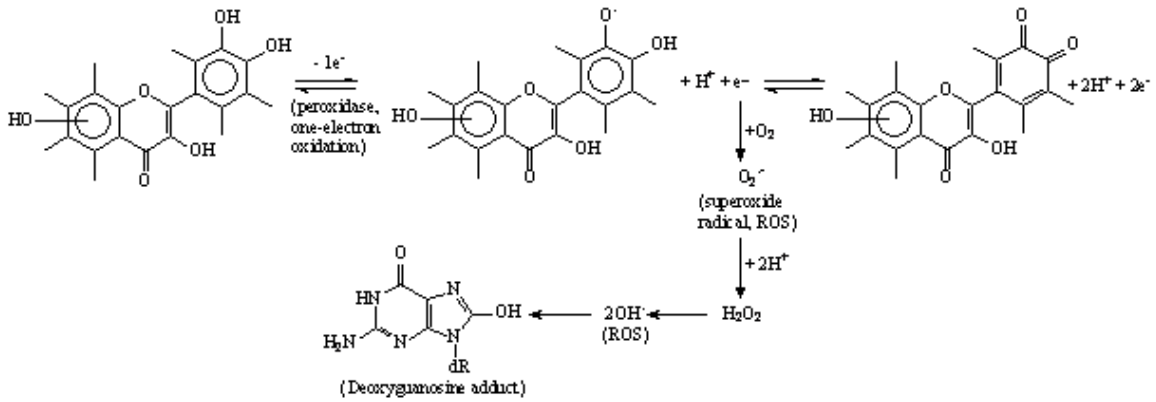
The mutagenicity of quercetin is assumed to be partly due to the generation of such active metabolites. One possible mechanism for the formation of DNA adducts from *o*-quinones could

involve depurination, due to Michael addition, according to the following scheme [8]:



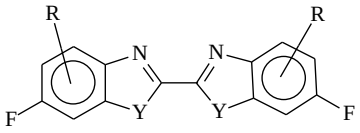
dR - desoxyribose phosphate fragme:

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:

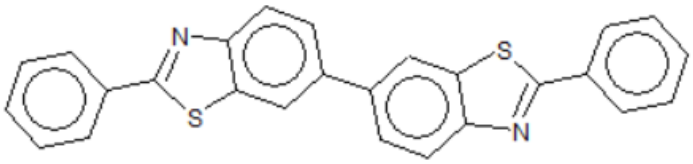


Set of chemicals used for profile development	Flavonoids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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	Fluoro bis-benzothiazole derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is S{V2} or S{V4}=O; R is -H (all) or -OH (maximum one in each benzenoid ring; the rest are H)
Mechanism	Non-covalent interactions

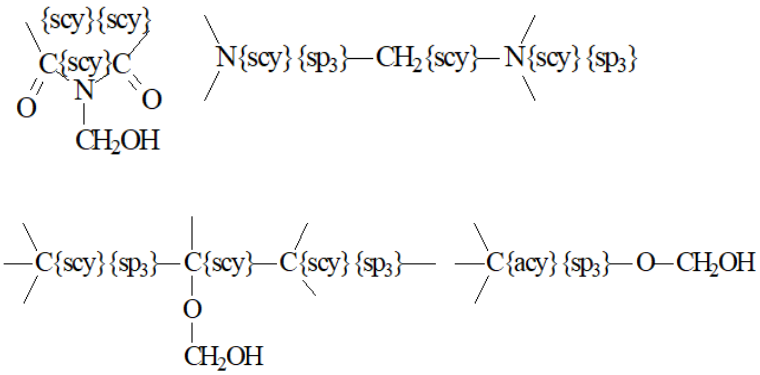
A series of novel bis-benzothiazole derivatives of the structural type:



have been synthesized, and evaluated for their anti-proliferative activities on various cells and in vitro anti-cancer activities. Molecular modeling, fluorescence, and viscosimetry studies revealed that these compounds could bind into the minor groove of DNA [3]. In other words, it is assumed that the target chemical KIF -230-I-6, structurally close to Compound A would also exhibit DNA intercalative properties, due to the planar benzothiazole rings, and the electron-withdrawing fluorine atom as a good hydrogen bond acceptor.

Generation of reactive oxygen species (ROS), due to, presumably, the principal oxidative metabolism of the target chemical, which may cause DNA damage, is also possible. For instance, phase I Aromatic Ring C-hydroxylation, and S-Oxidation as well as phase II O-Glucuronidation are expertly assumed, plausible in vitro metabolic transformations of 6,6'-Difluoro-2,2'-bibenzo[d]thiazole (KIF -230-I-6). Nevertheless DNA intercalation appears to be the predominant mode of action, determining mutagenicity.

Set of chemicals used for profile development	Fluoro bis-benzothiazole 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. EFSA (2018) Public consultation on the active substance benthiavalicarb-isopropyl. https://www.efsa.europa.eu/en/consultations/call/180309. Last visited: March, 2024. 2. Conclusion regarding the peer review of the pesticide risk assessment of the active substance benthiavalicarb, *EFSA Scientific Report* (2007) 107, 1 – 81. 3. Yang, M.-Li, H. Zhang, W. W. Wang, X.-Jun Wang, J. Heterocycl. Chem. 2017; DOI 10.1002/jhet.3041.

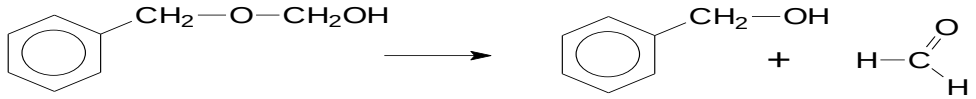
Individual profile/alert	
Name	Formaldehyde Releasers
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Mechanistic Domain: SN2 Mechanistic Alert: Alkylation, nucleophilic substitution on activated hydroxymethyl or methylene group, attached to N{sp3}- or O-atoms

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:

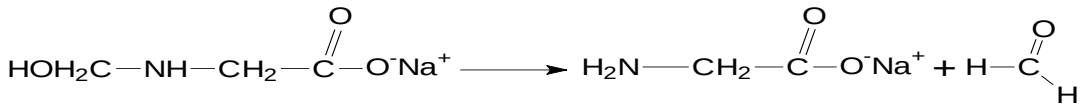
A. Benzylhemiformal:

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



B. Sodium Hydroxymethyl Glycinate

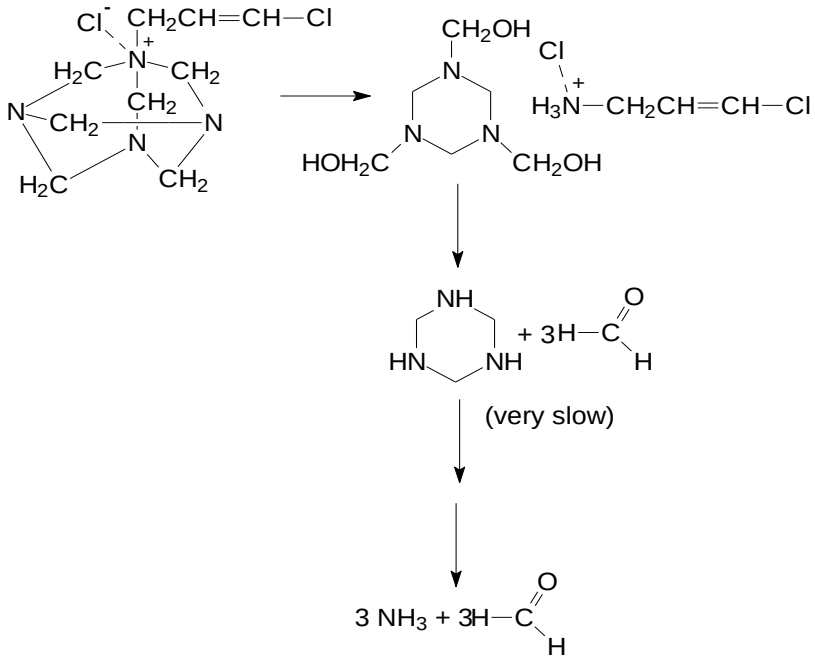
In an aqueous solution, sodium hydroxymethyl glycinate is decomposed to release formaldehyde. One molecule of formaldehyde is formed by the decomposition of each molecule of sodium hydroxymethyl glycinate, as described below:



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

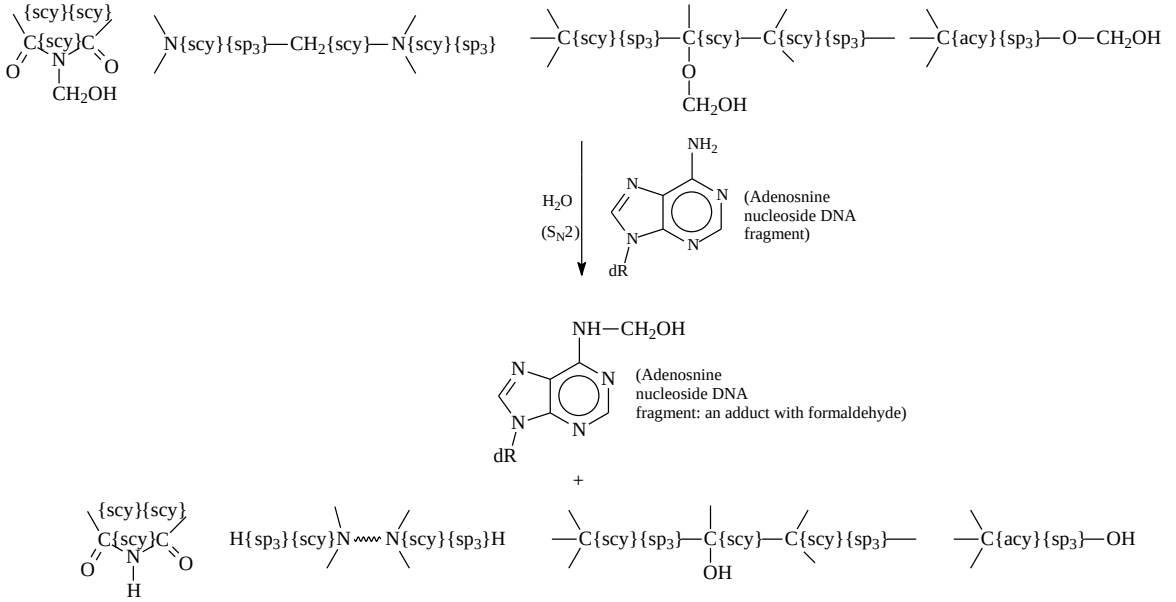
Such compounds may also release formaldehyde above pH6; hydrolysis can be slow, non-stoichiometric, and less complete at higher pH. The compound in 24 hours only released one-third of the potential formaldehyde [3]. The following stepwise abiotic (non-enzymatic) hydrolysis pathway can be assumed:



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].

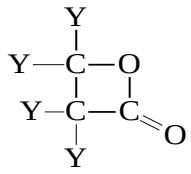
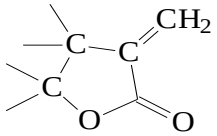
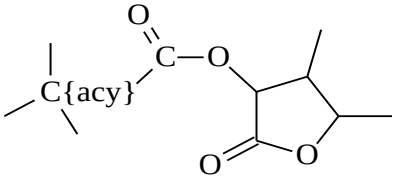
According to another publication, N-hydroxymethyl lesions have been instrumentally proved for cytosine, guanine and adenine nucleobases. The most persistent adducts resulting in pronounced mutagenicity were reported for adenine [5].

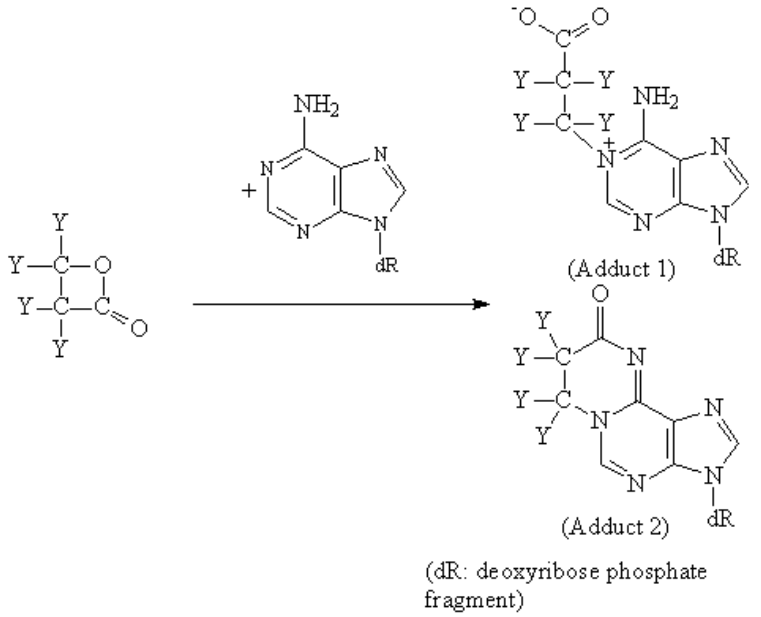
In these cases, formaldehyde release may occur "in situ", i.e. the nucleobase acts as stronger nuclophile, which replaces water and attaches the hydroxymethyl fragment directly to the amino group by SN2-type reaction. Based on the above discussions, the following, rather simplified mechanistic scheme,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



Set of chemicals used for profile development	Formaldehyde Releasers

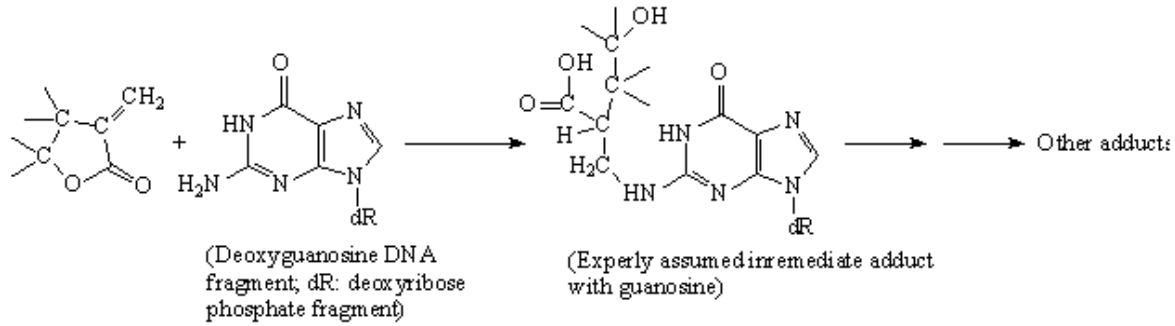
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Emeis, D., A. C. De Groot, J. Brinkmann, Determination 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:	

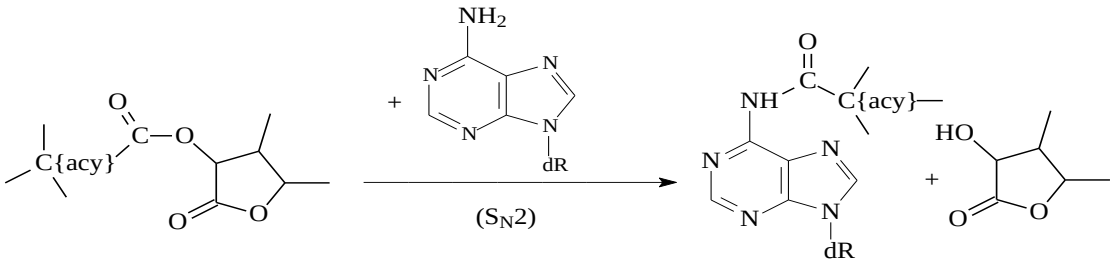


Scheme 1

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:

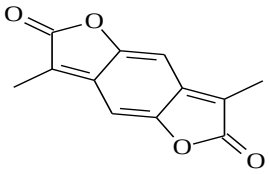
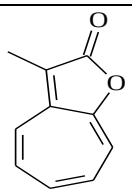


For some acylated five-membered hydroxylactones, another mechanistic scheme of interaction with DNA fragment, which is based on an expertly suggested activation of the acyloxy group attached to the lactone ring could be proposed:

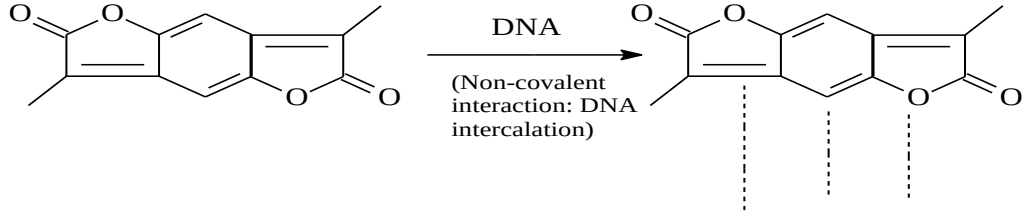


Set of chemicals used for profile development	Four- and Five-Membered Lactones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded

	in this profiler according to the references listed below.
References	1. 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.

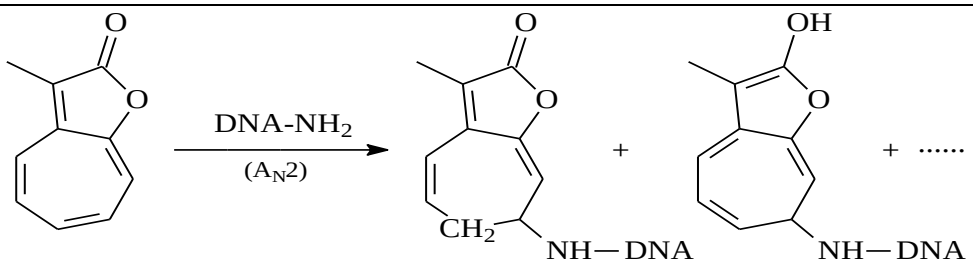
Individual profile/alert	
Name	Fused-Ring Conjugated Lactones
Type of profile	Structural alert
Description/applicability domain	 (Conjugated Benzodifurandione)  (Fused-Ring Conjugated Bicyclic Lactone)
Mechanism	Mechanistic Domain: AN2 Mechanistic Alert: Michael-type nucleophilic addition to conjugated 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)

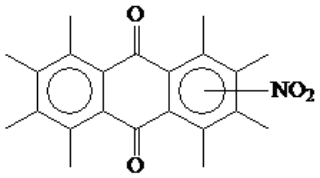
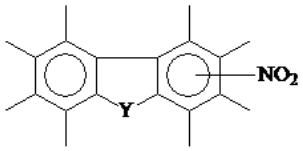
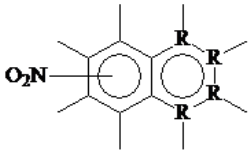
For more compact and less sterically hindered conjugated fused ring lactone systems, AN2 Michael-type additions with formation of covalent adduct(s) are also plausible (Scheme 2):

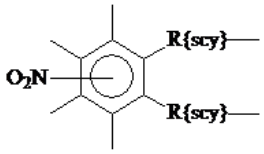
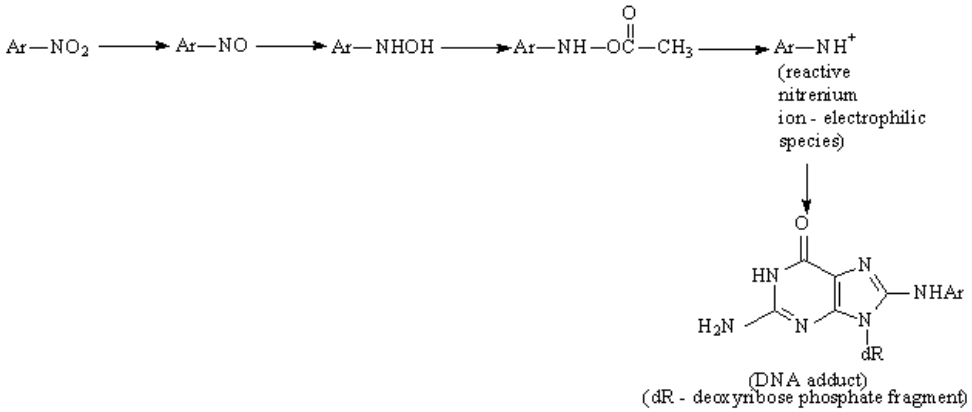


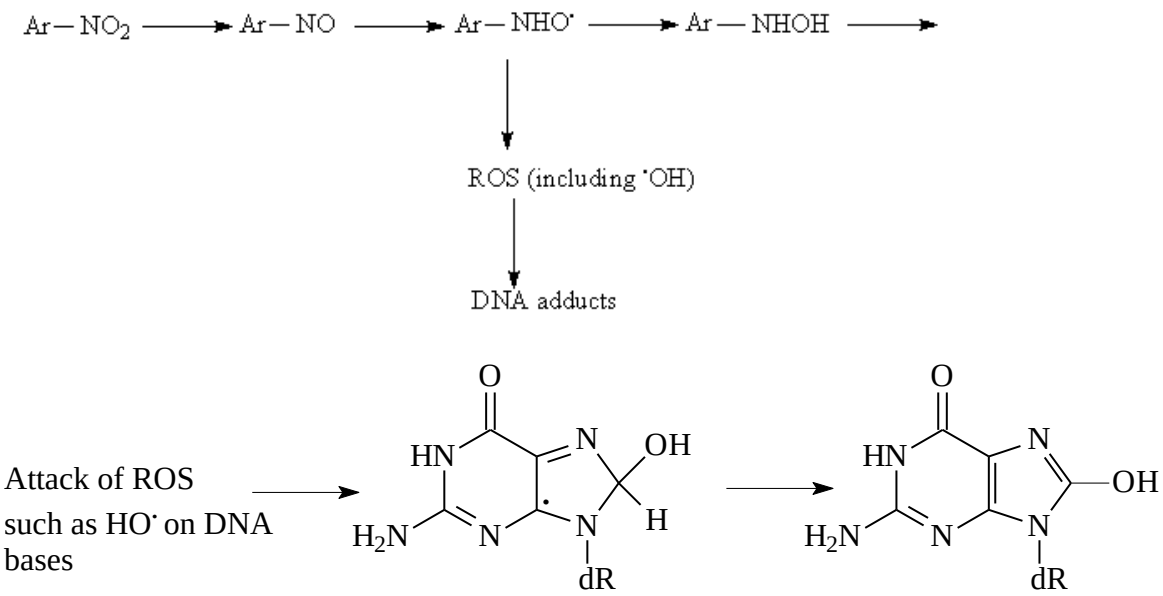
(Some possible DNA adducts)

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

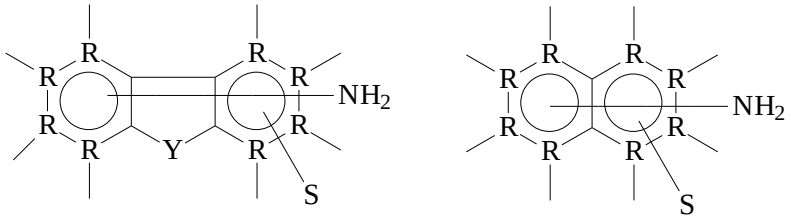
Set of chemicals used for profile development	Fused-Ring Conjugated Lactones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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**	

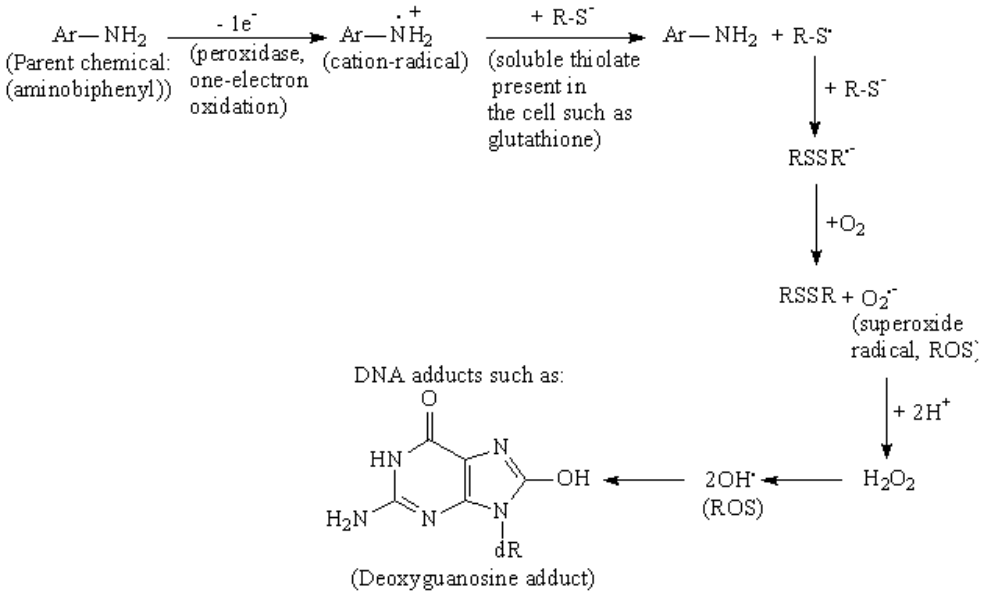


Set of chemicals used for profile development	Fused-Ring Nitroaromatics
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

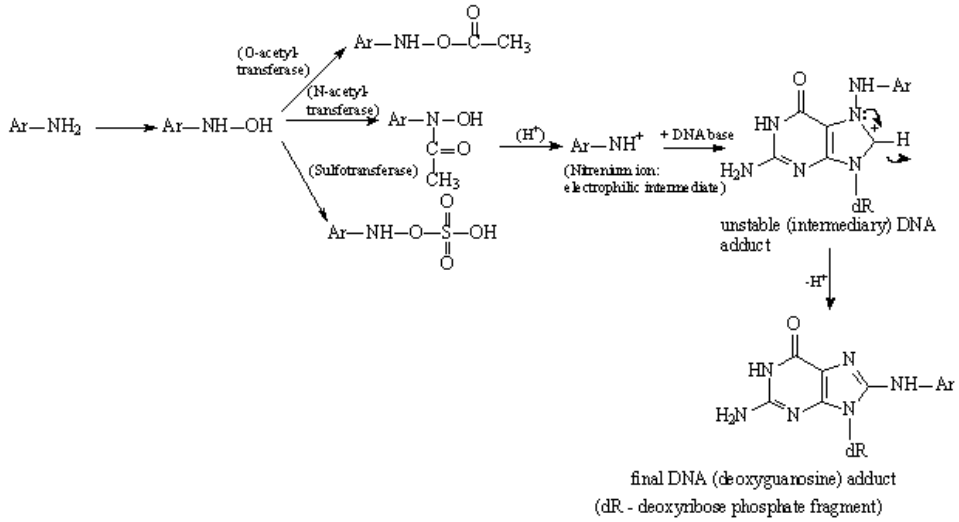
Mechanism	S_N1 Nucleophilic attack after metabolic nitrenium ion formation, Radical ROS generation (indirect) & Non-covalent interactions DNA intercalation

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

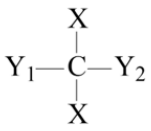
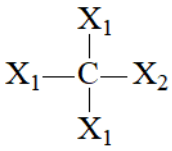
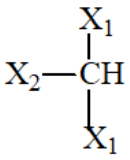
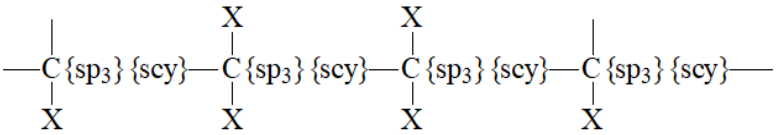
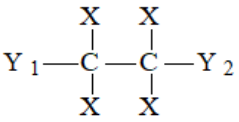
For all sub-classes of primary aromatic amines, including the polycyclic ones, there is strong evidence that, in many cases, metabolic activation with the external microsomal S9 system is required for eliciting mutagenicity and carcinogenicity. According to an excellent review on the bioactivation pathways 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]:

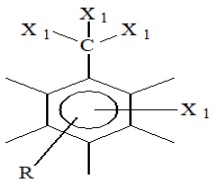
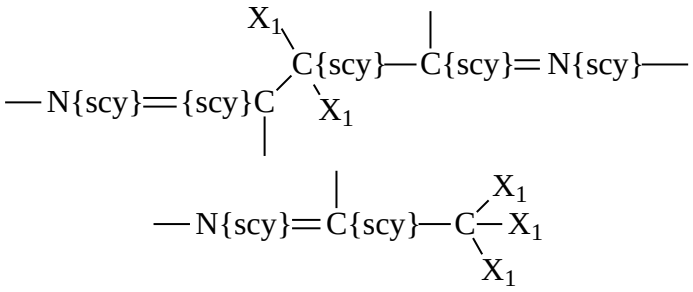
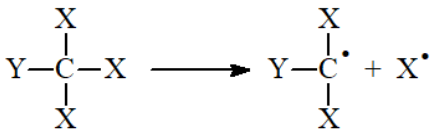


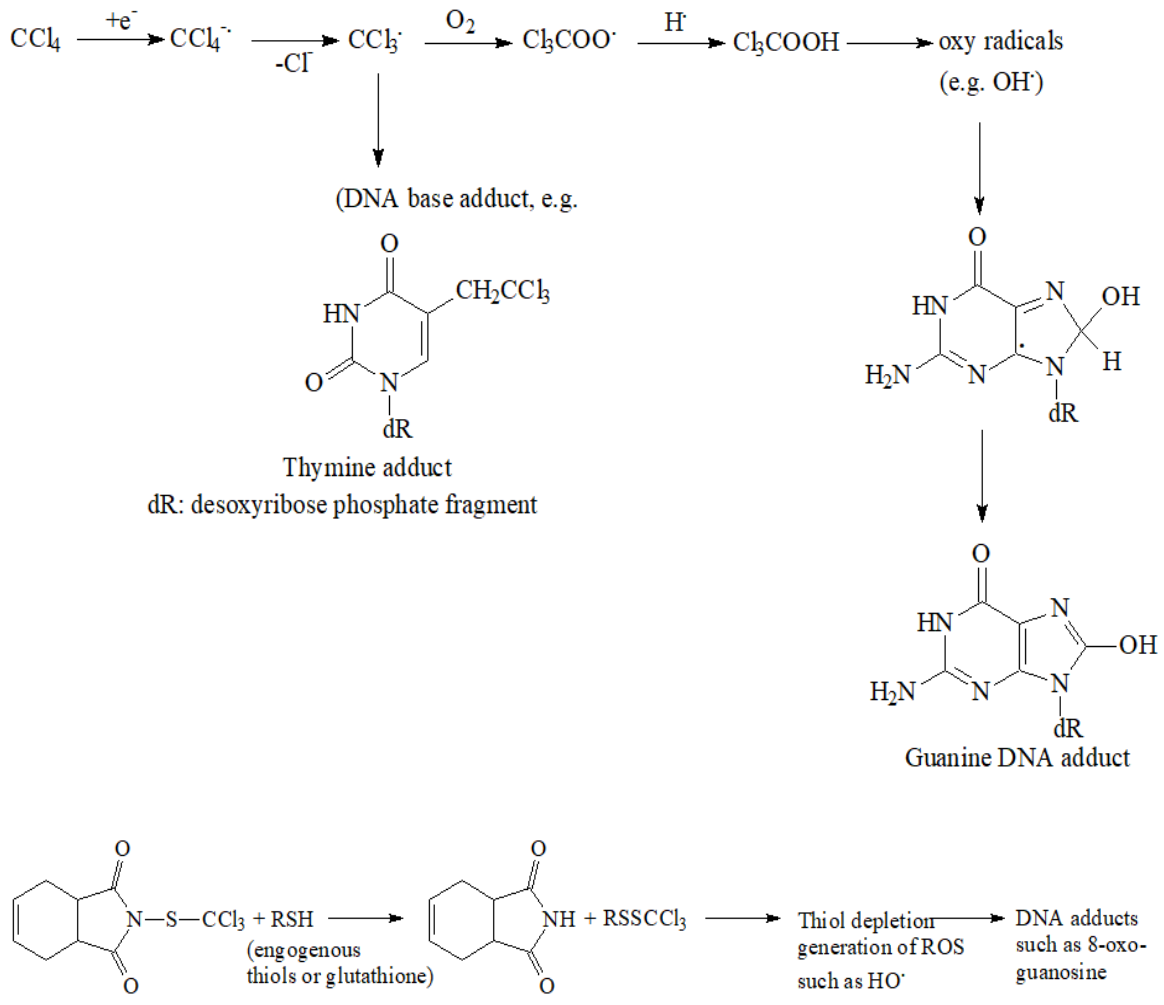
Scheme 2

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.

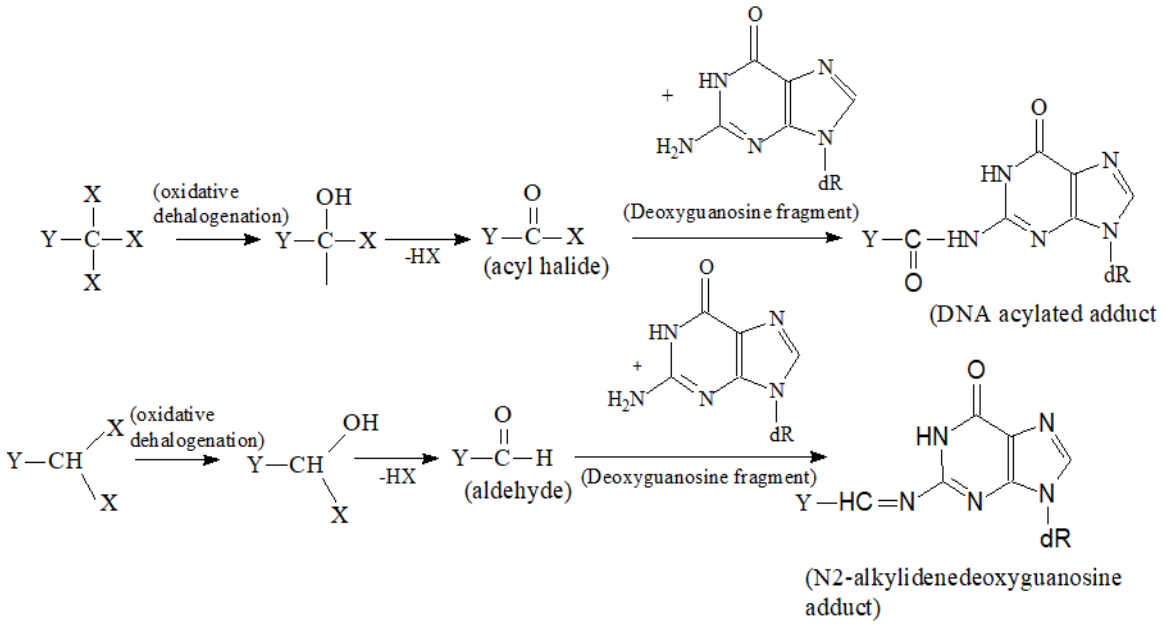
Individual profile/alert	
Name	Geminal Polyhaloalkane Derivatives
Type of profile	Structural alert

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

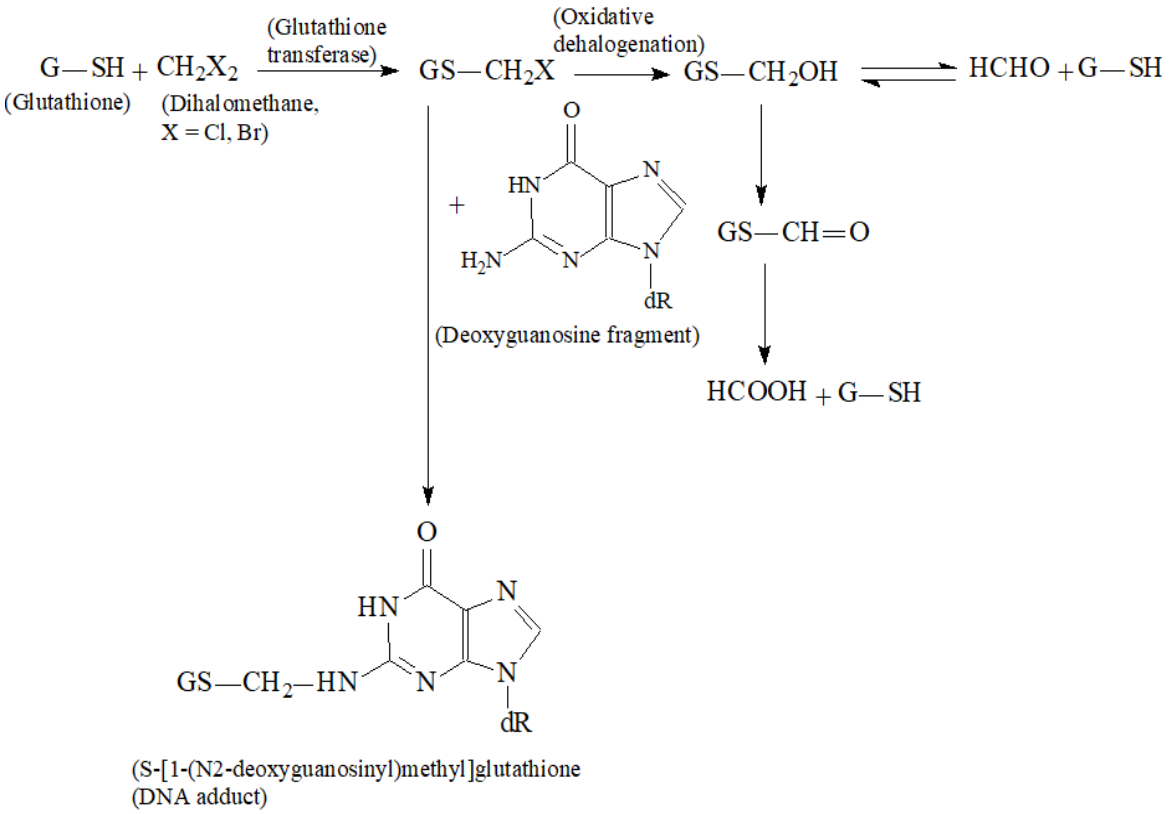
	 (X is Cl or Br; R is –CH_3 or –C_2H_5; No more than two X_1; No more than totally four substituents on phenyl ring)  (X_1 is Cl or Br)
Mechanism	S_N2 Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation, Radical ROS generation, S_N2 Acylation involving a leaving group after 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: 	



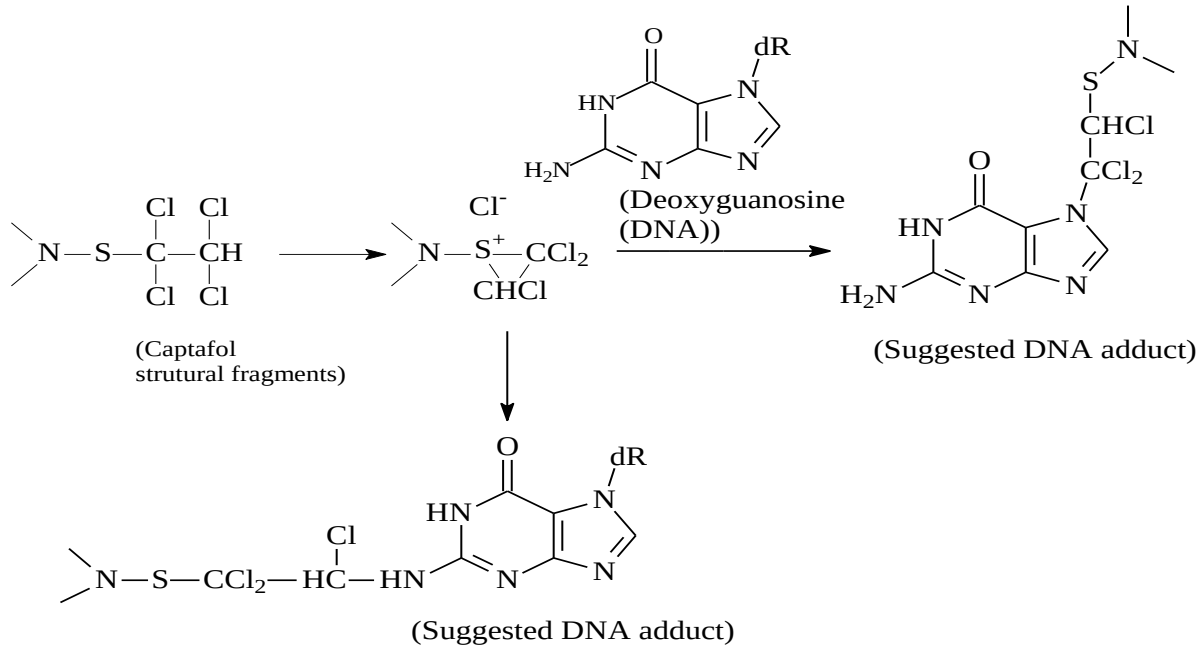
Non-Radical Mechanisms: Phase I metabolic activation –Oxidative dehalogenation



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

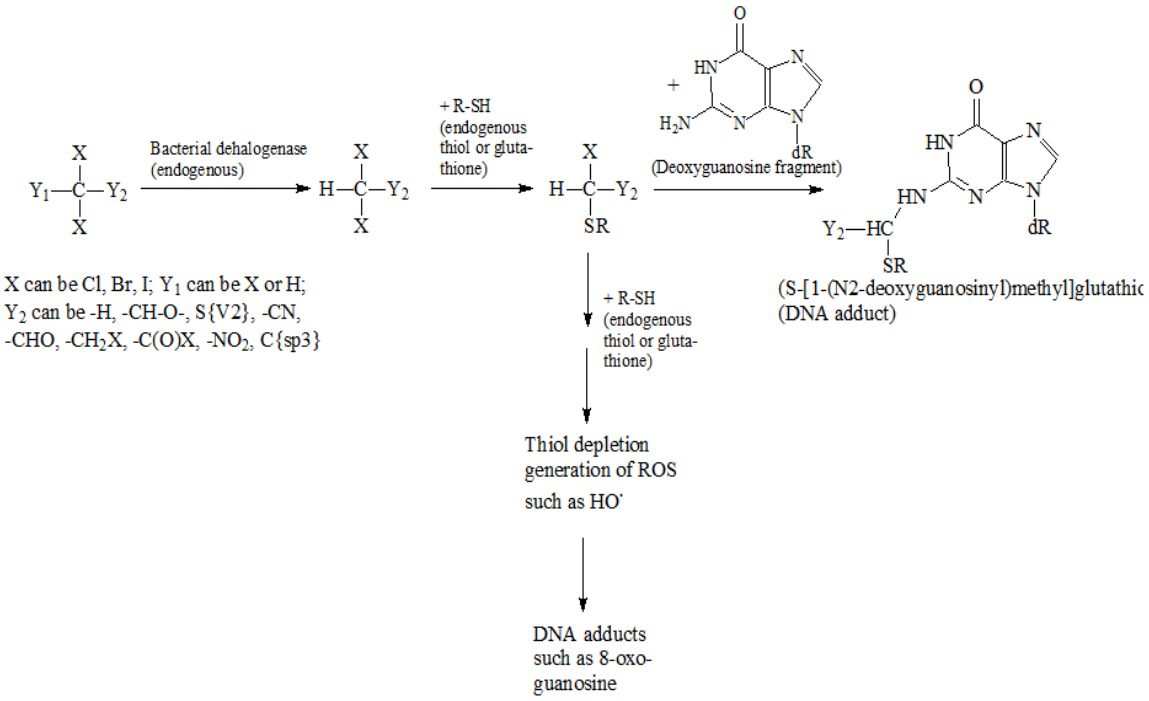


Episulfonium ion, which is known carcinogenic electrophile and derives from the glutathione-dependent bioactivation of some geminal polyhaloalkane derivatives such as captafol has been suggested as an active electrophilic species, which could explain the toxicity and mutagenicity of this chemical [30]:



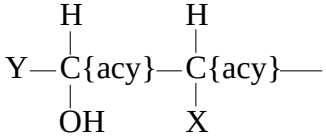
Non-Radical or Radical Mechanisms: Reductive dehalogenation and thiol/glutathione-dependent

bioactivation:



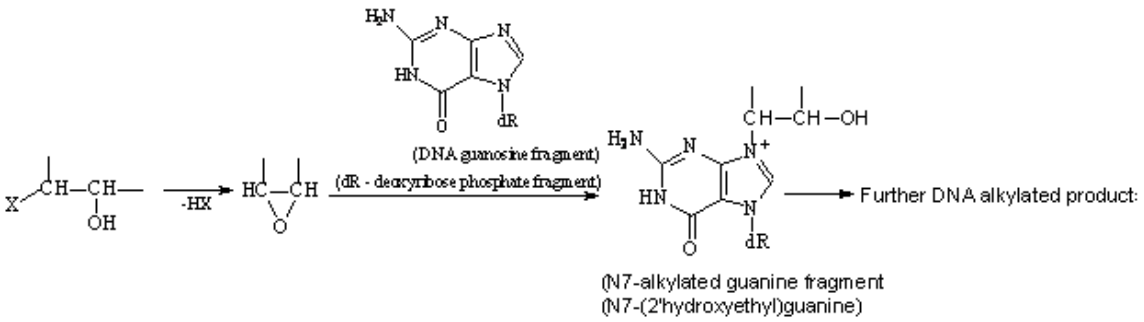
Set of chemicals used for profile development	Geminal Polyhaloalkane Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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. 5. Dodd, D.E., *Inhal. Toxicol.*, **1997**, 9(2), 111 – 131. 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). 7. CCRIS: Trifluoroiodomethane RN: 2314-97-8, Toxicology Data Network, U.S. National Library of Medicine**;** https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=2314-97-8. Last visited: June, 2021. 8. CCRIS: 1,1,1-Trichloroethane CASRN: 71-55-6, Toxicology Data Network, U.S. National Library of Medicine**;** https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=71-55-6. Last visited: June, 2021. 9. Schrader, T.J., *Mutat. Res.*, **1998**, **413**(2), 159 - 168. 10. Mortelmans, K., *Environ. Mutagen.*, **1986**, 8 (Suppl. 7), 1 - 119. 11. Hosey, K. M. Quinn, J. Environ. Protection **3** (21012), 902 – 914. 12. Captafol CASRN: 2425-06-1, CCRIS, Toxicology Data Network, U.S. National Library of Medicine; https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=2

	425-06-1. Last visited: June, 2021. 13. Barrueco, C., Mutagen. **3**(6) (1988), 467 – 480. 14. Sims, J. L., J. M. Suflita, H. H. Russel, Reductive Dehalogenation of Organic Contaminants in Soils and Ground Water, EPA/540/4-90/054, January 1991, 1 – 12. 15. Ruiz, M. J., Mutat. Res. **390** (1997), 245 – 255. 16. DeBaun, J. R., Xenobiotica **4**(2) (1974), 101 - 119. 17. D. Morte, Boll. Soc. Ital. Biol. Sper. **70**(8 - 9) (1994), 185 – 192 (Abtsract); http://www.ncbi.nlm.nih.gov/pubmed/7893475. Last visited: July, 2021. 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)

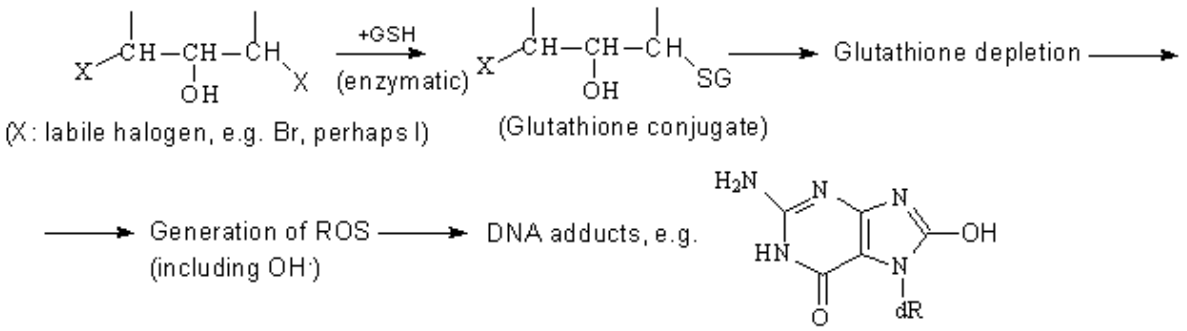
	$X—(CH_2)_n—OH$ (X is Cl, Br, n = 3 - 10) HC{scy}—X CH{scy}—OH Y X is Cl, Br or I; Y can be one of the following: C{sp3} or -H
Mechanism	S_N2 Alkylation, direct-acting epoxide formed after E2 reaction and Radical ROS formation after GSH depletion (indirect)

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:



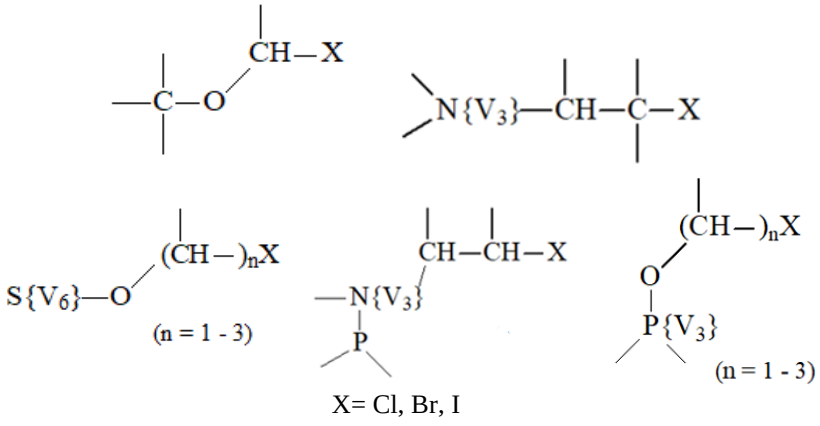
Such mechanistic scheme could also apply to haloalcohols of the type:

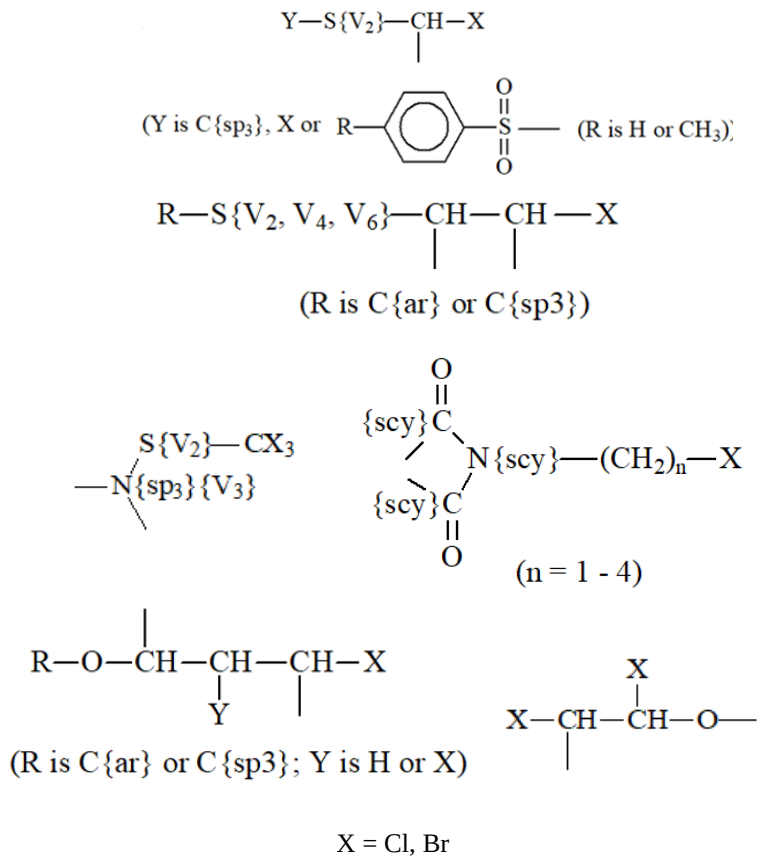
$$X—(CH_2)_n—OH$$

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

Scheme 2

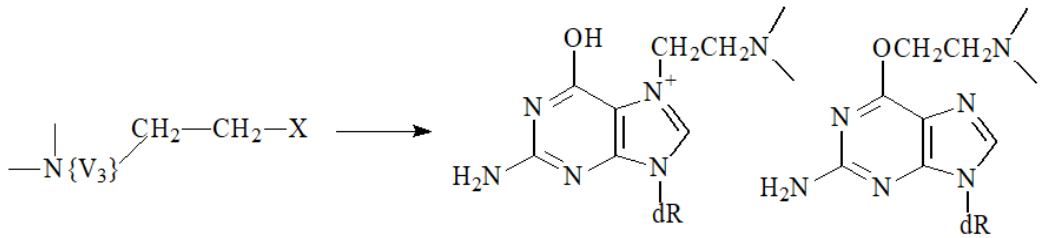
Set of chemicals used for profile development	Haloalcohols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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



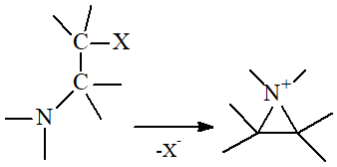
Mechanism	S_N2 Alkylation, nucleophilic substitution at sp3 carbon atom & Radical Generation of ROS by glutathione depletion

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

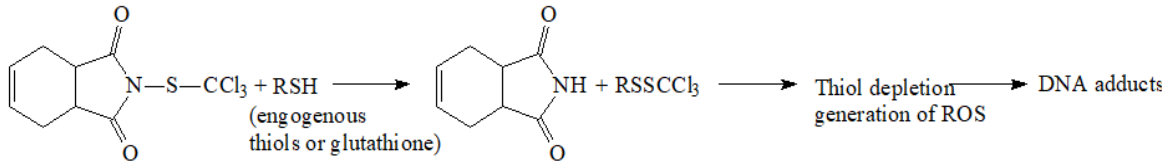


N7-alkylated adduct O6-alkylated adduct

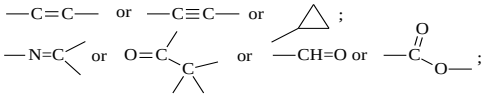
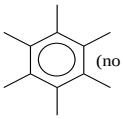
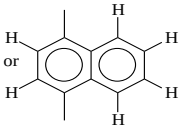
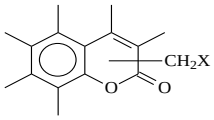
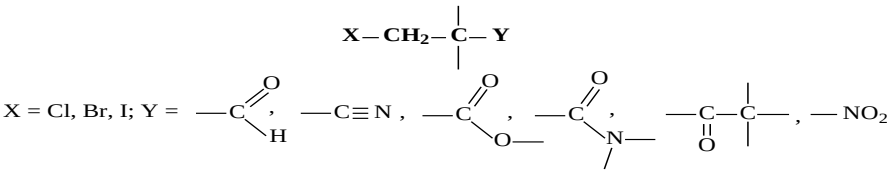
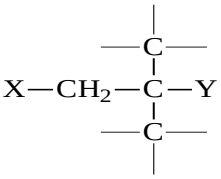
(dR - deoxyribose phosphate fragment)

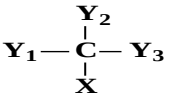
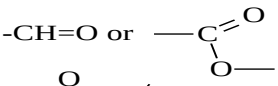
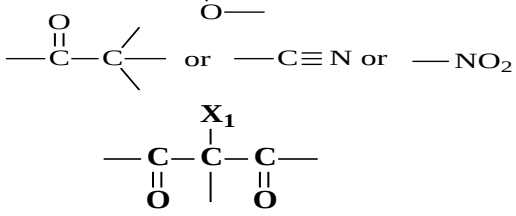


2. Compounds with halogen in α-position with respect to a heteroatom

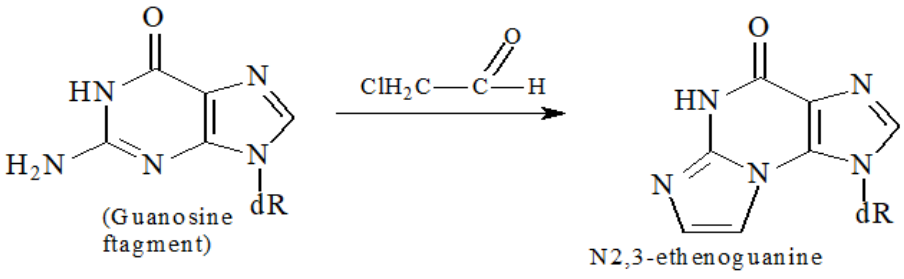


Set of chemicals used for profile development	Haloalkane Derivatives Containing Chain Heteroatom
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the 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.

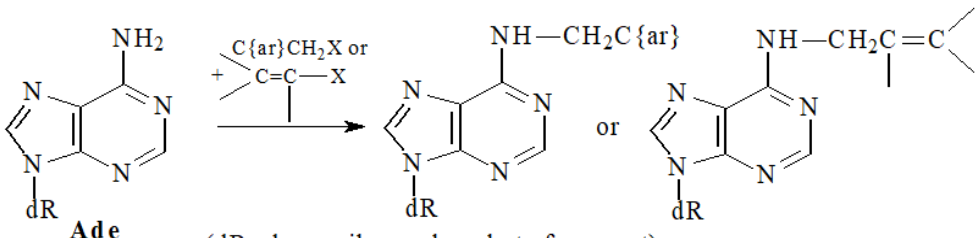
Individual profile/alert	
Name	Haloalkane Derivatives with Labile Halogen
Type of profile	Structural alert
Description/applicability domain	A. Primary haloalkane derivatives with labile halogen and alpha-activating group: **Y—CH_2X (Y is attached *via* Catom)** X is Cl, Br, I and Y can be one of the following: —C=C— or —C≡C— or ;  —C≡N or —NO_2;  (no X and no -SO_3H attached, single ring, no more than two substituents attached) or  "Masks":  B. Primary haloalkane derivatives with labile halogen and beta-activating group  "Mask":  *(Note: If additional one or two more –CH_2X-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 Y_1 is C; Y_2 is H or X or CH_3; Y_3 is -CH=O or  or  (X_1 is Cl, Br, F)
Mechanism	S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom, A_N2 Shiff base formation for aldehydes & S_N2 Acylation involving a leaving group

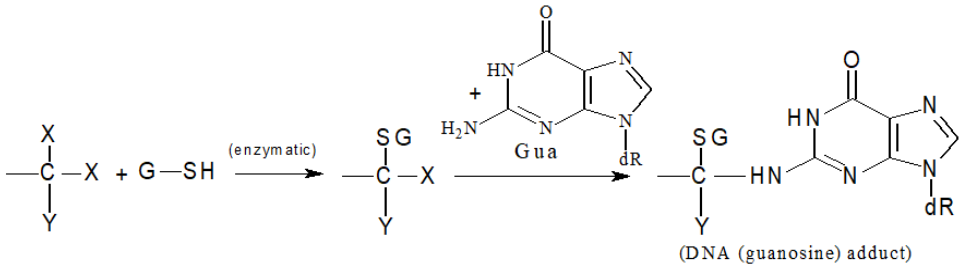
A. Primary haloalkane derivatives with labile halogen and alpha-activating group:



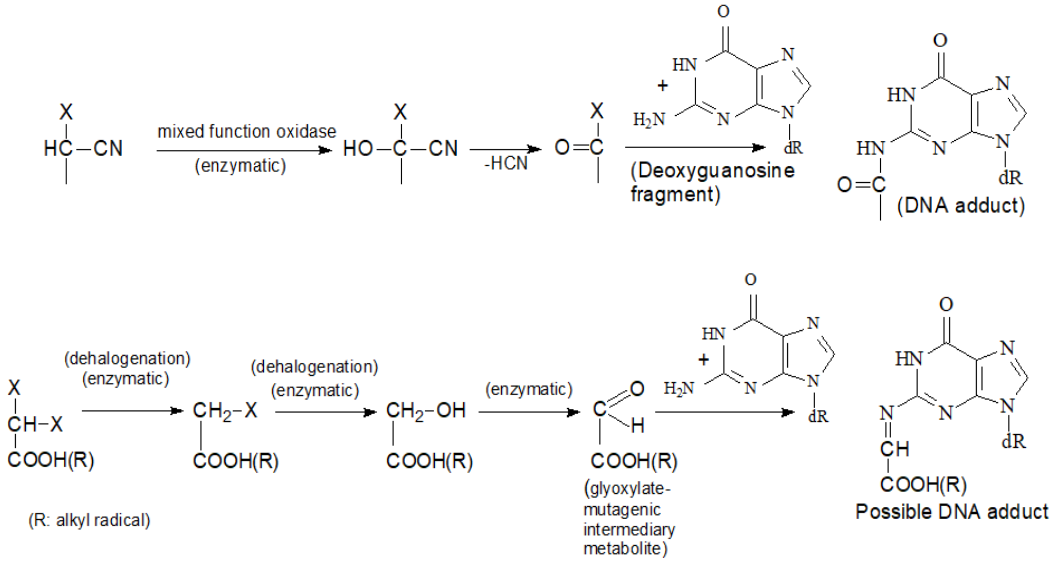
(dR - deoxyribose phosphate fragment)



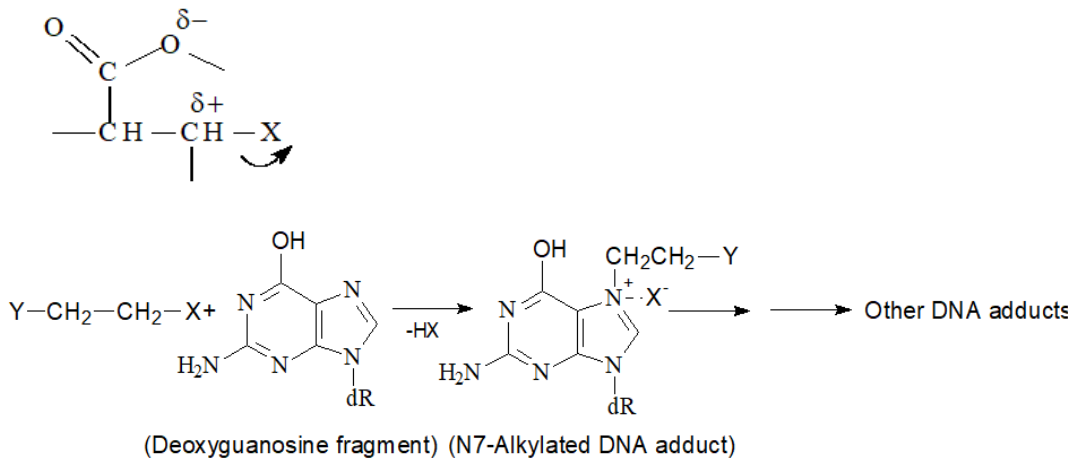
(dR- deoxyribose phosphate fragment)



(Y is $-NO_2$, X is halogen or -H)



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

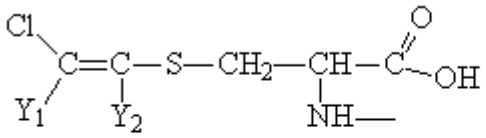
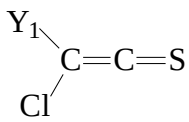


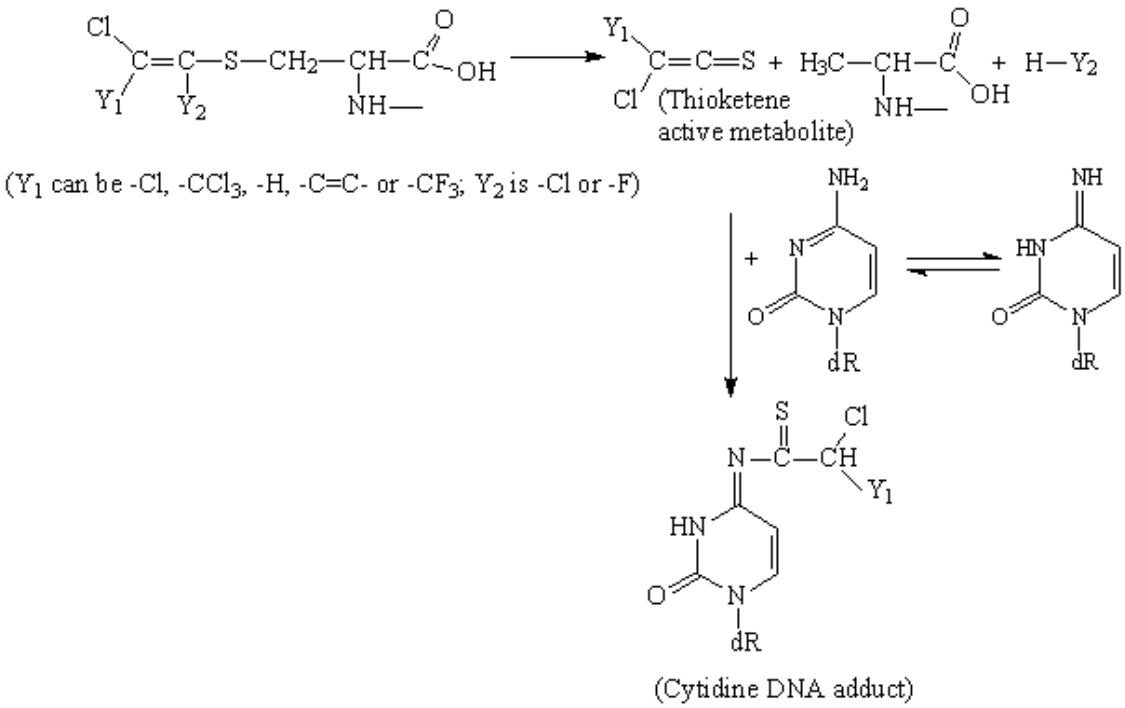
C. Secondary haloalkane derivatives with labile halogen and alpha-activating group

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.

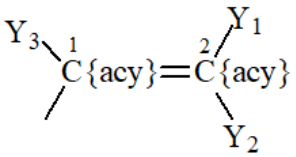
Set of chemicals used for profile development	Haloalkane Derivatives with Labile Halogen
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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

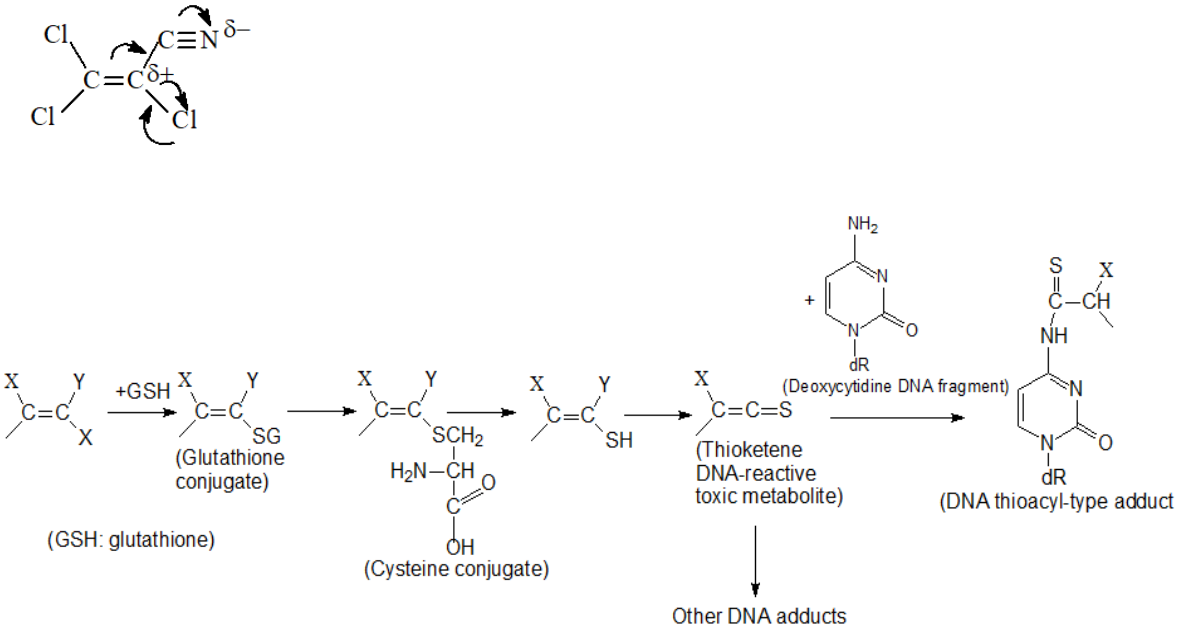


Set of chemicals used for profile development	Haloalkene Cysteine S-Conjugates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

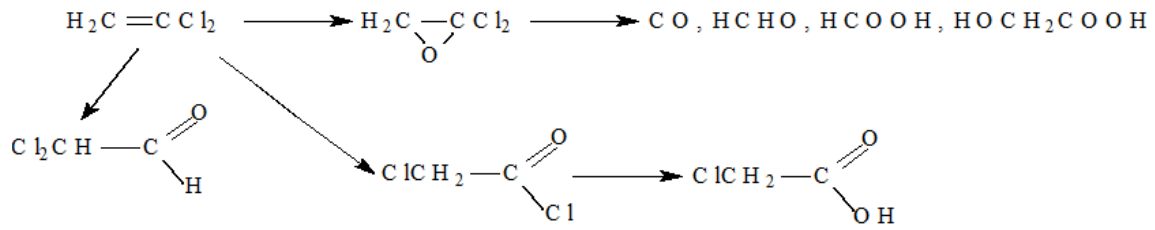
Individual profile/alert	
Name	Haloalkene Derivatives with Electron-Withdrawing Groups
Type of profile	Structural alert
Description/applicability domain	 Y_2 can be $–NO_2$ or –CN or **-C=C-** or Cl or Br or -C(O)O- (attached *via* the carbon of carbonyl group C(O)), or -C(O)C (attached *via* the carbon 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

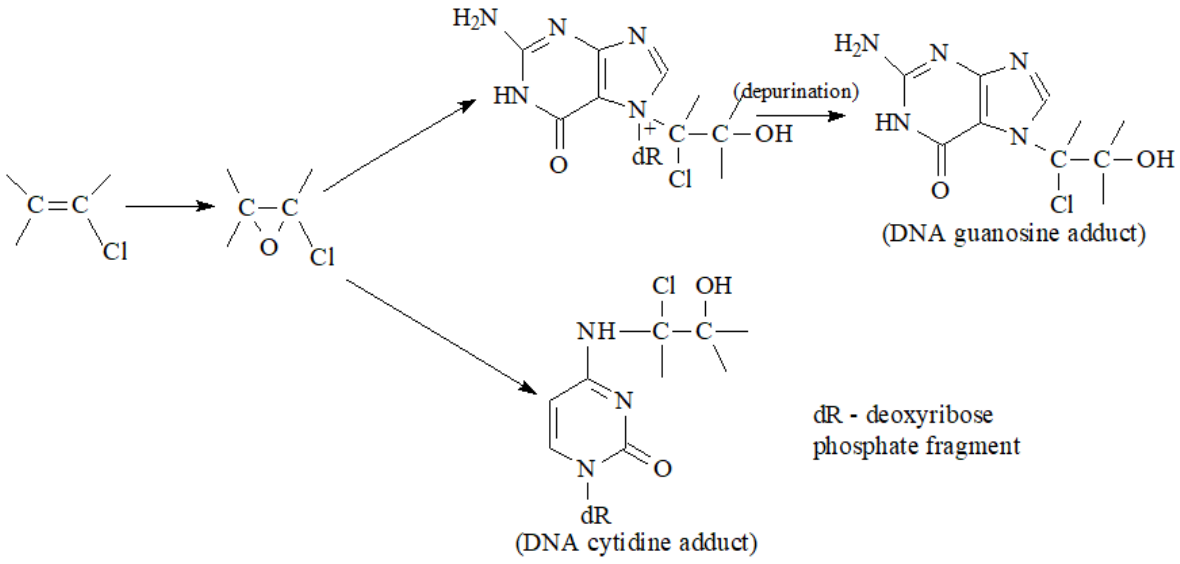
1. Haloalkenes containing halogen(s) and other electron-withdrawing group(s) (EWG).



2. Vinyl-type haloalkenes, not containing other EWGs

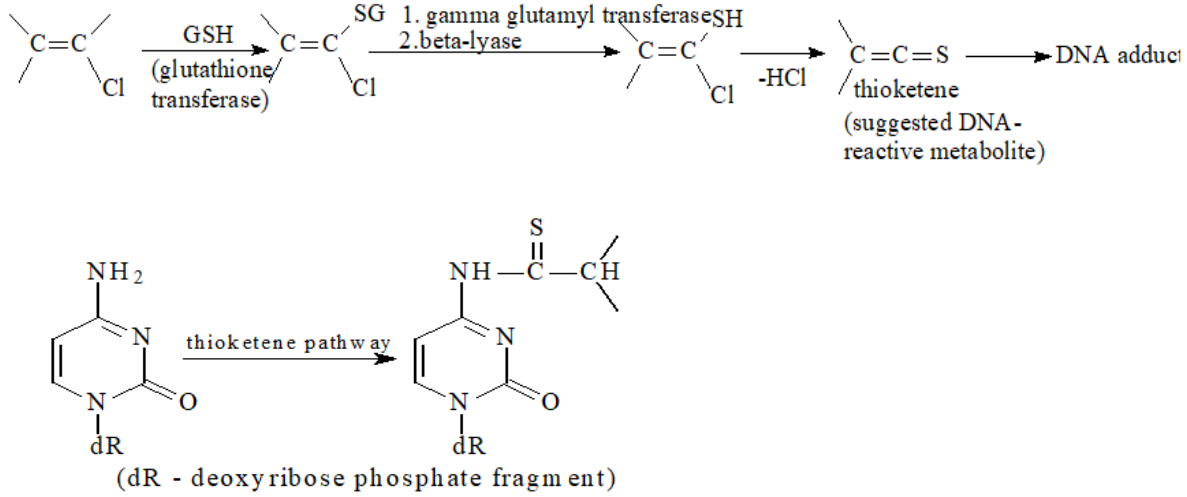


3. Formation of epoxide intermediate that binds covalently to DNA *via* electrophilic mechanism of alkylation towards the biological macromolecule:



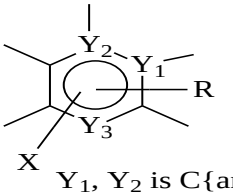
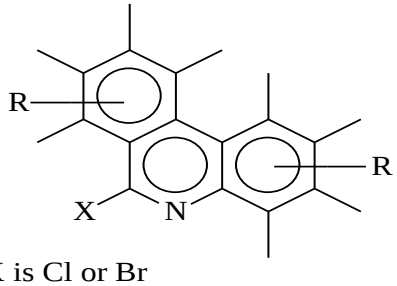
4. Glutathione or thiol activation pathway. In this case, the formation of reactive product that binds to

DNA *via* electrophilic mechanism [11] takes place:

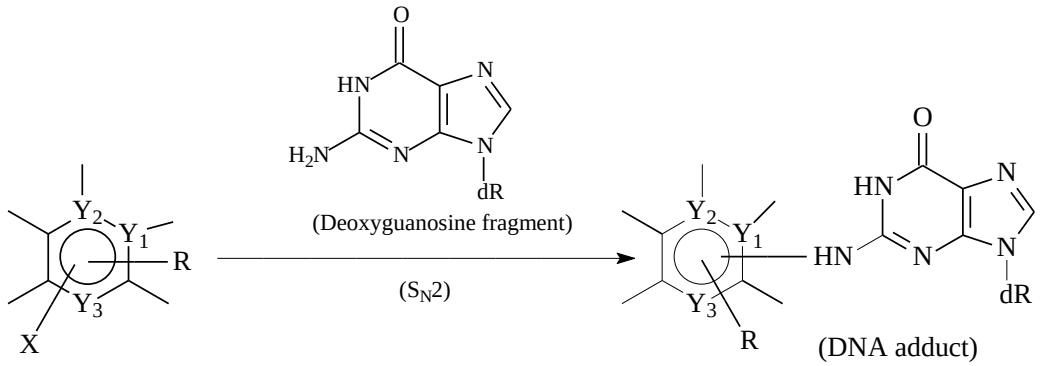


Set of chemicals used for profile development	Haloalkene Derivatives with Electron-Withdrawing Groups
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according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

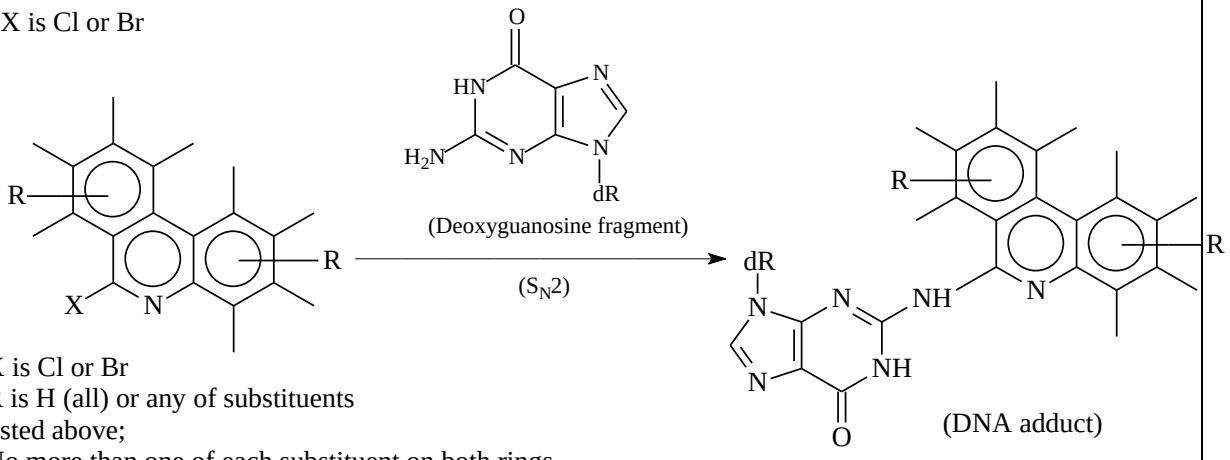
	15. Dichlorvos, ChemPlus, A Tooxnet Database, U.S. National Library of Medicine; https://chem.nlm.nih.gov/chemidplus/rn/62-73-7 Last visited: June, 2021. 16. Bucher, J. R., *NTP Technical Report on Toxicity Studies of β-Bromo-β-Nitrostyrene (CAS No. 7166-19-0) Administered by Gavage to F344/N Rats and 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:



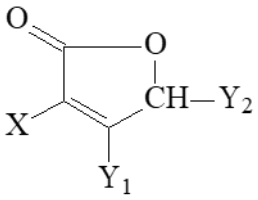
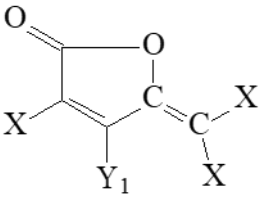
Y_1, Y_2 is C{ar} (both) or N{ar} (one only, the other is C{ar});
Y_3 is N{ar} or N{ar}=O;
R is $-NO_2$ or —C≡N or
$-CH_3$ or $-OCH_3$ or N{V3}{sp3}
or $-CCl_3$ or Cl;
X is Cl or Br

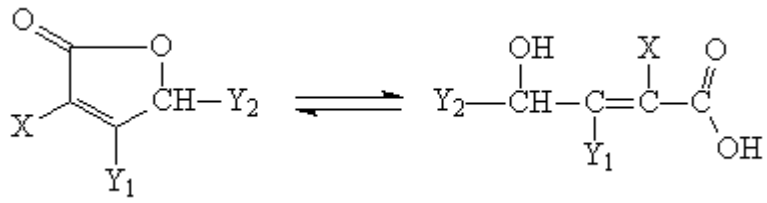


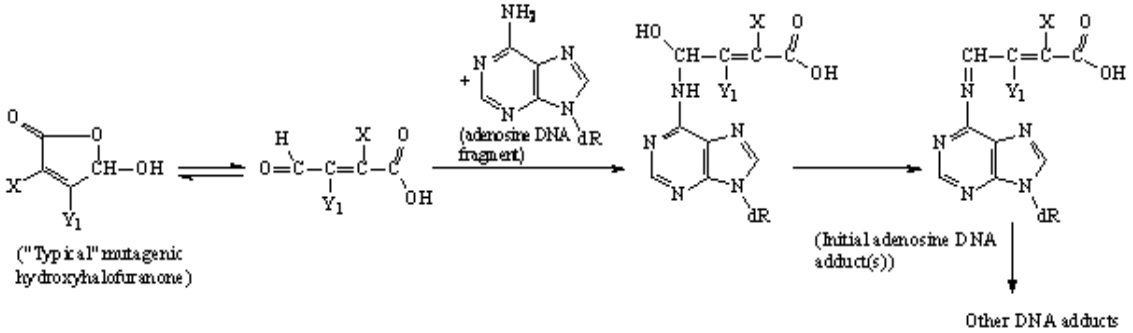
X is Cl or Br
R is H (all) or any of substituents listed above;
No more than one of each substituent on both rings

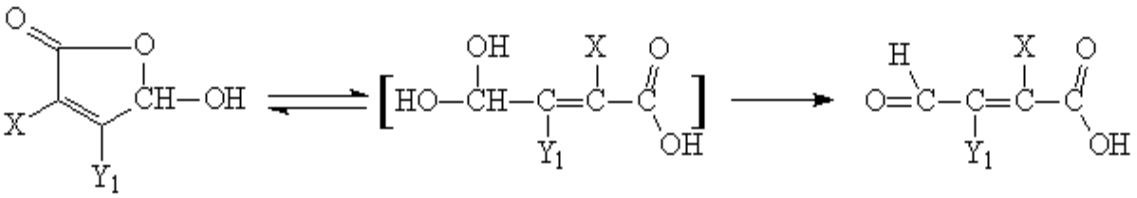
Set of chemicals used for profile development	Haloazaarene and Fused-Ring Haloquinoline Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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,

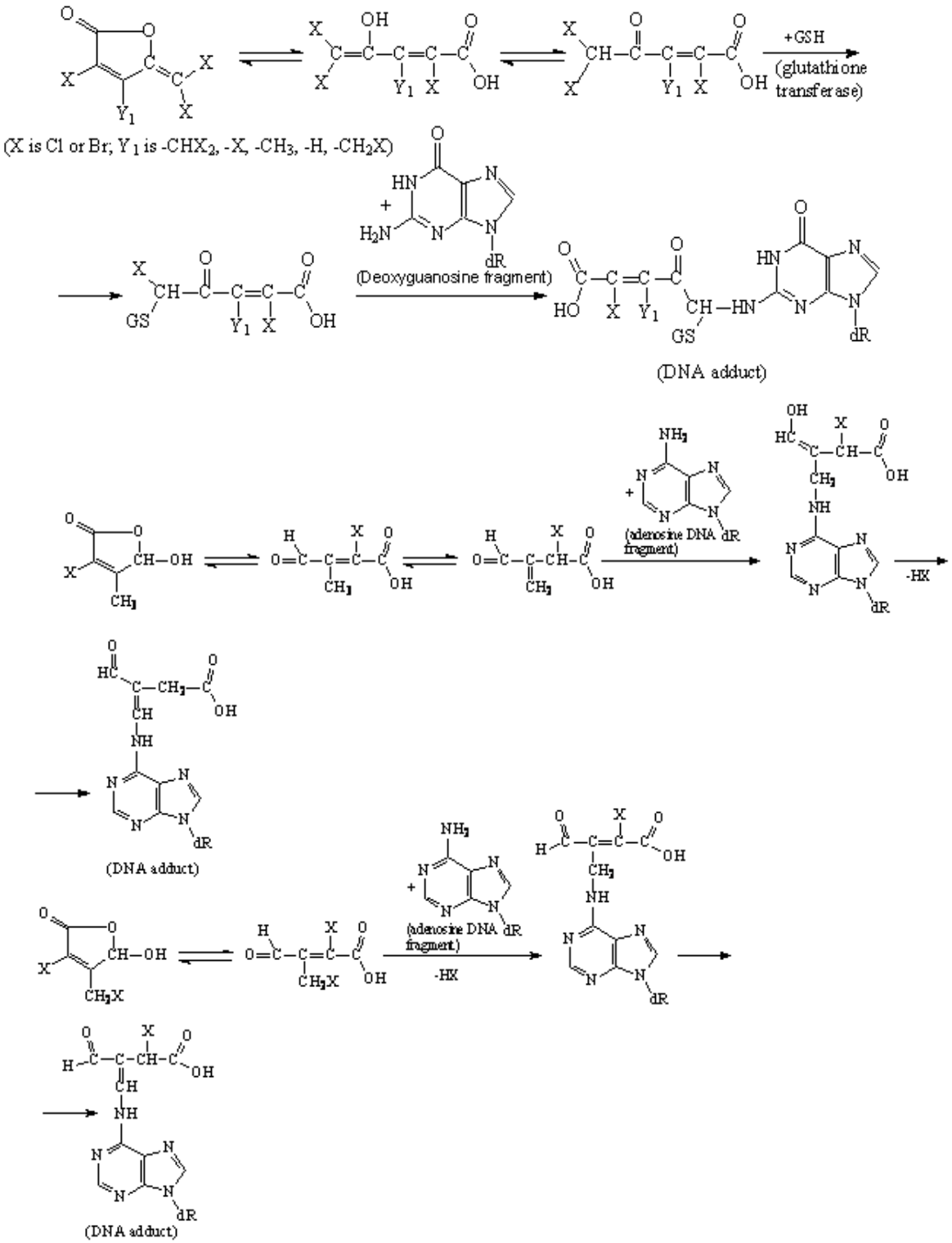
	Mutat. Res. 176 (1987), 185 – 198.

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









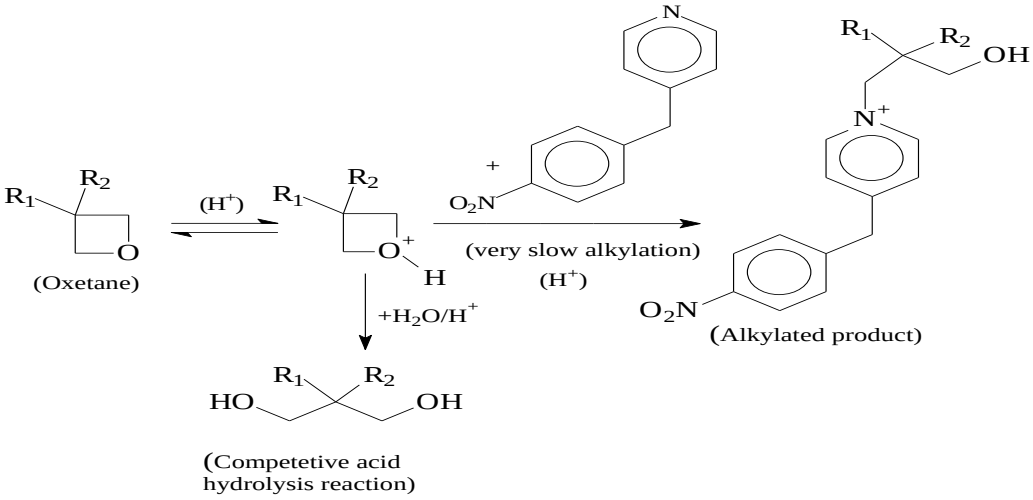
Set of chemicals used for profile development	Halofuranones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

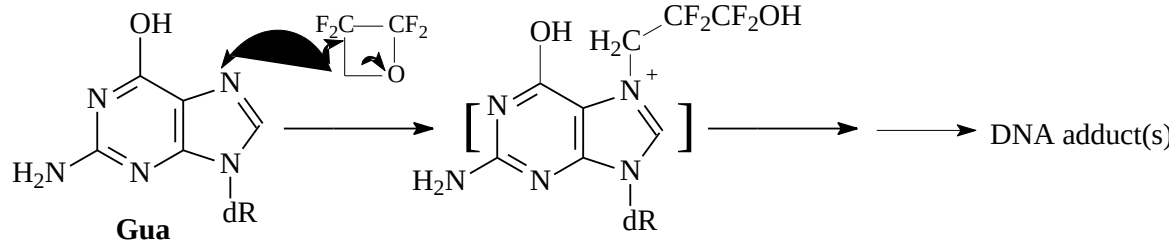
I. <u>Halogenated Oxetanes</u>

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):

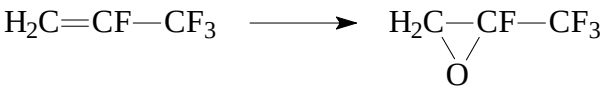


(dR - deoxyribose phosphate fragment;
Gua: Guanine nucleosides)

(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):

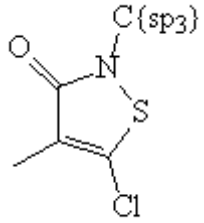


2,3,3,3-Tetrafluoropropene (HFO-1234yf)

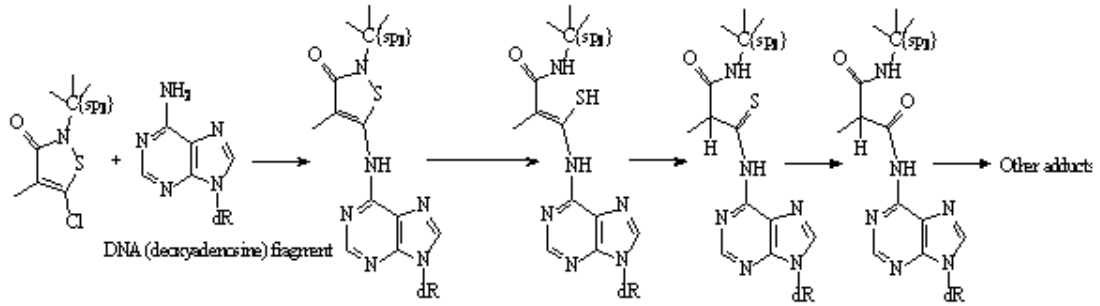
2,3,3,3-Tetrafluoropropene epoxide (active metabolite)

(Scheme 3)

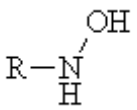
Set of chemicals used for profile development	Halogenated Oxetanes and Haloepoxides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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**

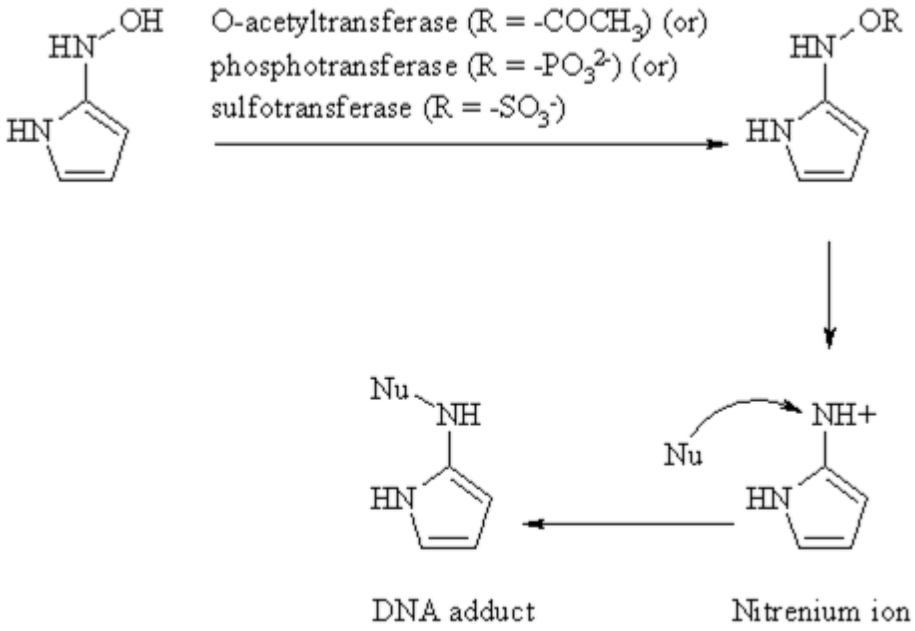
Despite the fact that no mechanistic schemes for DNA adduct formation with this class of chemicals have been found in the literature so far, it may be suggested that some potential DNA reactivity and adduct formation are possible. For example, the adenine base in DNA would perhaps react as nucleophile *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	Haloisothiazolinones
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.

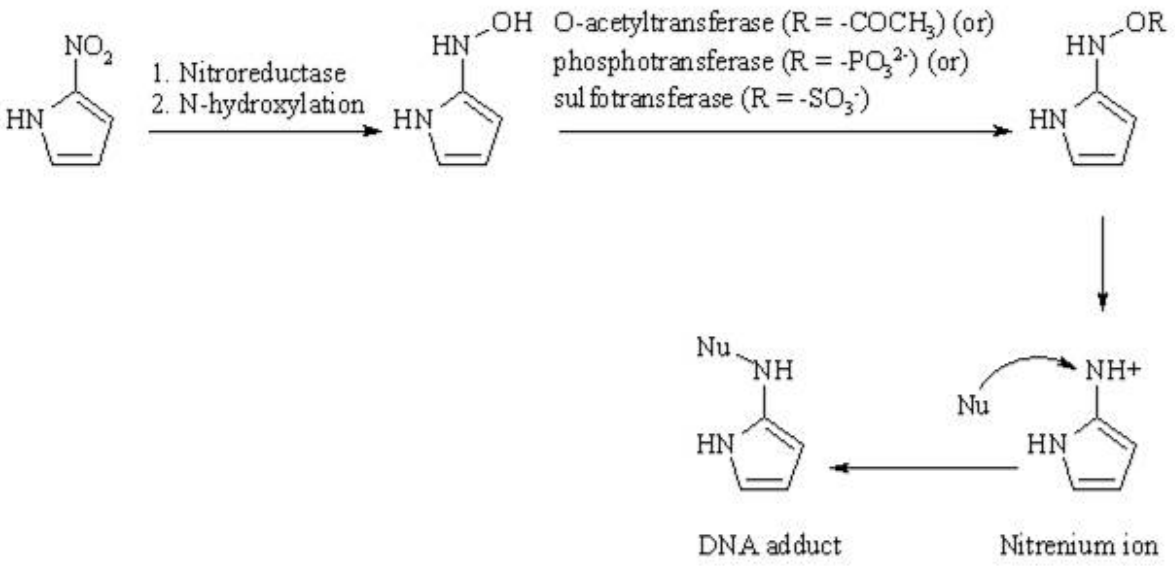
Individual profile/alert	
Name	Heterocyclic N-Hydroxylamines
Type of profile	Structural alert
Description/applicability domain	 R = aromatic carbon atom
Mechanism	SN1 reaction Nitrenium ion formation

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

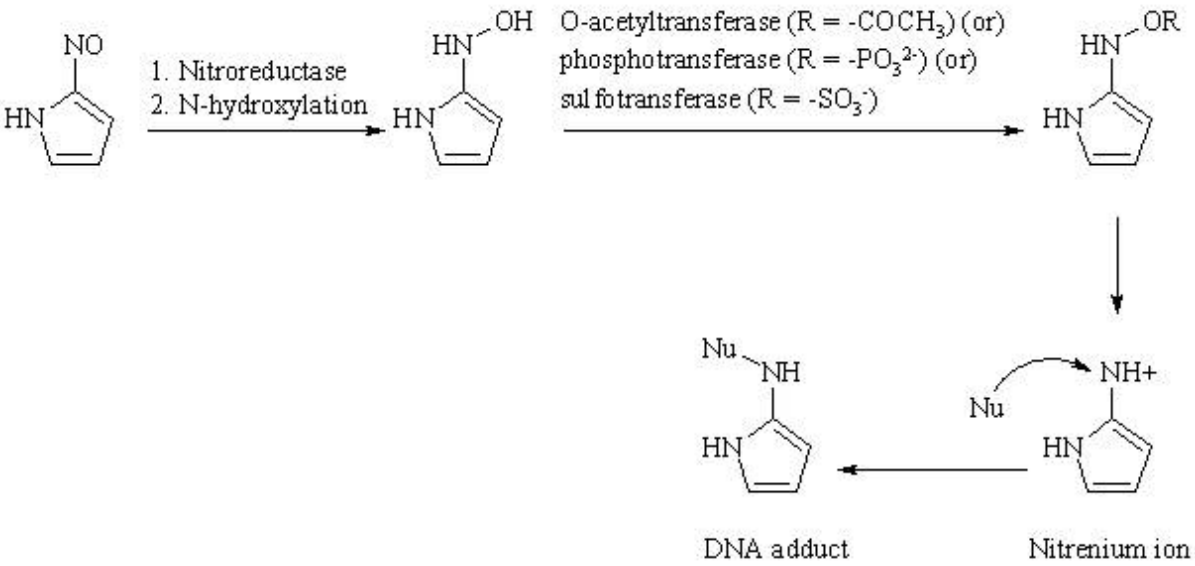
Individual profile/alert	
Name	Heterocyclic nitro compounds
Type of profile	Structural alert
Description/applicability domain	$R—NO_2$ R = aromatic carbon atom
Mechanism	SN1 reaction Nitrenium ion formation
Heterocyclic nitro groups can be metabolised into an N-hydroxylated intermediate which subsequently 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

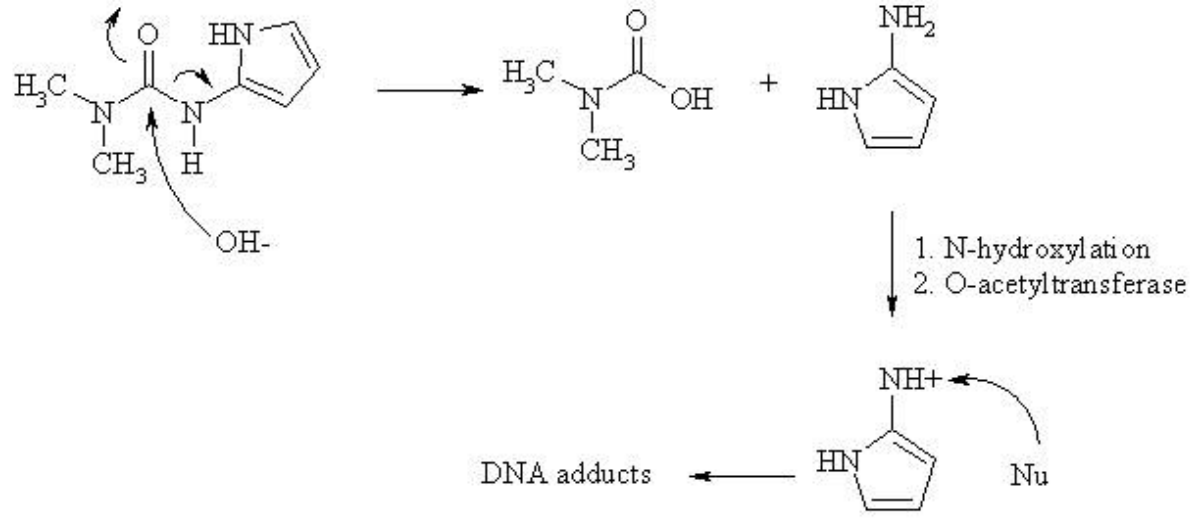
Heterocyclic nitroso compounds have the potential to be reduced, and then hydroxylated to an N-hydroxylamine intermediate. This species is then further metabolised by one of three potential transferases, which themselves produce the reactive nitrenium ion which can bind DNA via an S_N1 mechanism (Kalgutkaer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



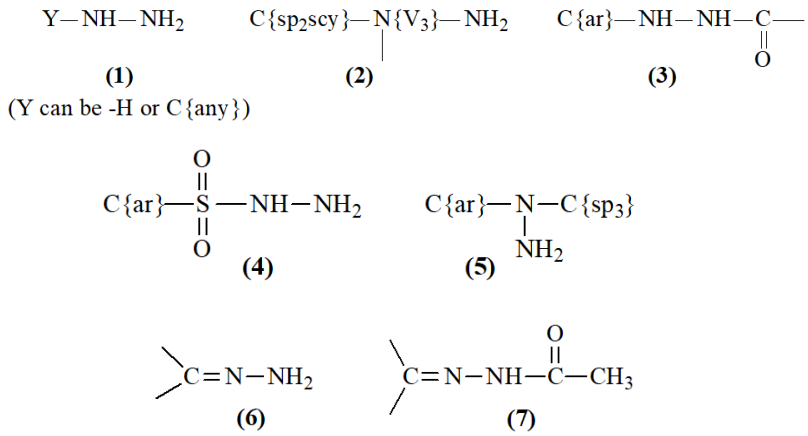
Set of chemicals used for profile development	Heterocyclic Nitroso compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

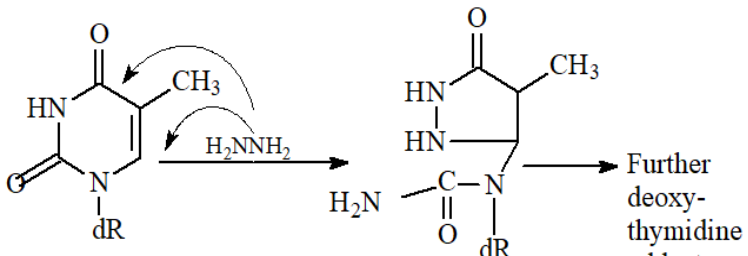
Hydrolysis of the amide bond to produce an aromatic amine moiety has been suggested to be responsible for the toxicity of chemicals containing this alert. The formation of the nitrenium ion results in DNA binding via an SN1 mechanism (Guengerich et al 1997).



Set of chemicals used for profile development	Heterocyclic urea derevatives
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

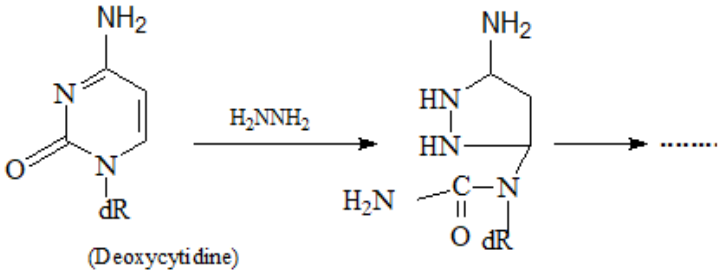
The mechanism of the direct formation of the initial DNA adduct with hydrazine is complex, accompanied by an array of DNA adducts [3]:



(Thy)

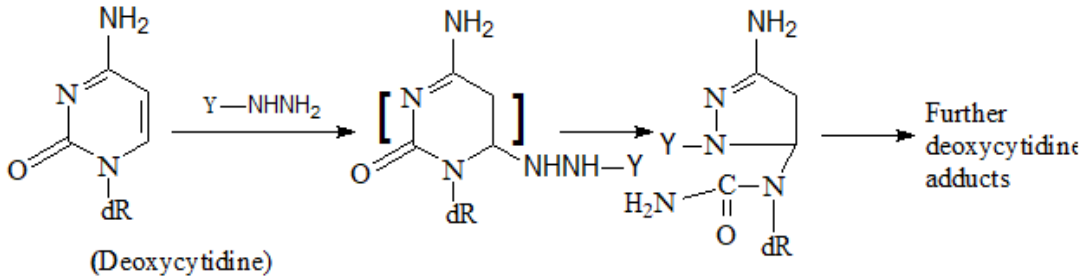
(dR - deoxyribose phosphate fragment)

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:



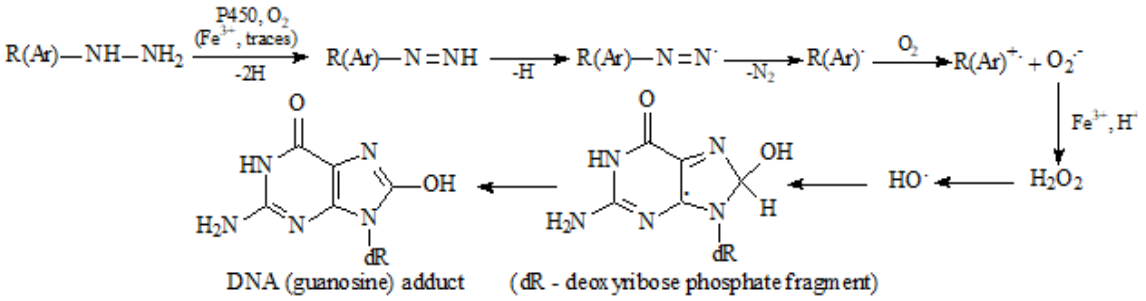
(Deoxycytidine)
(dR - deoxyribose phosphate fragment)

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:

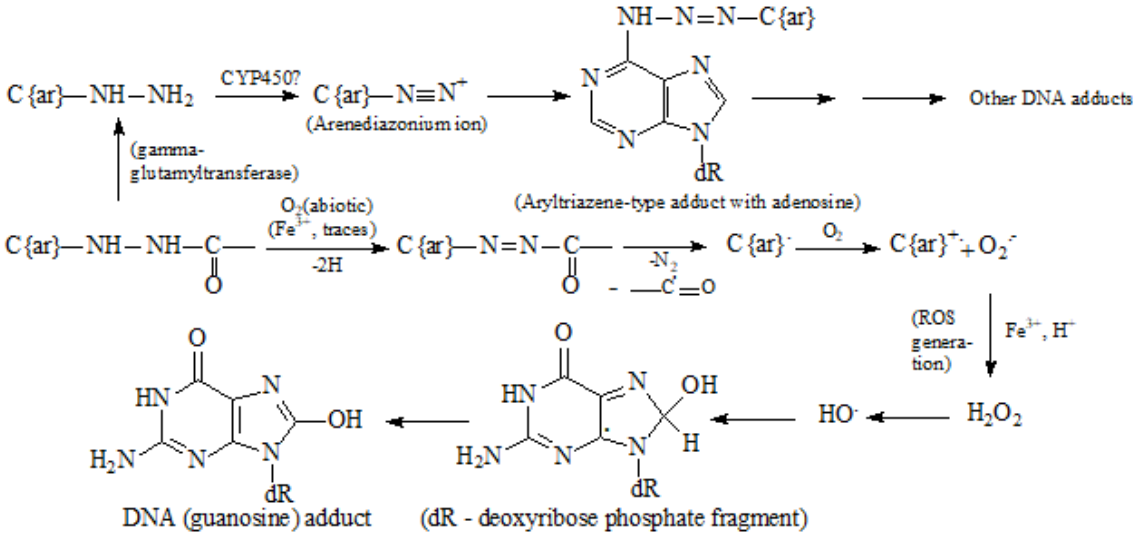


(Deoxycytidine)
(dR - deoxyribose phosphate fragment)
(Y can be alkyl, aryl or -H)

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]:

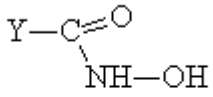


Based on the established abiotic oxidative consumption of agaritine and structurally similar chemicals, the following mechanistic scheme for the explanation of its mutagenicity can be expertly suggested:

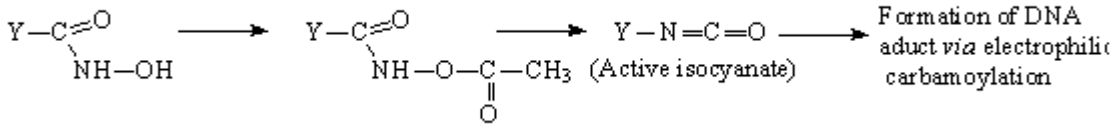


Set of chemicals used for profile development	Hydrazine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

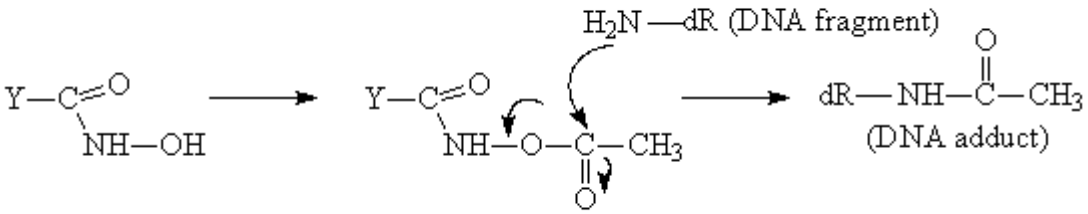
Individual profile/alert	
Name	Hydroxamic Acids
Type of profile	Structural alert

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].

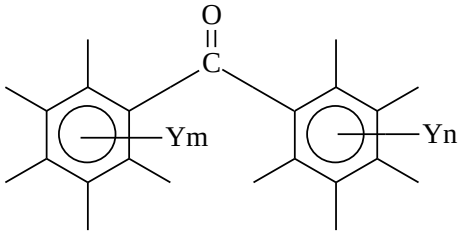


Another possible mechanism may involve enzymatic activation (O-acylation) and subsequent acylation reaction with DNA for acetohydroxamic acid derivatives (Y is alkyl, O-alkyl or N-alkyl) [5]:

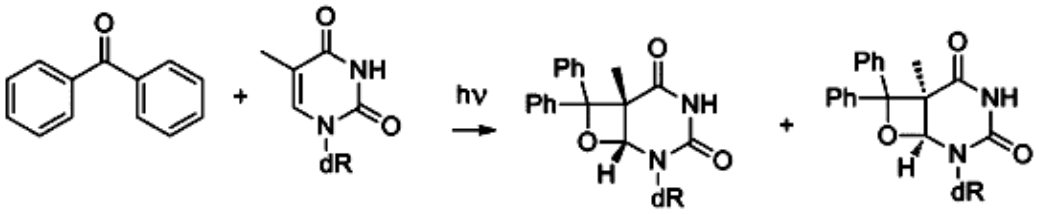


Set of chemicals used for profile development	Hydroxamic Acids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

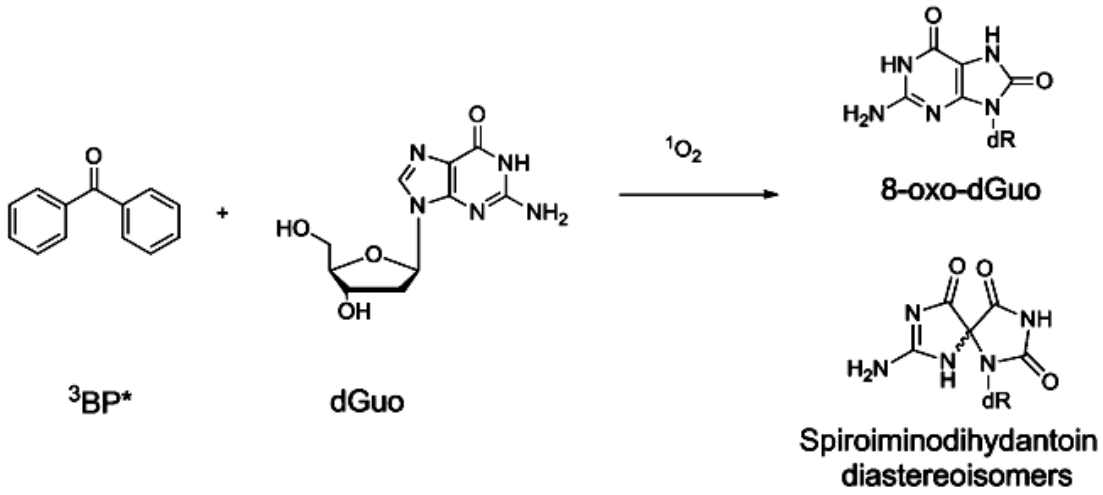
Individual profile/alert	
Name	Hydroxybenzophenone Derivatives
Type of profile	Structural alert

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

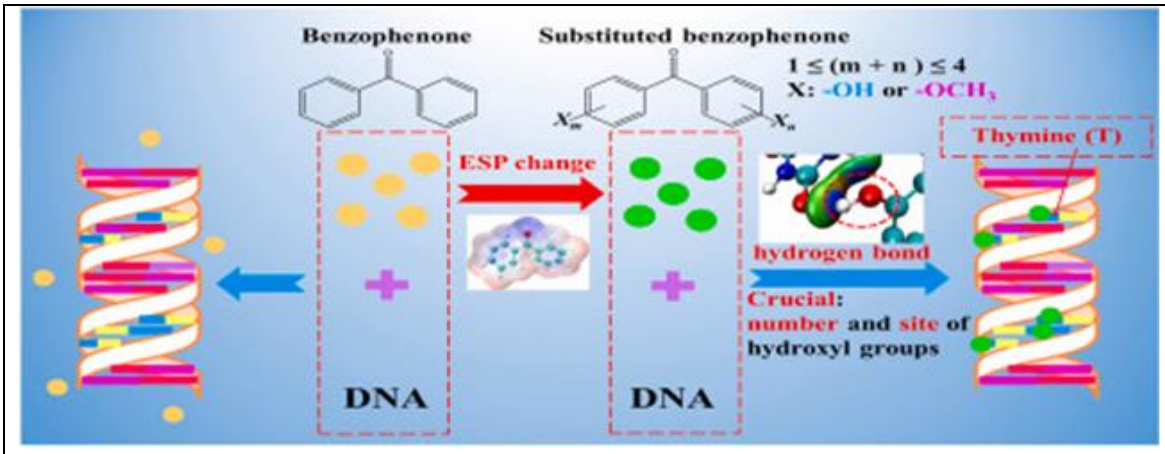
• Carbonyl compounds may react with olefins through a [2+2] AN2-type photocycloaddition giving rise to oxetane derivatives. Thus upon irradiation of Benzophenone (BP) in the presence of thymidine DNA nucleobase (Thd), two stereoisomeric oxetane derivatives have been isolated:



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:

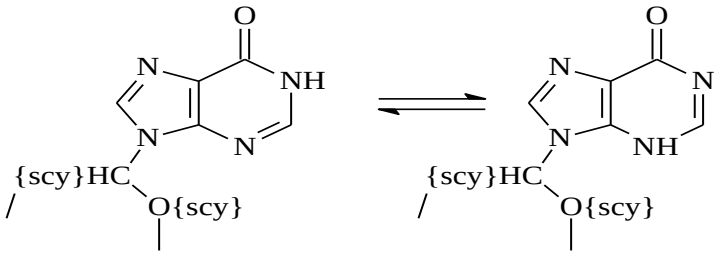


The following scheme of interaction of BPs with DNA has been finally proposed:

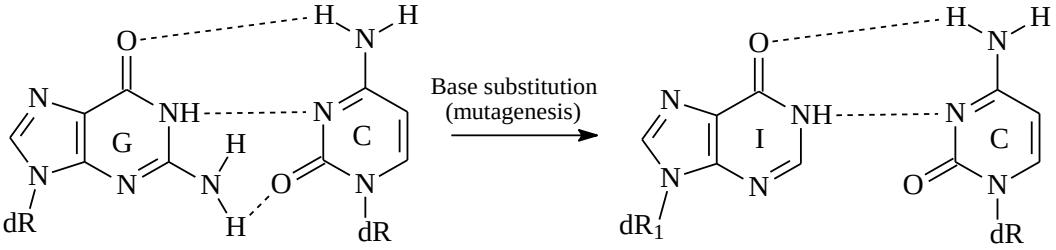


Set of chemicals used for profile development	Hydroxybenzophenone Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

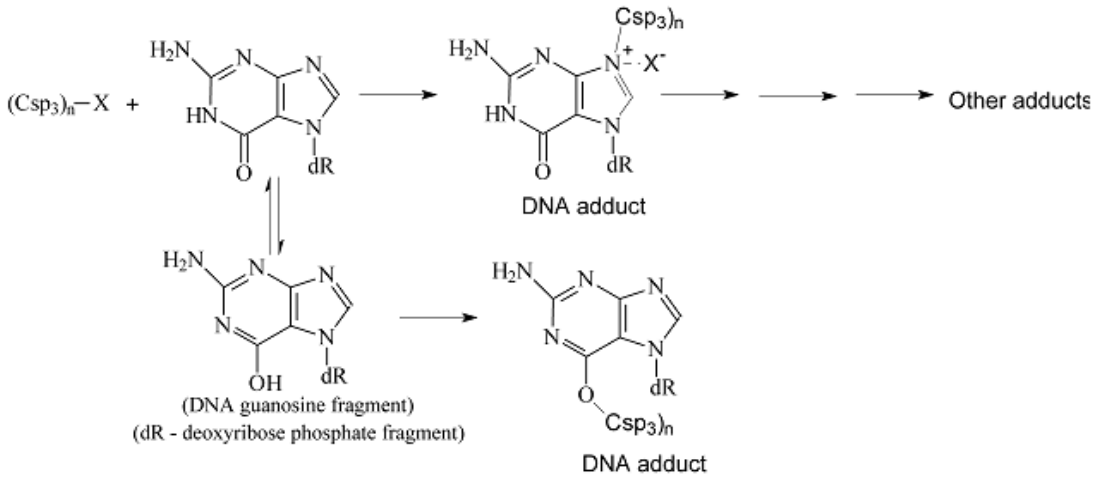


(G: guanine; C: cytosine; I: inosine; dR: deoxyribose fragment; dR_1: ribose fragment)

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.

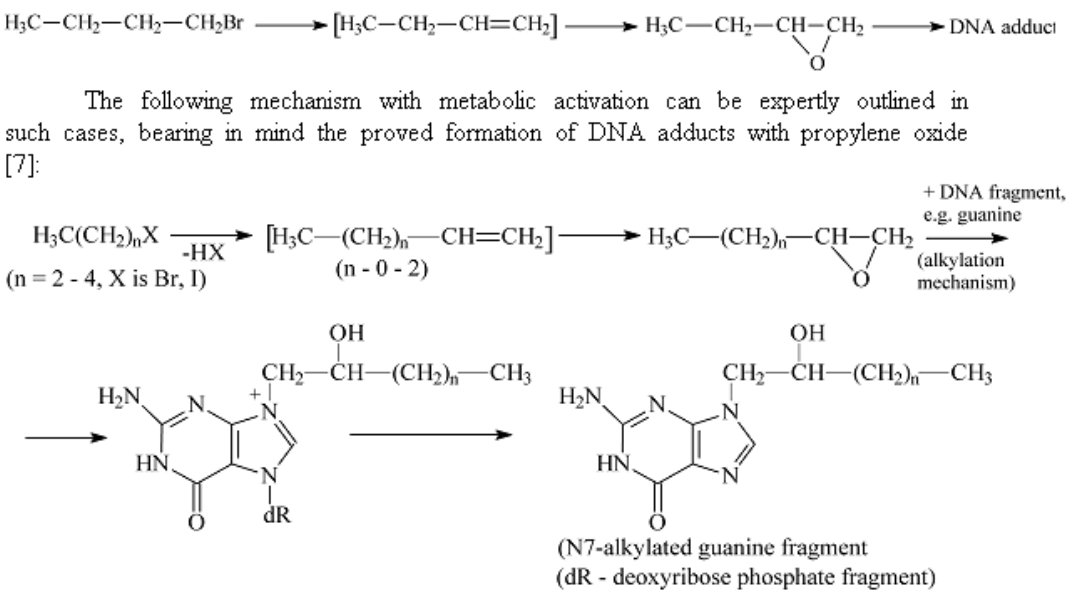
Individual profile/alert	
Name	Monohaloalkanes
Type of profile	Structural alert
Description/applicability domain	$(C(sp^3)(acy))_n$-X(n=1-4; X=-Cl, -Br, -I)
Mechanism	S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom, S_N1 Nucleophilic substitution after carbenium ion formation and S_N2 Alkylation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

Direct-Acting Mutagens – DNA alkylation in Scheme 1:



Scheme 1

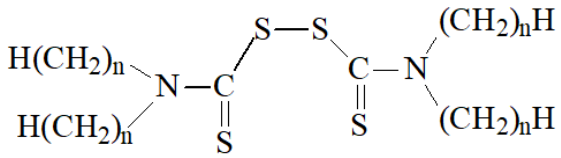
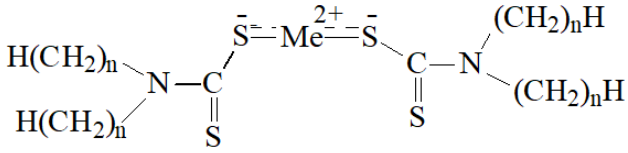
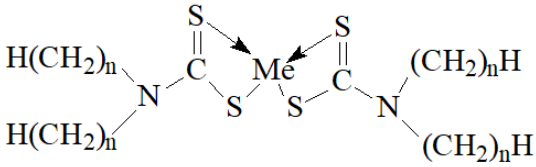
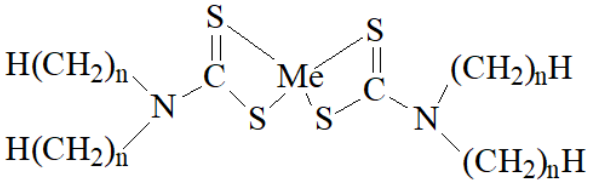
Metabolic Activation (Bioactivation) (Exogenous S9 System Added) in Scheme 2:

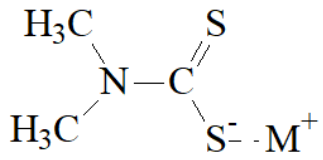


Scheme 2

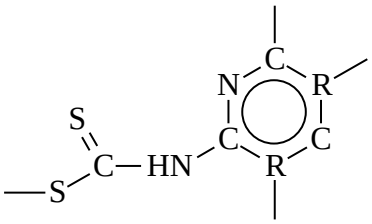
Set of chemicals used for profile development	Monohaloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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 B6C3F$_1$ 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))

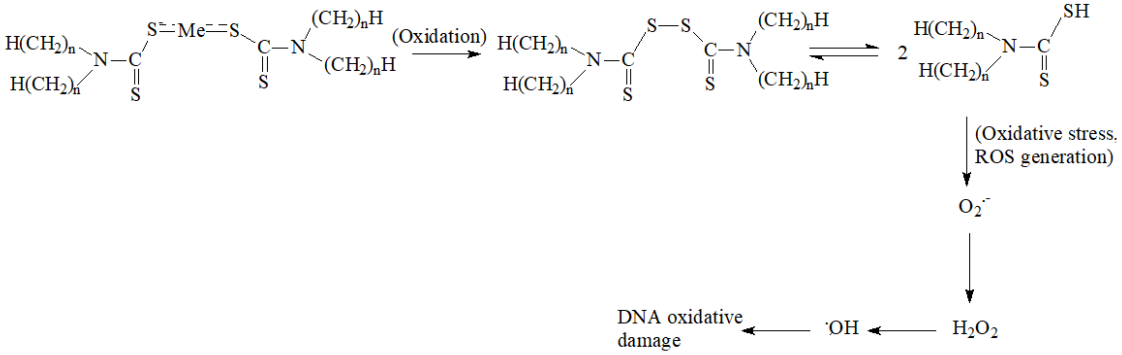


(M^+ can be Na^+, K^+, Li^+)

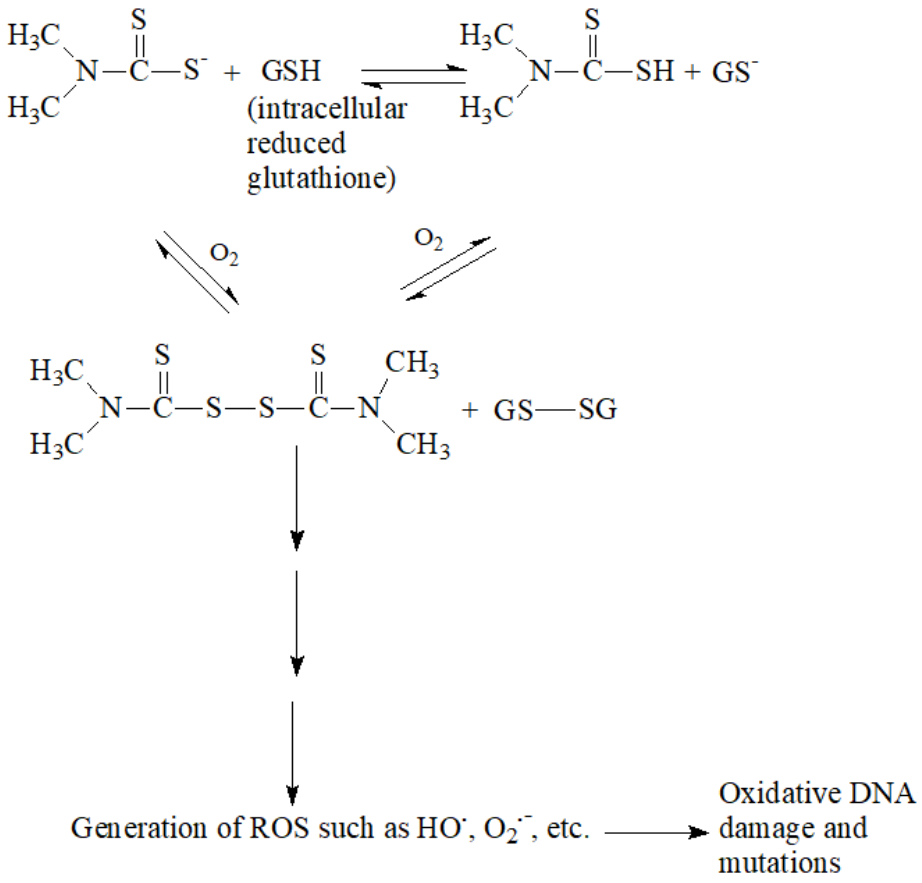


(R is Het_1: can be N or C-atom)

Mechanism	Radical ROS generation

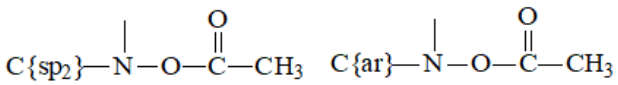


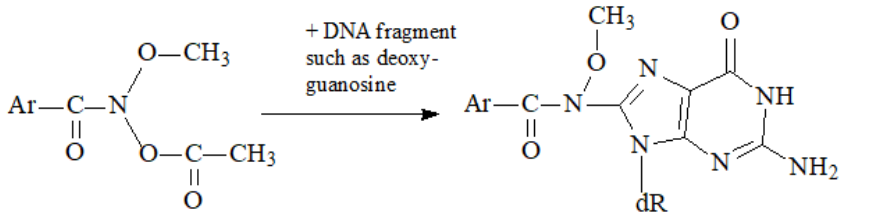
Mutagenicity of tetramethylthiuram disulfide (thiram), which can be obtained by mild oxidation of dimethyldithiocarbamate has been experimentally proved for both frameshift and base-substitution sensitive strains of Salmonella typhimurium. The following reversible equilibrium and redox cycling effects seem to be established for the interaction of sodium dimethyldithiocarbamate with endogenous (intracellular) thiols such as glutathione under biological conditions:

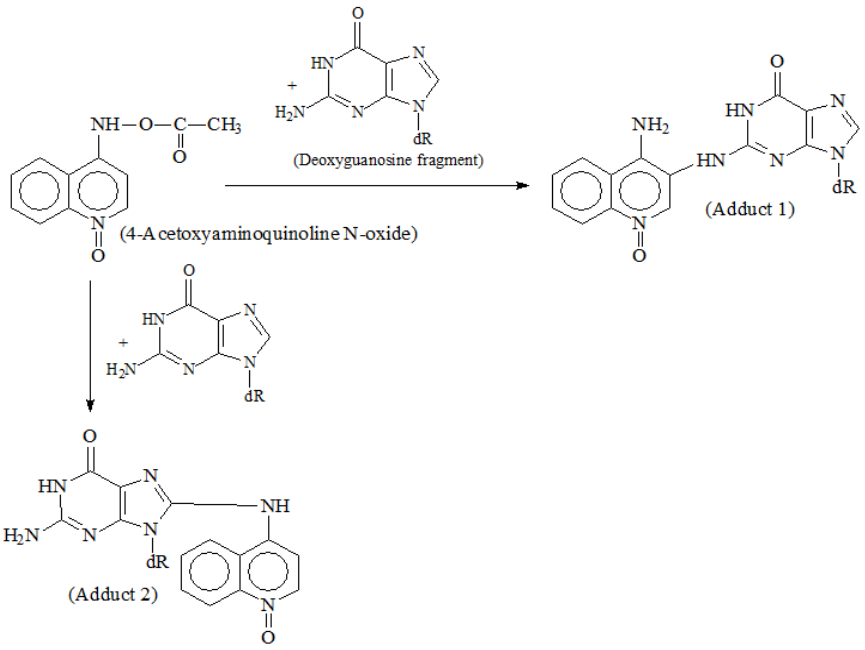


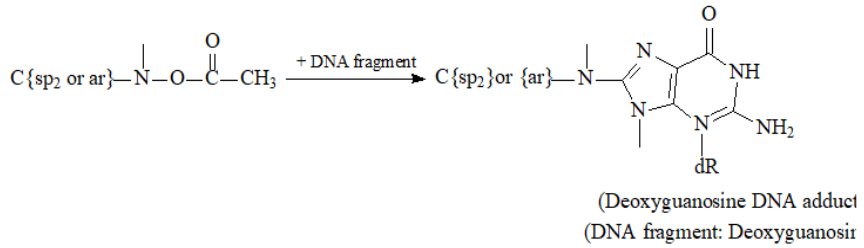
Set of chemicals used for profile development	N,N-Dialkyldithiocarbamate Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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

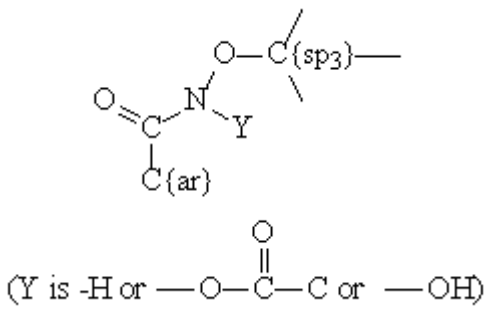
Description/applicability domain	
Mechanism	S_N2 reaction on a nitrogen-atom bound to a good leaving group

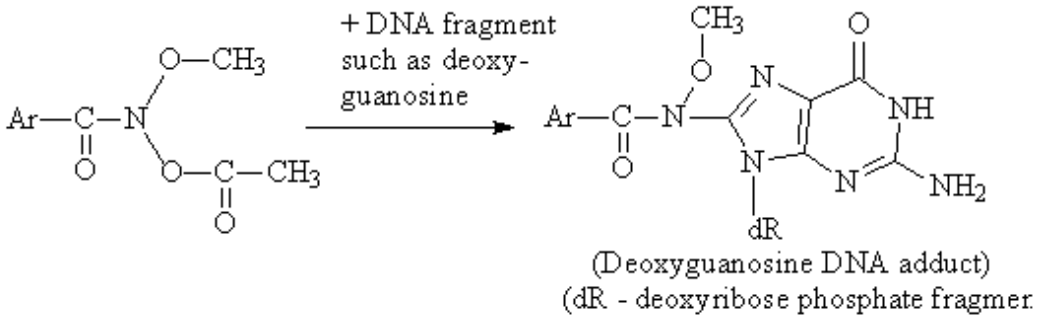




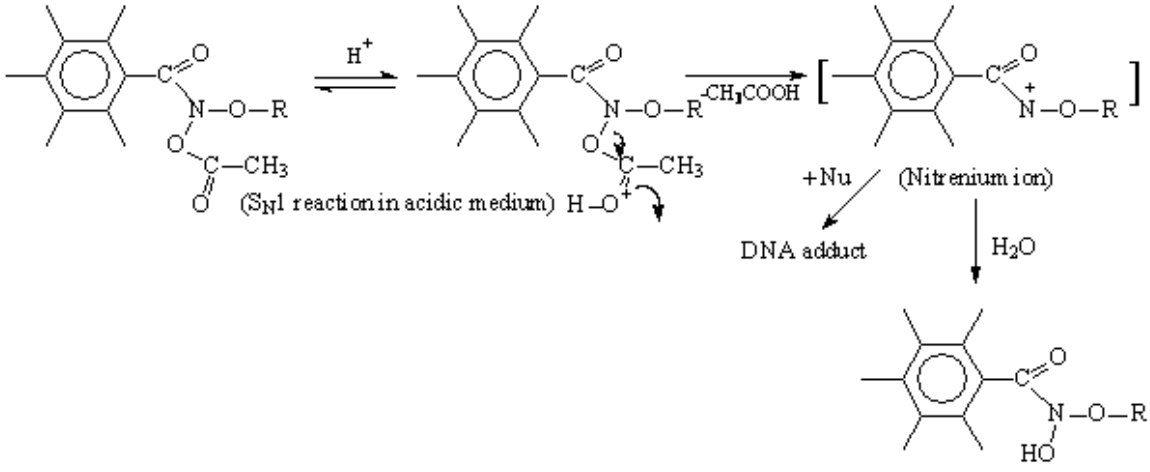


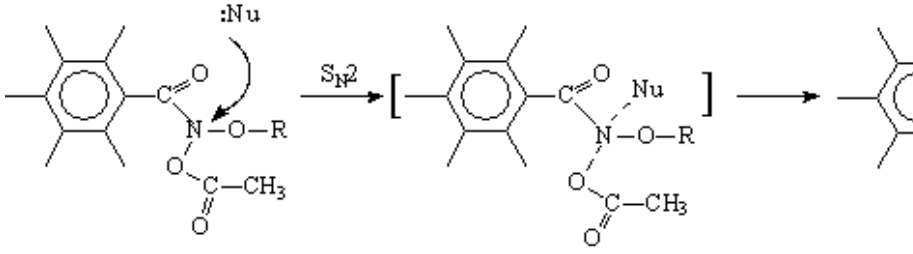
Set of chemicals used for profile development	N-Acetoxyamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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



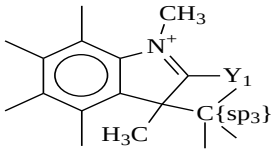
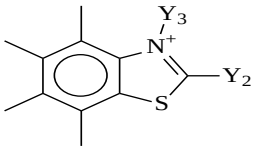
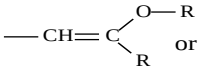
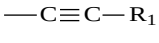
(Deoxyguanosine DNA adduct)
(dR - deoxyribose phosphate fragmer





(S_N2 or S_N1 reactions with nucleophile Nu such as primary amine and amino group in DNA purine bases such as adenine)

Set of chemicals used for profile development	N-Acyloxy(Alkoxy) Arenamides-metadata
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.

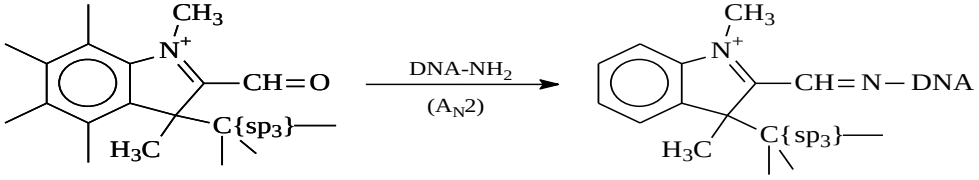
Individual profile/alert	
Name	N-Alkylindolinium and N-Alkylbenzothiazolium Salts
Type of profile	Structural alert
Description/applicability domain	 (Y_1 is $-CH_3$ or $-CH=O$)  (Y_2 is  or  R is $-CH_3$ or $-C_2H_5$) (R_1 is H or $-CH_3$) (Y_3 is $-(CH_2)_nSO_3^-$, n = 1 - 3)
Mechanism	AN2 Schiff base 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:

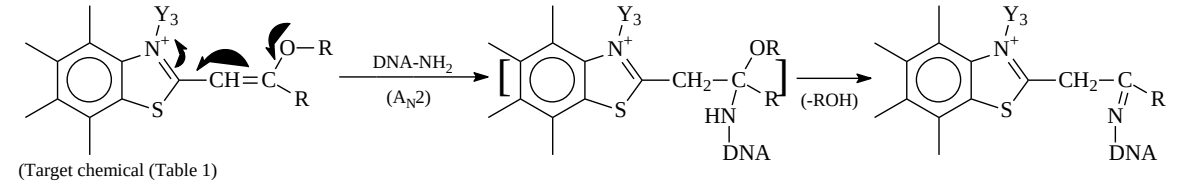


(Assumed active metabolite of the target chemical (Table 1)

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

(Expertly assumed DNA adduct, possibly eliciting mutagenicity)

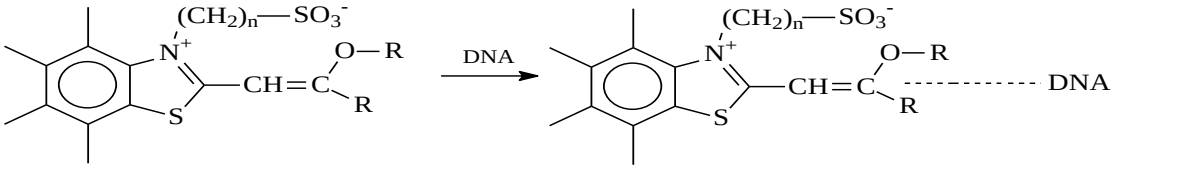
Mechanistic scheme B:



R is $-CH_3$ or $-C_2H_5$)

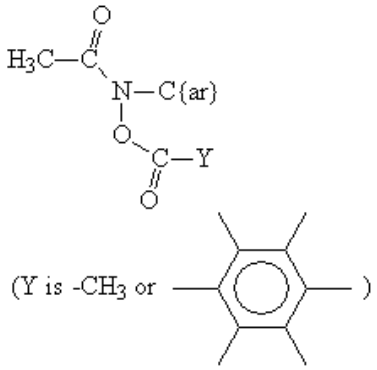
(Y_3 is $-(CH_2)_nSO_3^-$, n = 1 - 3)

Mechanistic scheme C:

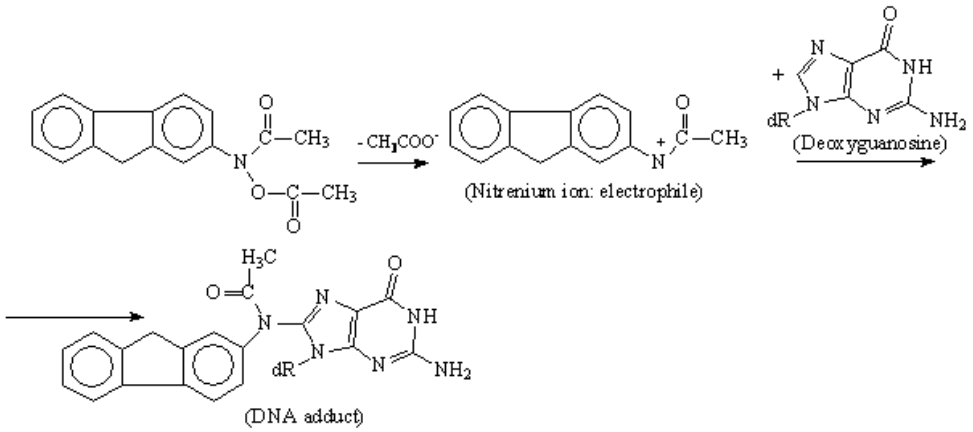


R is $-CH_3$ or $-C_2H_5$)

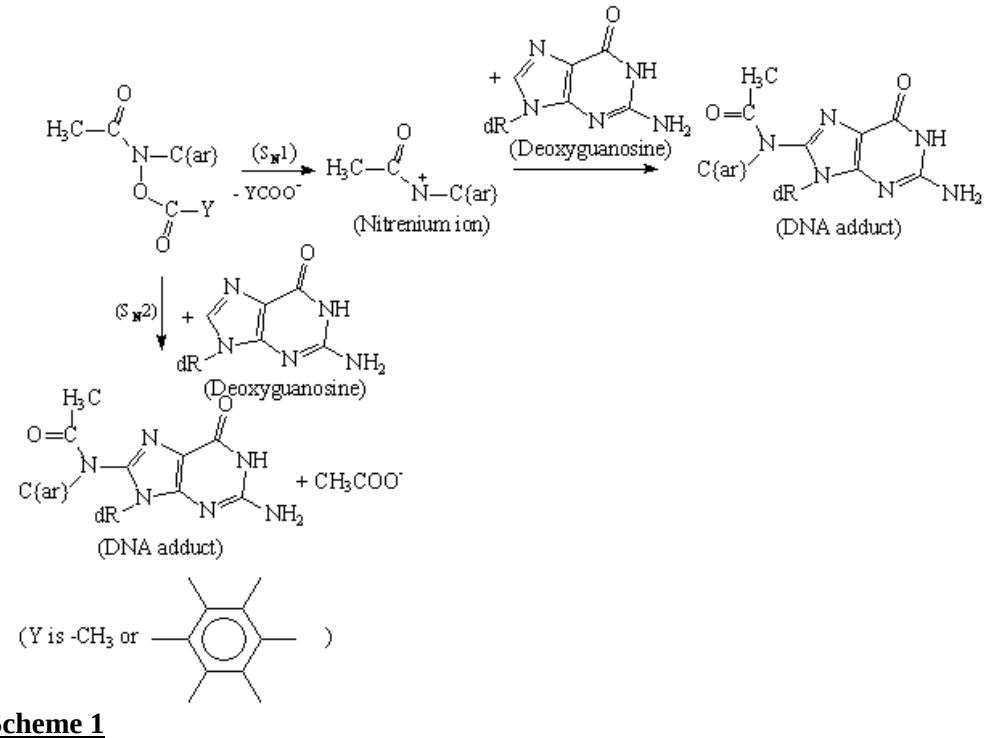
Set of chemicals used for profile development	N-Alkylindolinium and N-Alkylbenzothiazolium Salts
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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]:

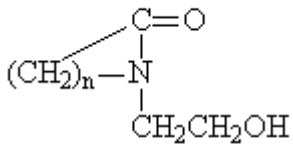
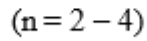


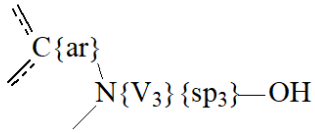
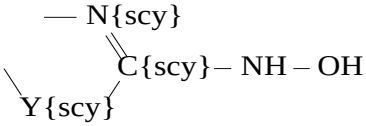
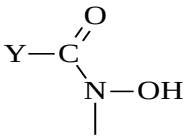
The general scheme for such interactions with DNA fragments could be outlined as follows:

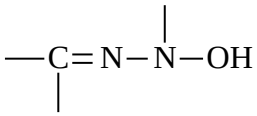


Scheme 1

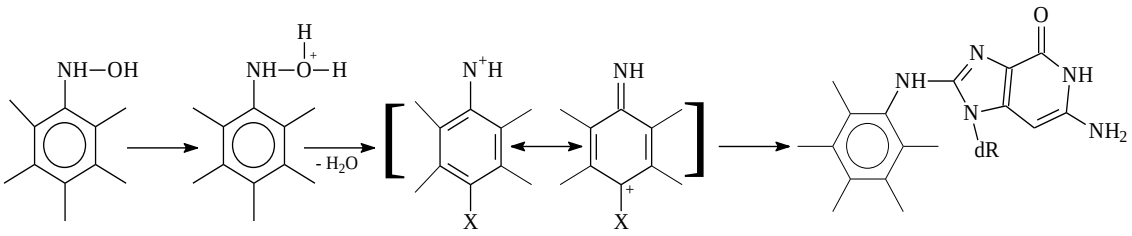
Set of chemicals used for profile development	N-Aryl-N-Acetoxy(Benzoyloxy) Acetamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references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

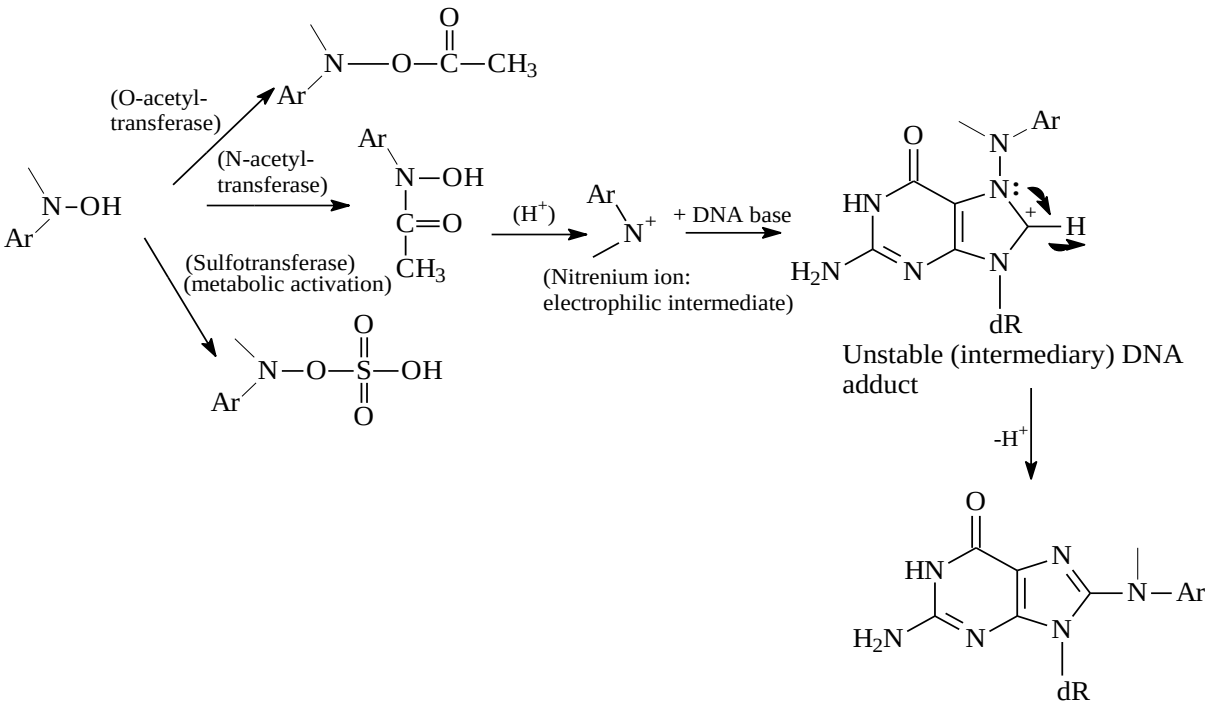
Individual profile/alert	
Name	N-Hydroxyethyl Lactams
Type of profile	Structural alert
Description/applicability domain	 
Mechanism	Non-covalent interactions DNA intercalation
Positive *in vitro* bacterial mutagenicity test results with *Salmonella typhimurium* strains TA100 and TA1535 were reported 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).

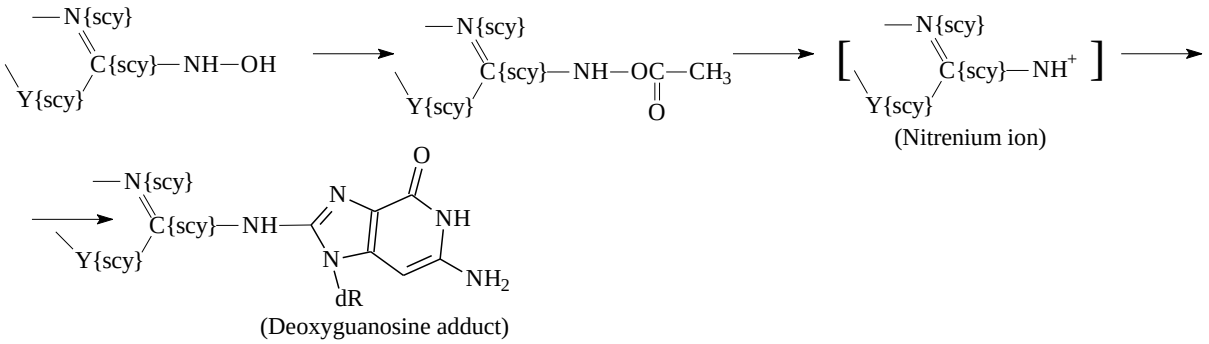
Individual profile/alert	
Name	N-Hydroxylamines
Type of profile	Structural alert
Description/applicability domain	  (Y is N{V3}{sp3} or S{V2})  (Y is C{ar} or C{sp2} or N{V3}{sp3})

	
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

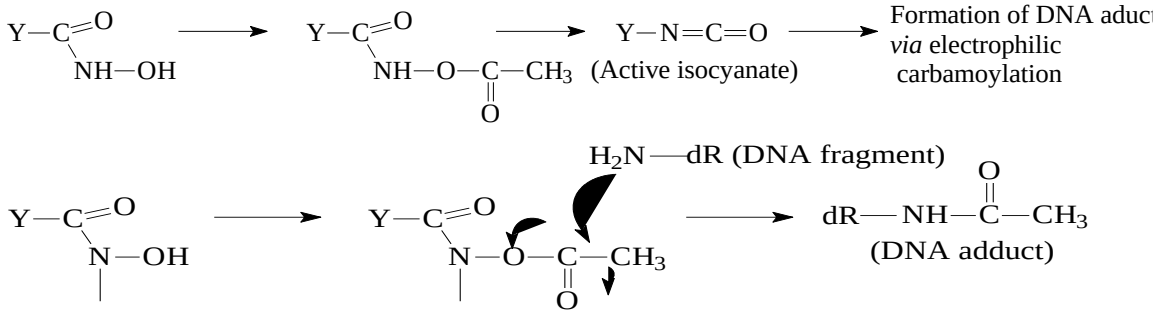
1. Aromatic and Heterocyclic N-Hydroxylamines



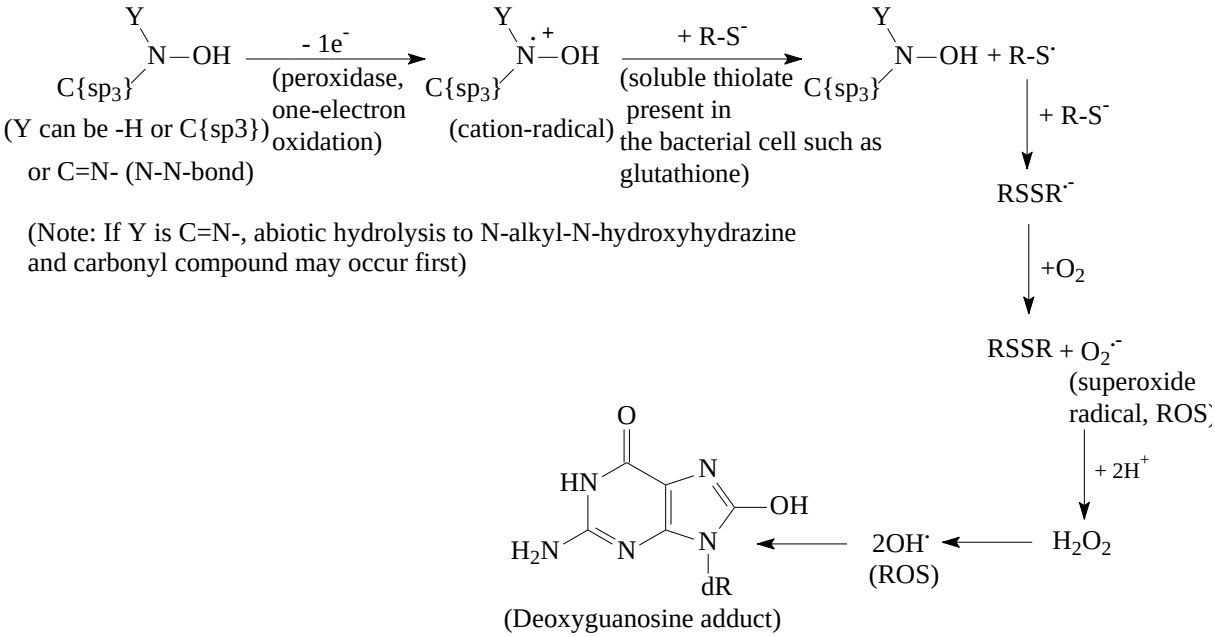




2 Hydroxamic Acids



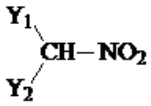
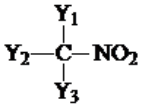
3. Other Non-Aromatic N-Hydroxylamines



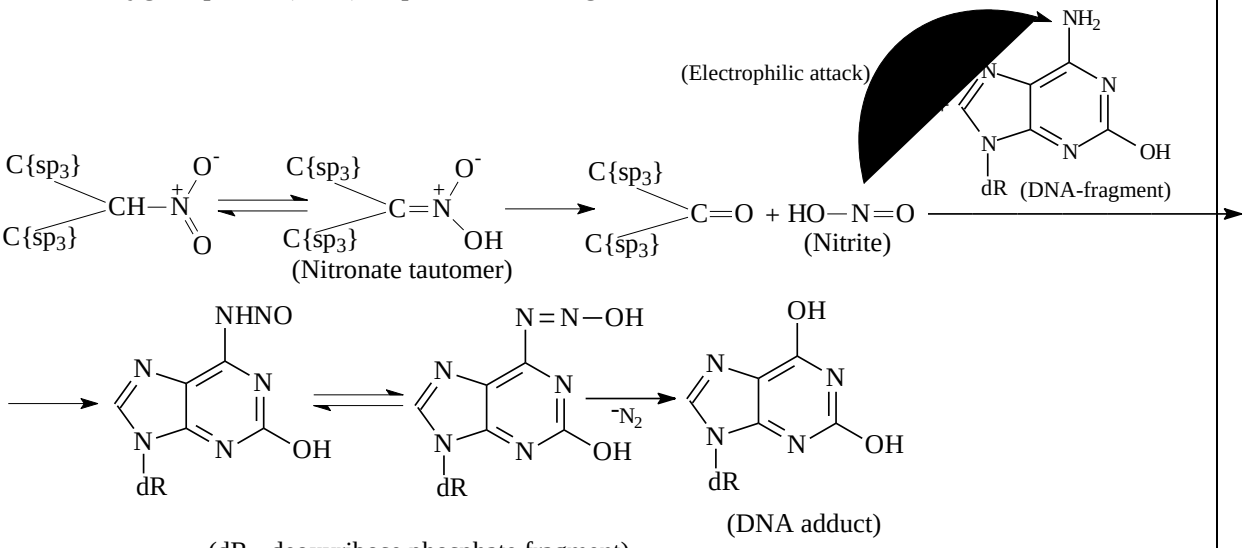
(Note: If Y is C=N-, abiotic hydrolysis to N-alkyl-N-hydroxyhydrazine and carbonyl compound may occur first)

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.

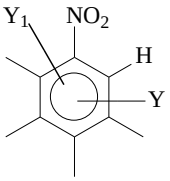
Individual profile/alert	
Name	Nitroalkanes
Type of profile	Structural alert
Description/applicability domain	Monoalkanes  Y_1- Me or H Y_2- Me or CH_2OH or CH_2COOH Low Molecular weight germinal Polynitroalkanes  Y_1, Y_2, Y_3 can be NO_2(all) or a combination between $–CH_3$, -H, $-NO_2$. The number 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	

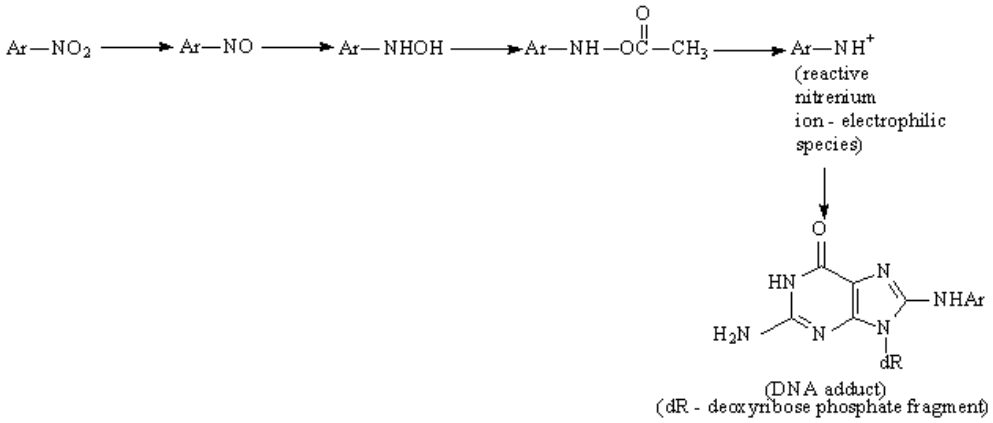
a potent protein nitrating agent and has been proposed to have role in the deamination of DNA (deamination of cytosine resulting in base mispair). However, there is insufficient information on the precise mechanism of mutagenicity/carcinogenicity of this compound [6, 7]. According to some publications, 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].



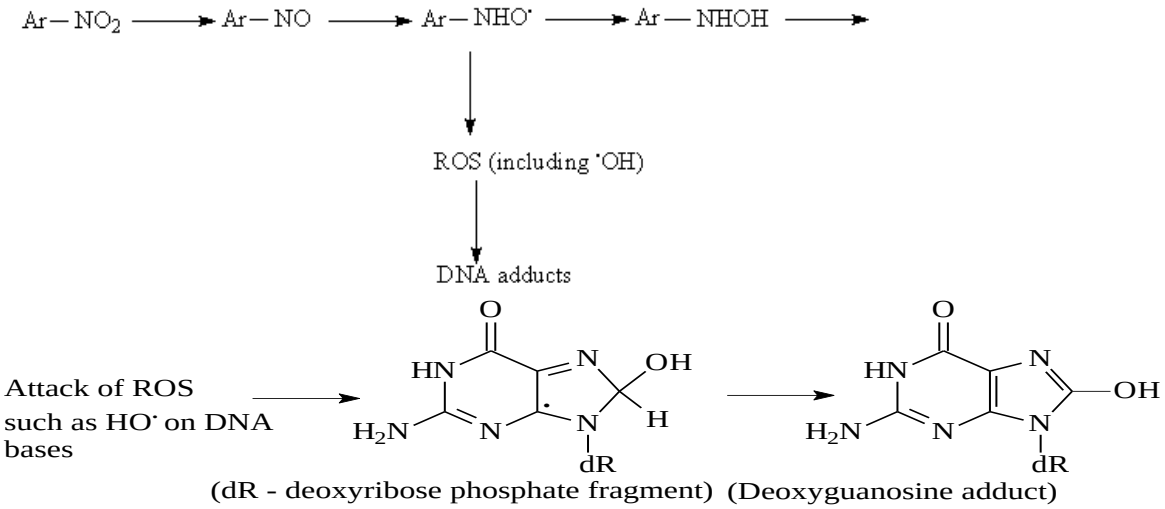
Set of chemicals used for profile development	Nitroalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

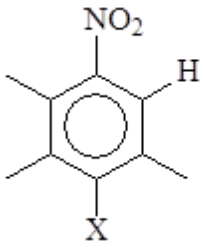
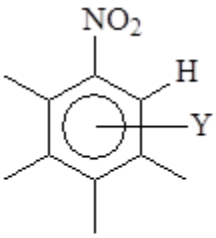
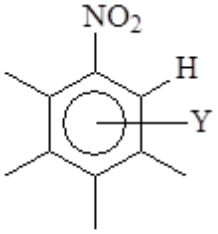
Individual profile/alert	
Name	Nitroaniline Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is N{V3}{sp3} (primary, secondary or tertiary amino group) Other substituents (Y_1) that may be present: 1. -NO_2, -NH{sp3}{V3}, -O-C{sp3} (no more than three C{sp3}); -OH, C, -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	

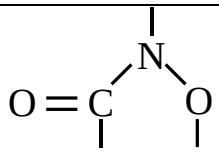


Homolytic

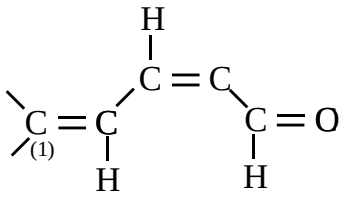


Set of chemicals used for profile development	Nitroaniline Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
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{V_3}{sp_3}(triazene) or —$N^+\equiv N$ (diazonium)); totally no more than four substituents) Additional activating substituents Y:



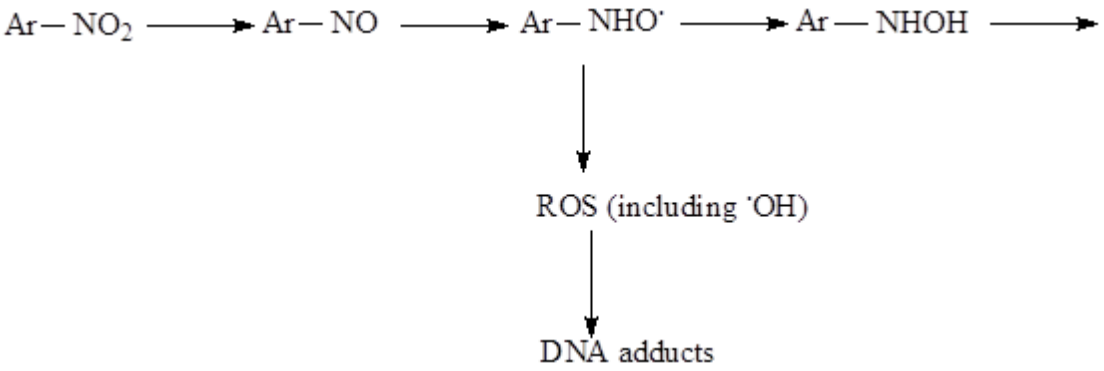
(Note: N, C and O could be atoms in a heterocycle, too)



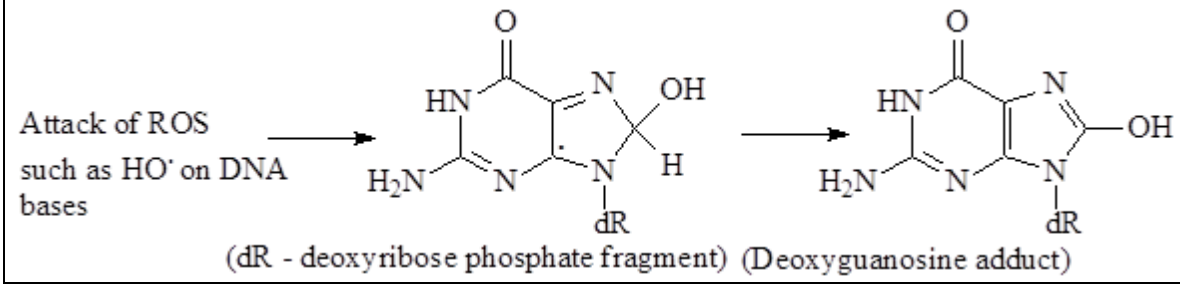
(attached to the ring via C(1))

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

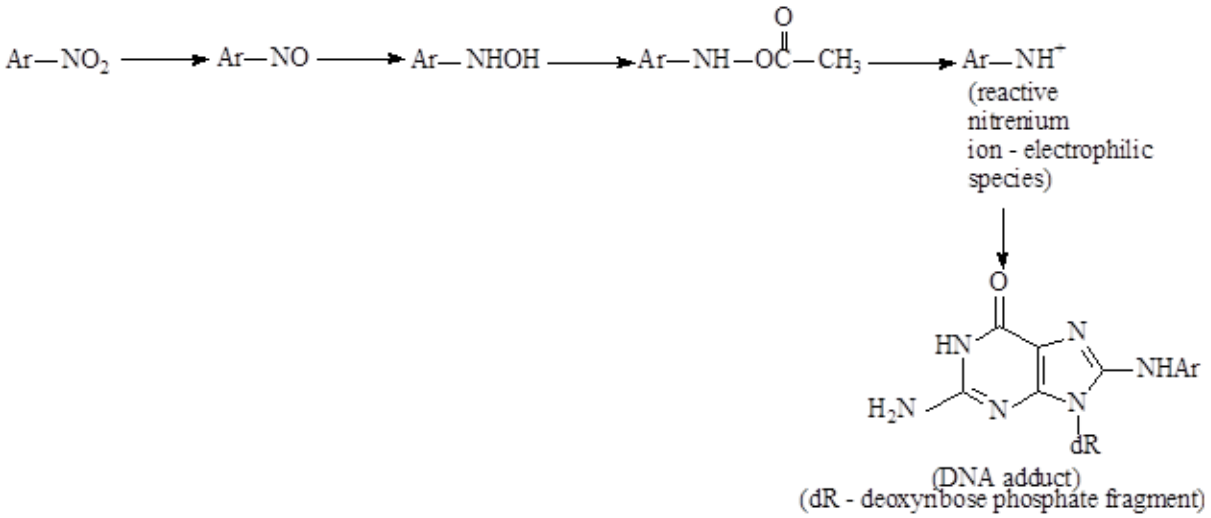
Radical (Homolytic) Mechanism**.** This is one of the mechanisms (*but not the most important*) for eliciting bacterial mutagenicity of nitro compounds. Certain monocyclic and polycyclic aromatic nitro compounds ($ArNO_2$) are implicated in carcinogenesis [5]. Reduction of the nitro to the nitroso intemediate 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]:



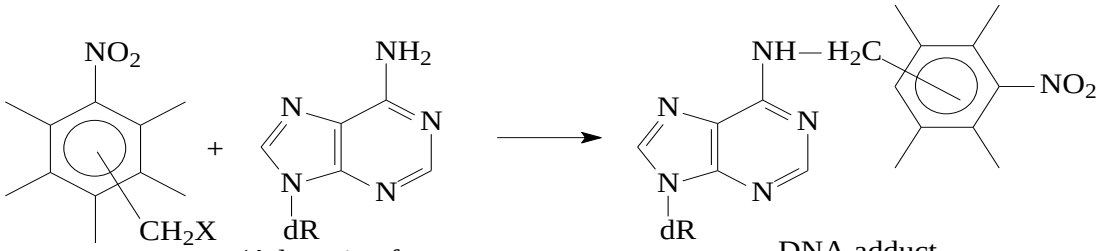
Heterolytic Mechanism. This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly from covalent adduct formation. It arises from several activated metabolites, 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].

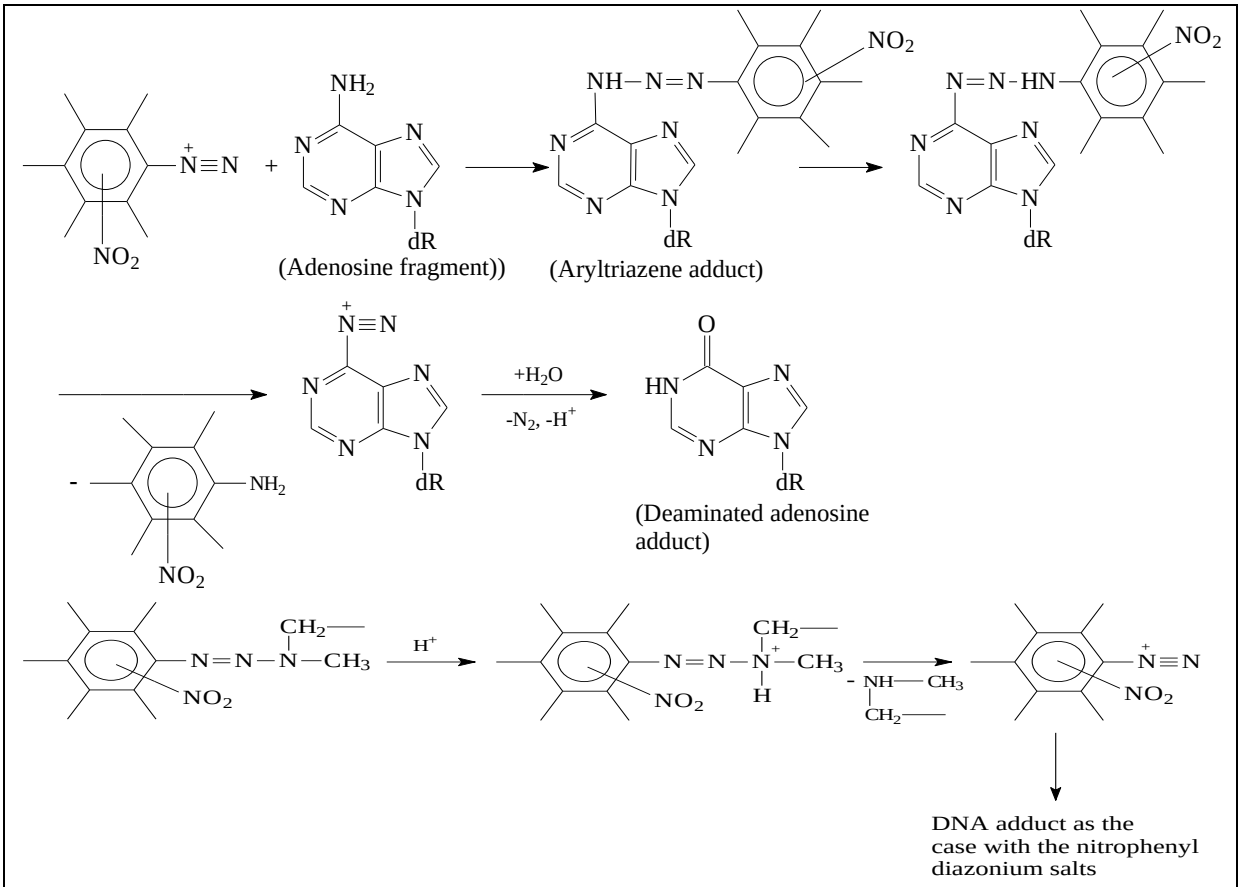
Additional chemical mechanistic schemes, other than those associated with nitro group reduction to N-hydroxylamine or generation of ROS (see above) are associated with some nitroarenes, containing other active functionalities and belonging to other classes of *Ames*-positive chemicals involved in the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

For nitrobenzyl and nitrobenzoyl halides – aralkylation [13]:



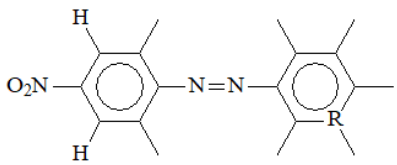
(Adenosine fragment; dR - deoxyribose phosphate fragment)

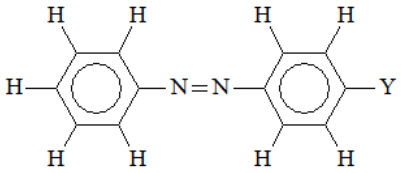
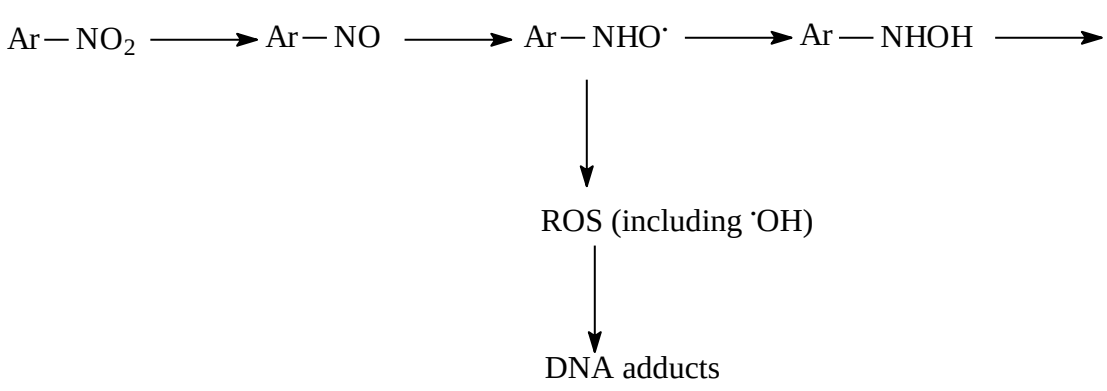
For nitrophenyl diazonium salts and triazenes [14, 15]:



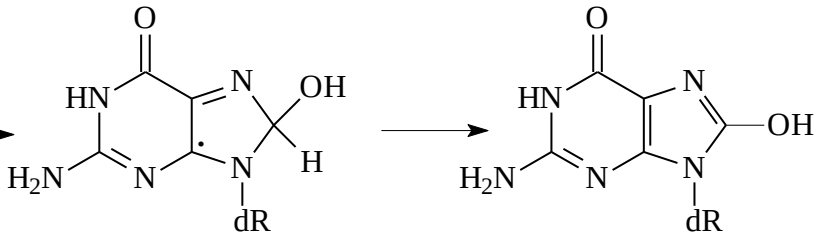
Set of chemicals used for profile development	Nitroarenes with Other Active Groups
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references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

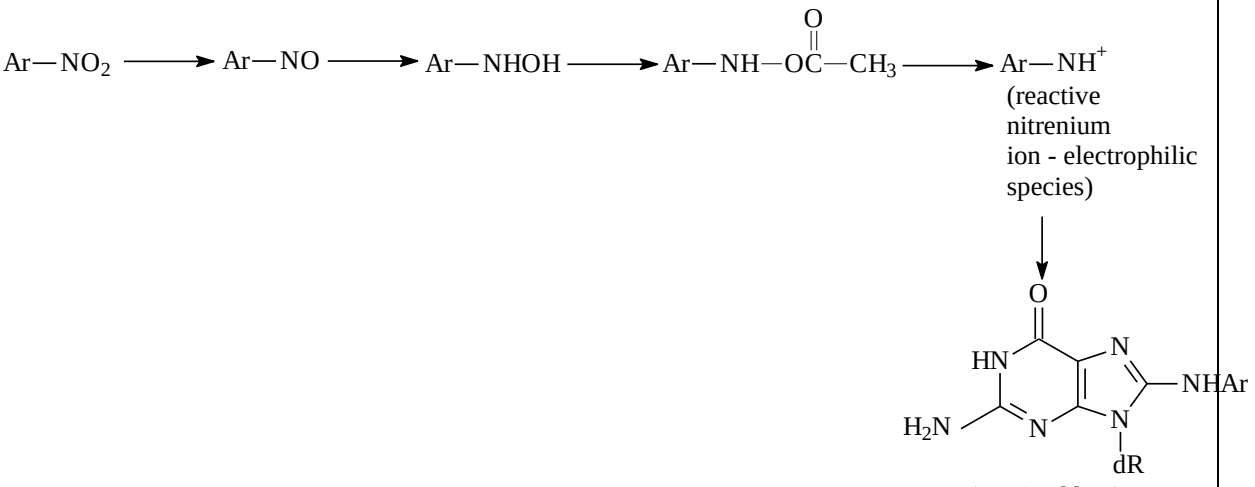
Individual profile/alert	
Name	Nitro Azoarenes and p-Monosubstituted Azobenzene Derivatives
Type of profile	Structural alert
Description/applicability domain	 R = any carbon or nitrogen, single arene ring only, no fused ring fragments in the molecular structure

	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  	

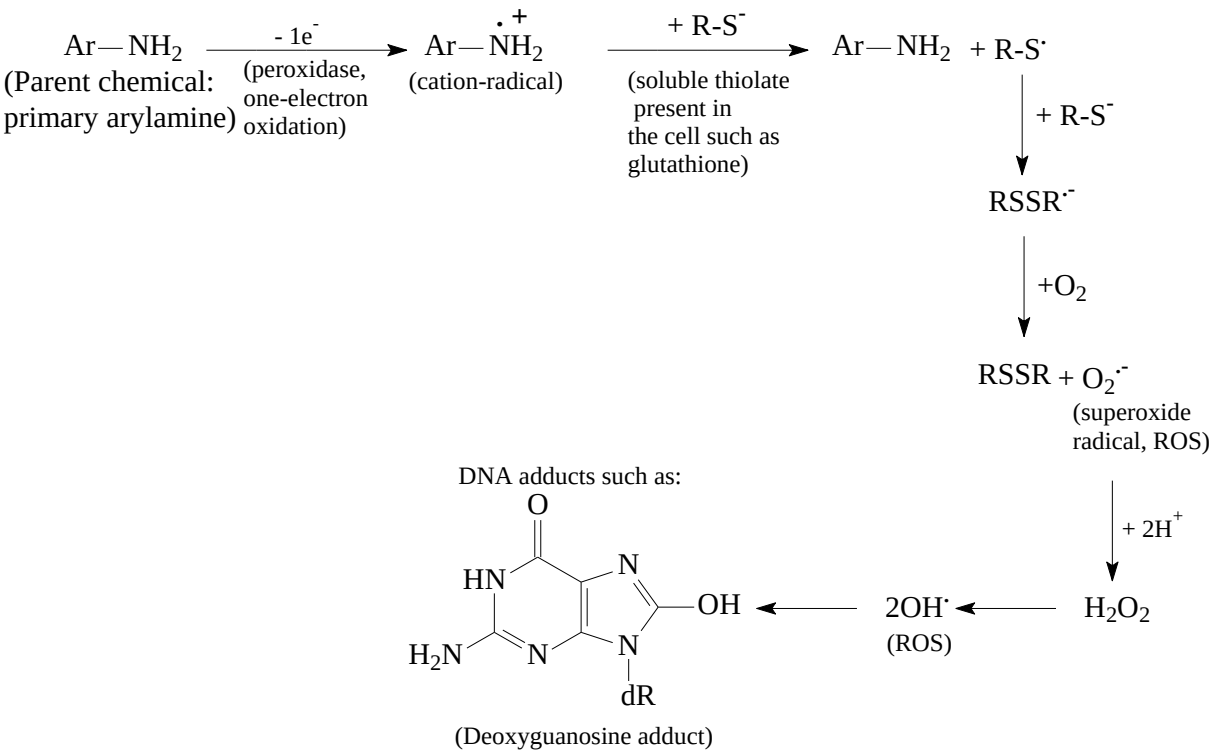
Attack of ROS such as HO· on DNA bases

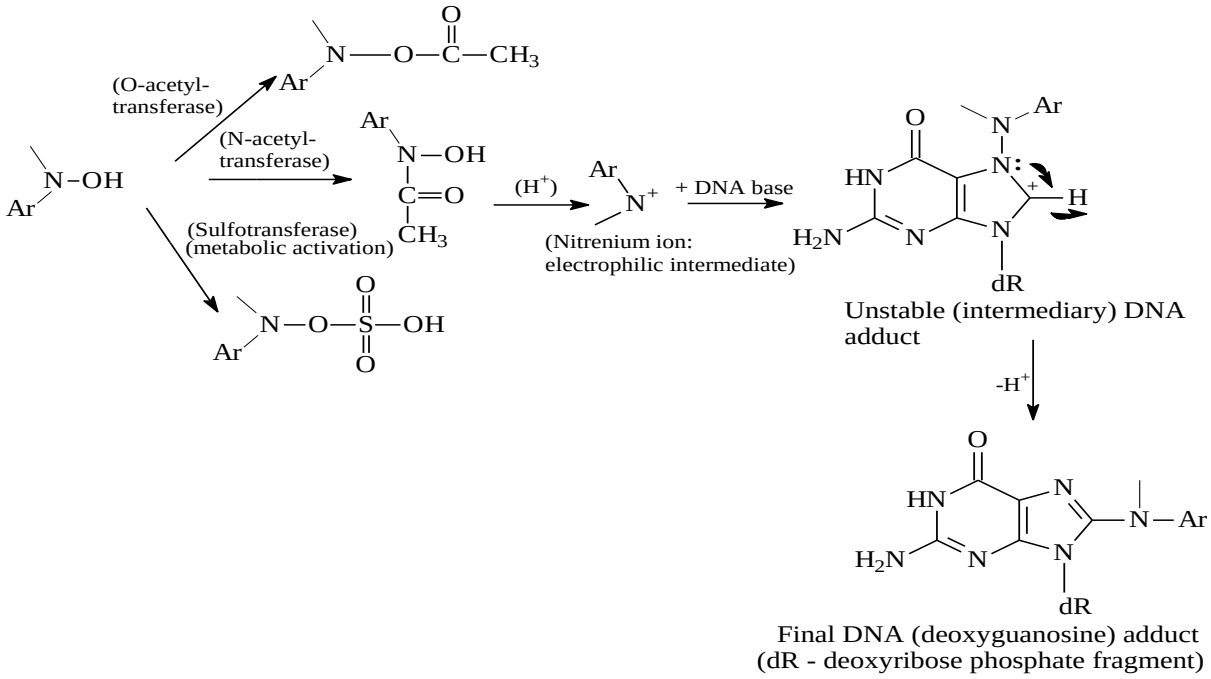


(dR - deoxyribose phosphate fragment) (Deoxyguanosine adduct)

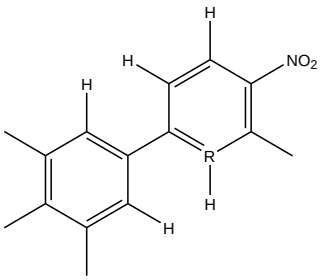
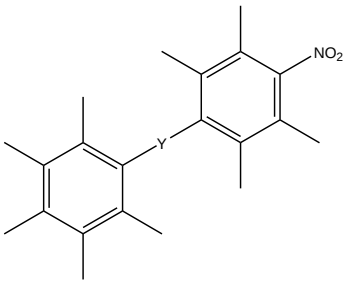


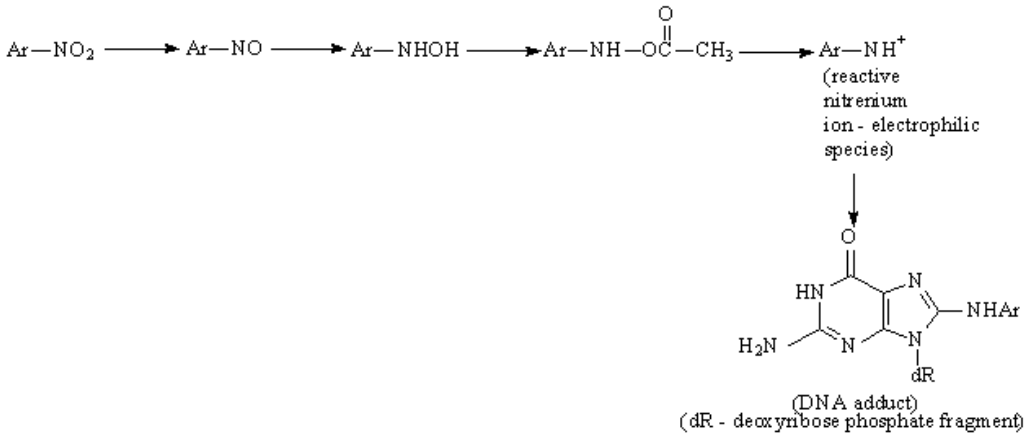
II. p-Monosubstituted Azobenzene Derivatives



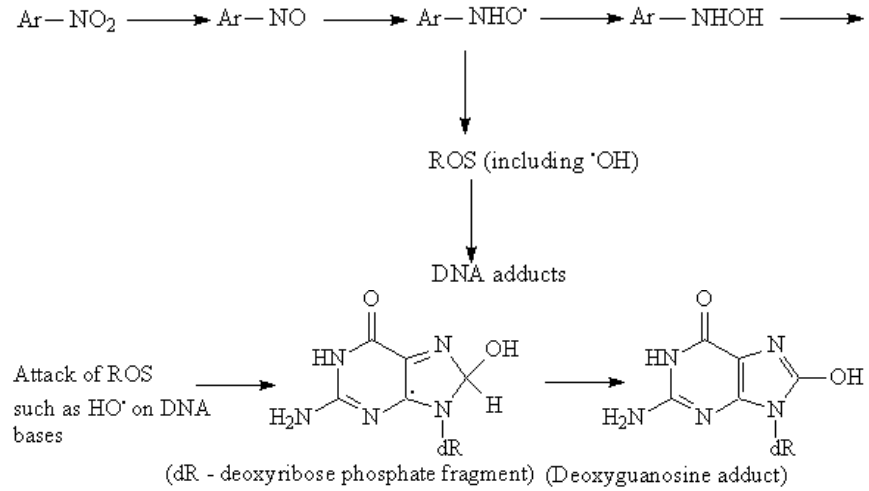


Set of chemicals used for profile development	Nitro Azoarenes and p-Monosubstituted Azobenzene Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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	

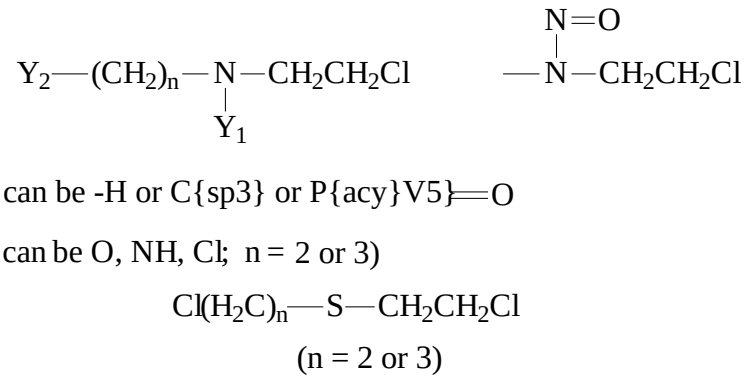


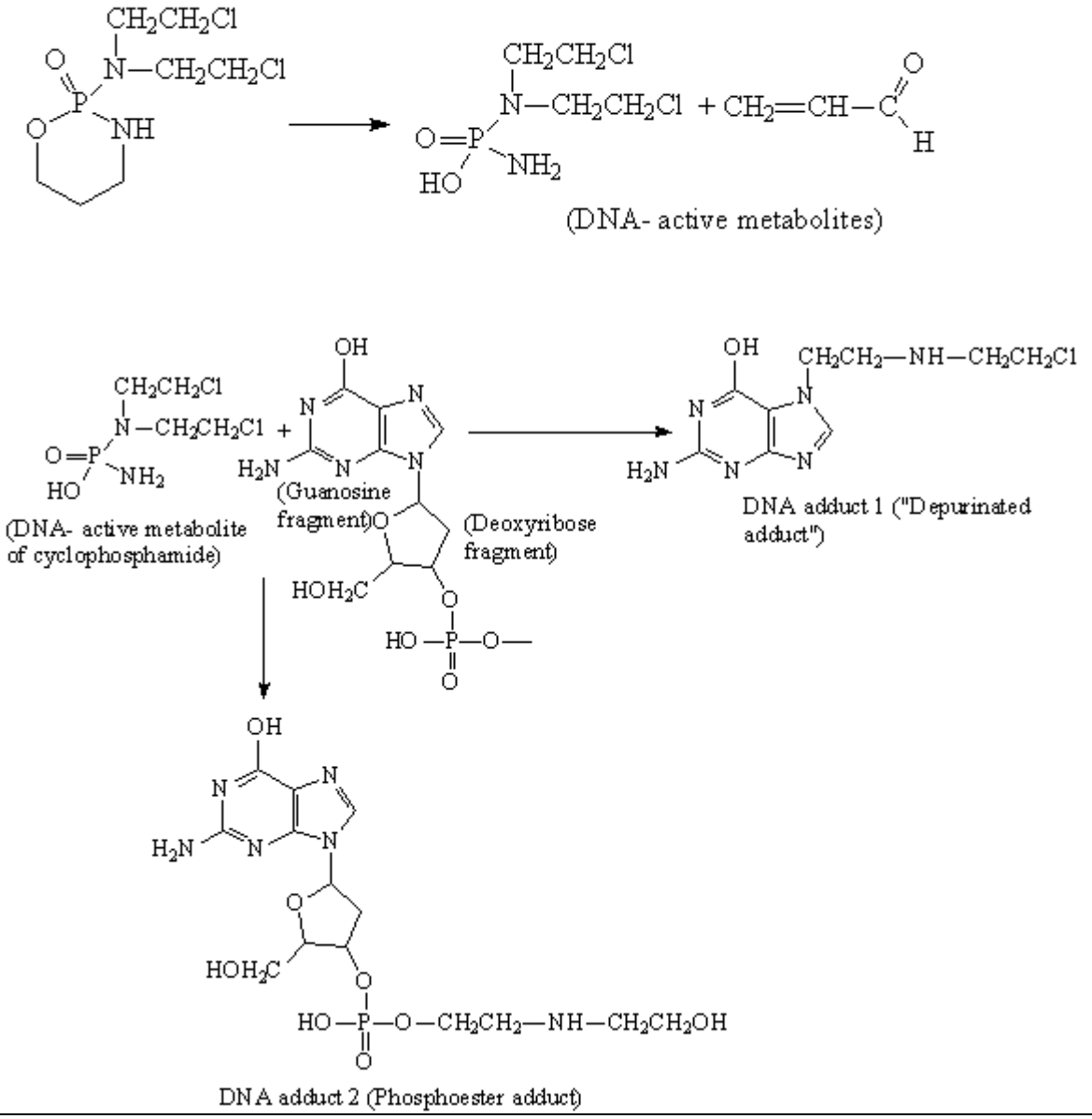
Homolytic

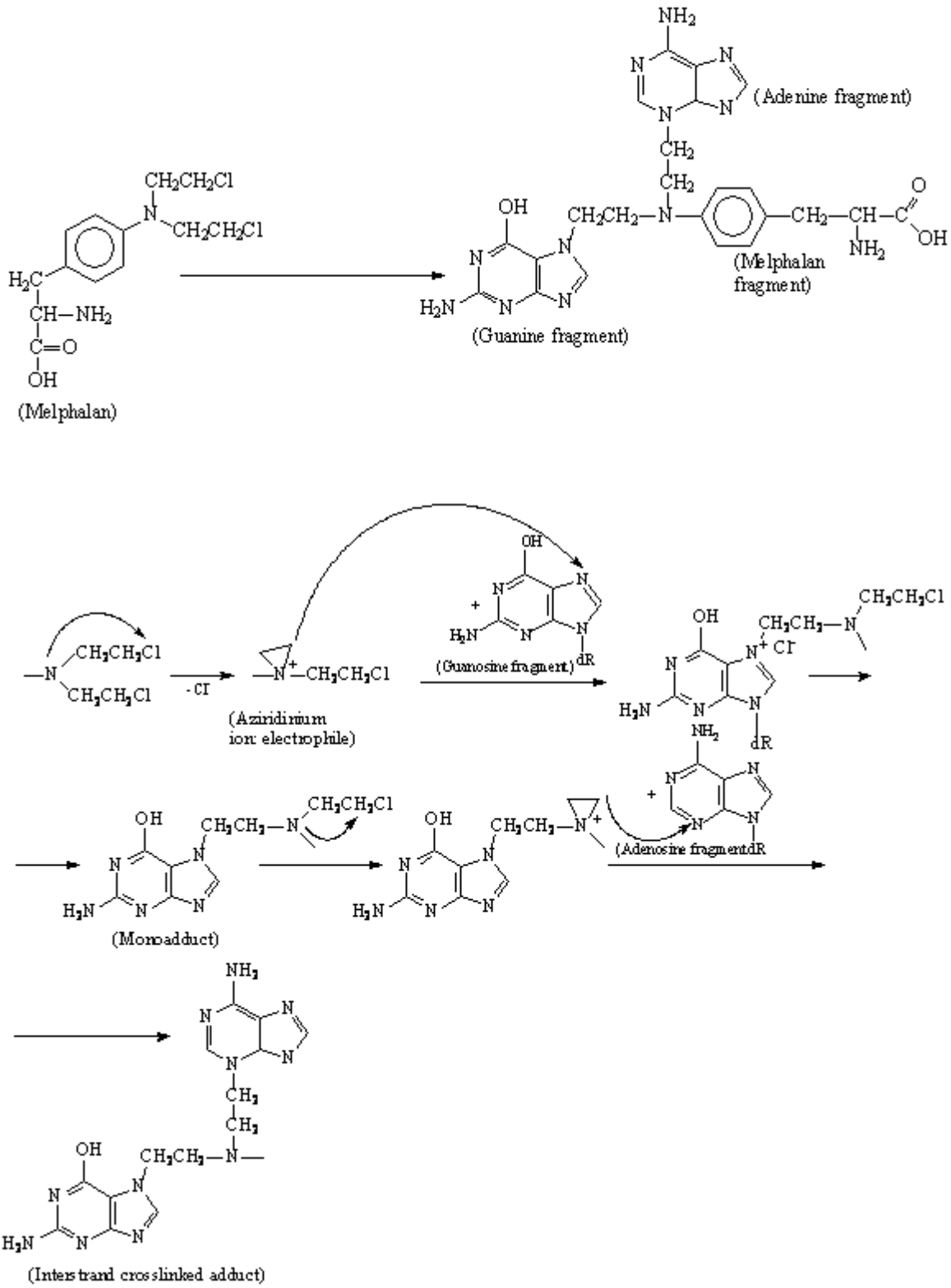


Set of chemicals used for profile development	Nitrobiphenyls and Bridged Nitrobiphenyls
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references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

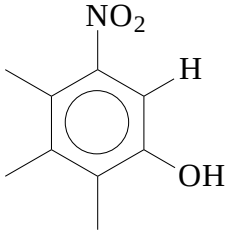
Individual profile/alert	
Name	Nitrogen and Sulfur Mustards
Type of profile	Structural alert
Description/applicability domain	 (Y_1 can be -H or C{sp3} or P{acy}V5}=O Y_2 can be O, NH, Cl; n = 2 or 3) (n = 2 or 3)
Mechanism	S_N2 Alkylation, direct acting epoxides and related after cyclization

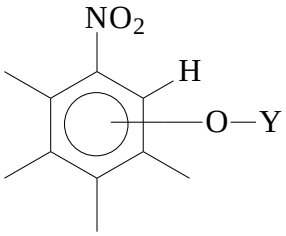
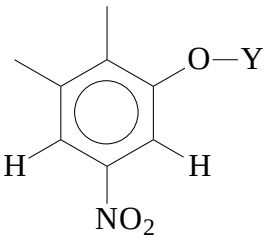
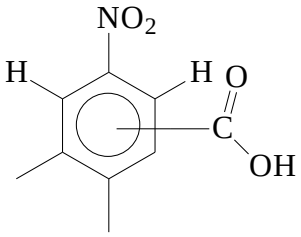




Set of chemicals used for profile development	Nitrogen and Sulfur Mustards
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.

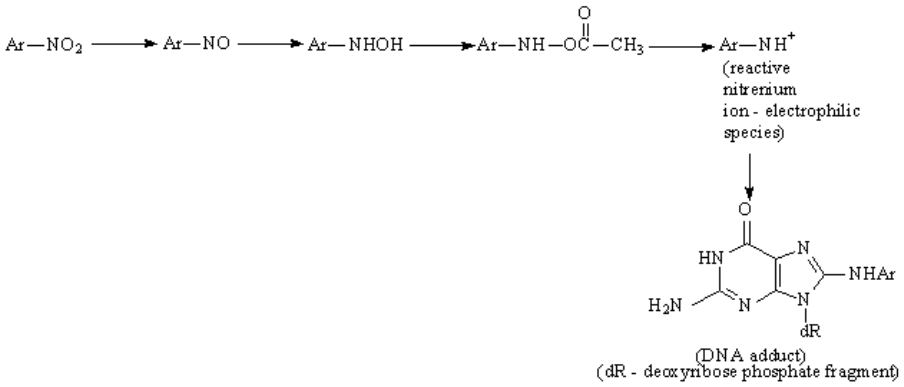
References	1. Kovacic, P., J. D. Jacinto, *Mechanism of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer*, Current Med. Chem. **8** (2001), 773 – 796. 2. Hartley, J. A., J. P. Bingham, R. L. Souhami, DNA *Sequence Selectivity of Guanine-N7 Alkylation by Nitrogen Mustards is Preserved in 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)

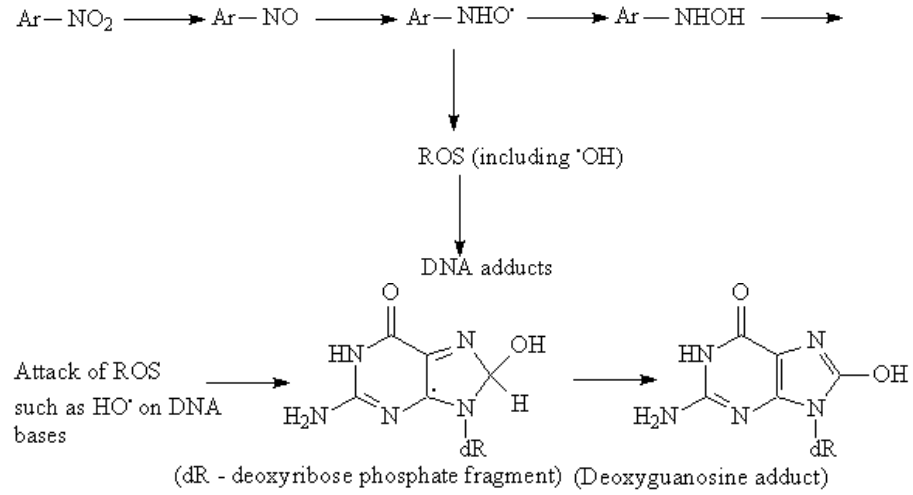
	Nitrophenyl ethers  Y is -CH_3 or -CH_2CH_3; no more than three substituents; no -SO_3H or -COO- "Mask":  Nitrobenzoic Acids  (No more than three substituents; no -SO_3H or additional -COO- groups) No more than three substituents No –SO_3H and –COO-
Mechanism	**Heterolytic Mechanism.** This is the most 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

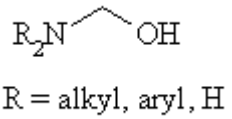
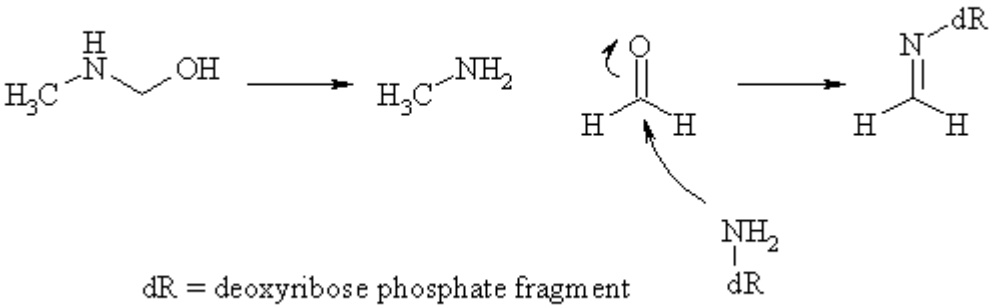


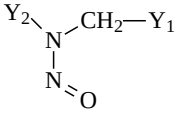
Homolytic

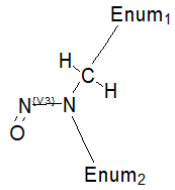
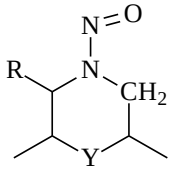
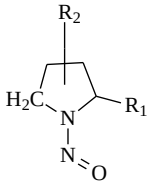


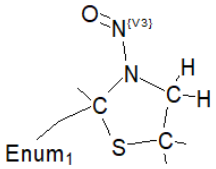
Set of chemicals used for profile development	Nitrophenols, Nitrophenyl Ethers and Nitrobenzoic Acids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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	
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	N-methylol 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. 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-Nitrosamines
Type of profile	Structural alert
Description/applicability domain	**A. Acyclic N-Nitrosamines**: *Metabolic activation mechanism starting with alpha-carbon atom hydroxylation*  (Y1 is –

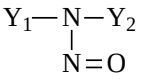
	$(CH_2)_nH$ (n = 0 – 14) Y_2 is C{ar} or $–(CH_2)_mH$ (m = 1 – 5) or $–(CH_2)_p$-C(O)-C{ar} (p = 2 – 4)  (**Y_1** is $–(CH_2)_nH$ (n = 0 – 14) or C{ar}-CH_2-; or $-(CH_2)nH$ (X is –OH or =O or their combination attached to any carbon; no more than two X on each branch attached to –N-N=O); or – CH(OH)-C{scy}- (attached to CH_2 *via* –CH(OH).  **Y_2** is C{ar} or $–(CH_2)_mH$ (m = 1 – 5) or C{ar}-CH_2-; or $-(CH_2)nH$ (X is –OH or =O or their combination attached to any carbon, except to the *alpha*-one, no more than two X on each branch attached to –N-N=O); or $–(CH_2)_p$-C(O)-C{ar} (p = 2 – 4). Coded depictions:  $Enum_1$: -C{ar}; – CH(OH)-C{scy}-; $–(CH_2)_nH$ (n = 0 – 14); -C{sp3}n=0-14, O{H}m=0 - 2; -C(=O)p=0 - 2 “Mask”: O=N{V_3}-N-$Enum_3$; $Enum_3$ = (-H, -O{H}, -C=O) – direct bonding to N-atom of - H, -O{H}, -C=O is forbidden. $Enum_2$: -C{ar}; - C{H_2}C[ar]; -C{H_2}H n = 1-5; -C{sp3}n=0 - 14, O{H}m=0 - 2; -C(=O)p = 0 – 2 **B. Cyclic N-Nitrosamines:** *Metabolic activation mechanism starting with alpha-carbon atom hydroxylation* - any of the following:  (Y is -CH_2- or –O- or –NH-) (R is C{sp3} (no more than one as substituent) or none); (one C=C double bond at most or none)  (R_1 is C{sp3} or C{ar}: one substituent only or none; R_2 is –OH (one substituent only or none) or combination of R_1 and R_2



(Enum$_1$: -H, -C{H2}O{H})

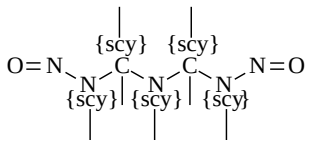
C. N-Nitrosamines as direct-acting mutagens:

- One N-nitroso group only:

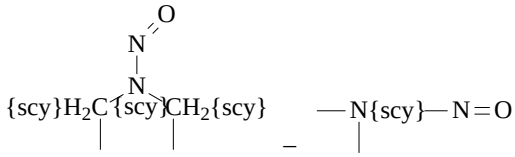


(Y_1, Y_2 are $–CH_2CH_2$-OH (both) or $–CH(OH)CH_3$ (both) or one of them in combination with C{ar})

- Two N-nitroso groups located closely to each other within cyclic structure:



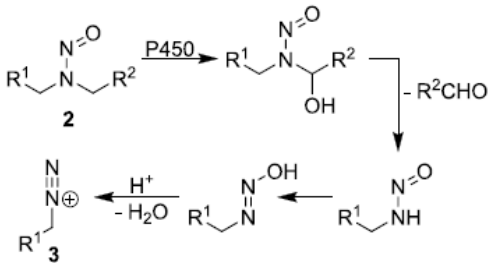
OR



(Combination of two fragments within one cyclic structure)

Mechanism	Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after carbenium ion formation Mechanistic Domain: SR (radical) Mechanistic Alert: Radical attack after nitroso radical formation Mechanistic Domain: SN1 Mechanistic Alert: Nucleophilic attack after nitrosonium cation formation

According to some publications, generally, the metabolic activation pathway to a highly reactive "ultimate carcinogen" such as diazonium cation 3 can be expressed as follows



Nitrosamine 2 first undergoes enzymatic α-hydroxylation with cytochrome P450 as biocatalyst and subsequently forms the dealkylated primary nitrosamine with aldehyde as a by-product. The unstable primary nitrosamine further decomposes to diazonium cation 3, which is the DNA alkylating agent via the carbenium ion generated from diazonim cation spontaneous decomposition. The resulting DNA damage can lead to in vitro bacterial mutagenicity, and, frequently, to cancer [1]. According to other publications [17], empirical evidence indicates that the presence of an oxidizable carbon center in α-position to the N-nitroso functionality is a critical requirement for N-nitrosamines

carcinogenicity.
It must be stressed that there are competing sites of CYP-450-mediated metabolism (e.g., hydroxylation at the β-carbon atom or at other carbons, more distant from the N-nitroso moiety than the α-carbon). However, these alternative pathways tend to decrease, rather than enhance carcinogenic potency. The bioactivation pathway, the oxidative N-dealkylation, is a common reaction in the drug metabolism, including the α-hydroxy-N-nitrosamines, and it has been proved that the carbonyl byproduct, e.g., formaldehyde, does not contribute to the toxic properties of N-nitrosamines [17].
In other cases, some N-nitrosamines do contain carbons in α-position to the N-nitroso group, bearing H-atoms, however, some specific structural features determine their partial mode of action as direct-acting mutagens. For instance, the compound Dinitrosopentamethylenetetramine:

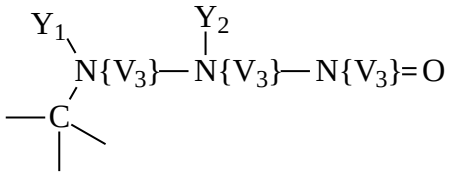
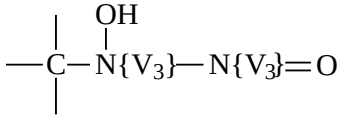
is also mutagenic as parent chemical with Salmonella typhimurium TA100. This is probably caused by the presence of two N-nitroso functionalities located relatively close to each other within a cyclic structure, which, together with steric hindrance effects could contribute to the observed direct mutagenicity [28]. N-nitrosamines, hydroxylated at the carbon atoms at alpha- or beta-position with respect to the N-nitroso group, if regarded as parent chemicals, can also be considered as direct-acting mutagens. This could be due to the –I-effect of the –OH group located closely to the –N-N=O functionality thus enhancing the N=O releasing capability. One example in this respect is another chemical, N-Nitrosodiethanolamine [28]:

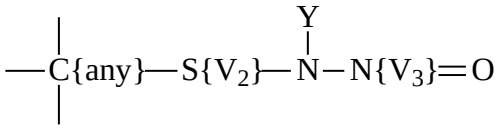
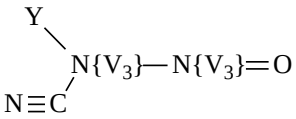
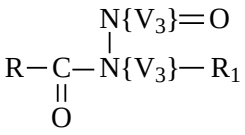
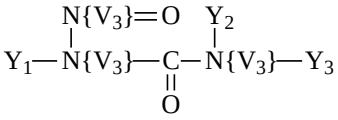
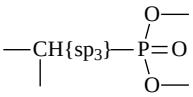
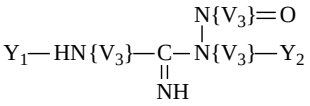
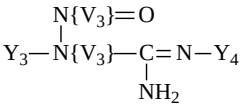
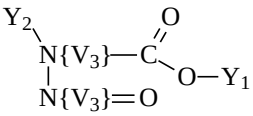
In conclusion, all chemicals listed in Table 4 contain alpha-carbon atoms next to the N-nitroso group, which is capable of metabolic C-hydroxylation as an initial step of bioactivation. This makes it possible for both modes of action to be involved in eliciting mutagenicity, with the direct-acting mechanism predominating.

Set of chemicals used for profile development	N-Nitrosamines
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. Beard, J. C., T. M. Swager, An Organic Chemist`s Guide to N-Nitrosamines: Their Structure, Reactivity, and Role as Contaminants, J. Org. Chem. 86 (2021), 2037 – 2057. 2. Guttenplan, J. B., N-Nitrosamines: Bacterial Mutagenesis and in Vitro Metabolism, Mutat. Res. 186 (1987), 81 – 134. 3. Kushida, H., K. I. Fujita, A. Suzuki, M. Yamada, T. Endo, T. Nohmi, T. Kamataki, Metabolic Activation of N-alkylnitrosamines in Genetically Engineered Salmonella Typhimurium expressing CYP2E1 or CYP2A6 Together with Human NADPH-Cytochrome P450 Reductase, Carcinogenesis 21(6) (2000), 1227 – 1232. 4. Maertens, L. A., P. Upadhyaya, St. S. Hecht, Ch. L. Zimmerman, Formation and Distribution of NNK Metabolites in an Isolated Perfused Rat Lung, Drug Metabol. Dispos. 38 (2010), 752 – 760. 5. Peterson, L. A., N. M. Thomson, D. L. Crankshaw, E. E. Donaldson, P. J. Kenney, Interactions Between Methylating and Pyridyloxobutylating Agents in A/J Mouse Lungs: Implications for 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone-Induced Lung Tumorigenesis, Canc. Res. 61 (2001), 5757 – 5763. 6. N-Nitrosomethylethylamine, Summaries & Evaluations, IARC, Vol. 17 (1978), p. 221; http://www.inchem.org/documents/iarc/vol17/nitrosomethylethylamine.html. Last visited: April, 2024. 7. Farelly, J. G., M. L. Stewart, J. E. Saavedra, W. Lijinski, Relationship Between Carcinogenicity and In Vitro Metabolism of Nitrosomethylethylamine, Nitrosomethyl-N-Butylamine, and

Nitrosomethyl-(2-Phenylethylamine) Labeled with Deuterium in the Methyl and α-Methylene Positions, Canc. Res. 42 (1982), 2106 – 2109.
8. Von Hofe, E., Fr. Grahmann, L. K. Keefer, W. Lijinski, V. Nelson, P. Kleihues, Methylation versus Ethylation in Target and Nontarget Tissues of Fischer 344 Rats Treated with N-Nitrosomethylethylamine, Canc. Res. 46 (1986), 1038 – 1042.
9. Rao, T.K., J. T. Cox, B. E. Allen, J. L. Epler, W. Lijinsky, Mutagenicity of N-Nitrosopyrrolidine Derivatives in Salmonella (Ames) and Escherichia coli K-12 (343/113) Assays, Mutat. Res. 89(1) (1981), 35 – 43.
10. Rao, T.K., D. W. Ramey, W. Lijinsky, J. L. Epler, Mutagenicity of Cyclic Nitrosamines in Salmonella typhimurium: Effect of Ring Size, Mutat. Res. 67(1) (1979), 21 - 26.
11. Padma, P.R., A. J. Amonkar, S. V. Bhide, Mutagenic and Cytogenetic Studies of N'-Nitrosonornicotine and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone. Cancer Lett. 46(3) (1989), 173 - 180.
12. 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: April, 2024.
13. Thomas, D.N., Wills, J.W. Tracey, H., Baldwin, S.J., Burman, M., Williams, A.N., Harte, D.S.G., Buckley, R.A. Lynch, A.M., Ames Test study designs for nitrosamine mutagenicity testing: qualitative and quantitative analysis of key assay parameters. Mutagenesis, 2023, gead033. doi: 10.1093/mutage/gead033.
14. Andrews, A.W., Thibault, L.H., Lijinsky, W., The relationship between mutagenicity and carcinogenicity of some nitrosamines. Mutat. Res., 1978, 51(3), 319-326. doi: 10.1016/0027-5107(78)90121-5.
15. Andrews, A.W., Lijinsky, W., The mutagenicity of 45 nitrosamines in the Salmonella typhimurium. Teratog. Carcinog. Mutagen., 1980,1(3), 295-303. doi: 10.1002/tcm.1770010306.
16. Umano, K., Shibamoto, T., Fernando, S.Y., Wei, C.I., Mutagenicity of 2-hydroxyalkyl-N-nitrosothiazolidines. Food Chem. Toxicol., 1984, 22(4), 253-259. doi: 10.1016/0278-6915(84)90002-4.
17. Snodin, D. J., A. T.-Martin, D. J. Ponting, Gr. F. Smith, A. Czich, K. Cross, L. Guster, J. Elloway, N. Greene, A. S. Kalgutkar, et al., Mechanisms of Nitrosamine Mutagenicity and Their Relationship to Rodent Carcinogenic Potency, Chem. Res. Toxicol. (2024); https://doi.org/10.1021/acs.chemrestox.3c00327. Last visited: April, 2024.
18. N-Nitrososarcosine; https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrososarcosine#section=Evidence-for-Carcinogenicity. Last visited: April, 2024.
19. N-Nitrosomethylvinylamine; https://pubchem.ncbi.nlm.nih.gov/compound/N-Nitrosomethylvinylamine#section=Special-Reports. Last visited: April, 2024.
20. Zielenska, M., J. B. Guttenplan, Mutagenic activity and specificity of N-nitrosomethylaniline and N-nitrosodiphenylamine in Salmonella, Mutat. Res. 202 (1988), 269 – 276.
21. N-Nitroso Ethyl Isopropyl Amine NEIPA [Genotoxic Impurity]; https://www.simsonpharma.com/product/n-nitroso-ethyl-isopropyl-amine-neipa-genotoxic-impurity. Last visited: April, 2024.
22. EFSA Nitrosamine Opinion TOX/2022/58, Last updated: 24 October 2022;

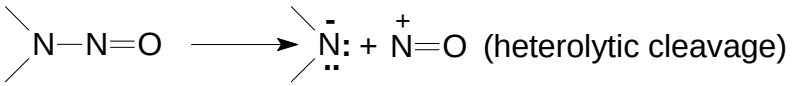
	https://cot.food.gov.uk/EFSA%20Nitrosamine%20Opinion. Last visited: April, 2024. 23. Zeiger, E., A. T. Sheldon, The mutagenicity of N-nitrosopiperidines for Salmonella typhimurium, Mutat. Res. 57 (1978), 85 - 89. 24. Toxicological Profile for N-Nitrosodiphenylamine, US Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Sisease Registry, April 1993; http://www.atsdr.cdc.gov/ToxProfiles/tp16.pdf. Last visited: April, 2024. 25. Miura, M., S. Sakamoto, K. Yamaguchi, T. Ohwada, Influence of Structure on N-NO Bond Cleavage of Aliphatic N-Nitrosamines, Tetrahedron Lett. 41 (2000), 3637 – 3641. 26. N-Nitrosodiphenylamine (86-30-6); https://cebs.niehs.nih.gov/cebs/test_article/86-30-6. 27. Appel, K. E., S. Gorsdorf, T. Scheper, H. H. Ruf, C. S. Ruhl, A. G. Hildebrandt, Metabolic denitrosation of diphenylnitrosamine: a possible bioactivation pathway, J. Cancer Res. Clin. Oncol. 113(2) (1987), 131 – 136; doi: 10.1007/BF00391434. 28. Chemical Carcinogenesis Research Information System (CCRIS); https://chem.nlm.nih.gov/chemidplus/ (for bacterial mutagenicity data for chemicals such as Dinitrosopentamethylenetetramine, N-Nitroso phenylhydroxylamine and N-Nitrosodiethanolamine).

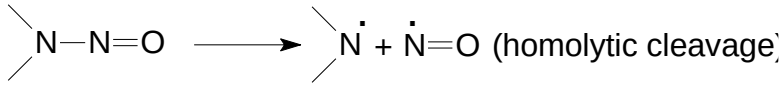
Individual profile/alert	
Name	N-Nitroso Compounds
Type of profile	Structural alert
Description/applicability domain	I. N-Nitrosohydrazine Derivatives  (Y1 is C{any} or H; Y2 is OH or O- or CH3 or H) (Note: In the case of Y2 being O- with Na+, K+, Li+ and NH4+ as counter-ions, spontaneous abiotic hydrolysis of the ionic bond occurs, and Y2 becomes OH) II. N-Nitroso-N-hydroxylamine derivatives  III. N-Nitrososulfenamide Derivatives

	 (Y is CH_3 or H) IV. N-Nitrosamide and N-Nitrosocyanamide derivatives A. N-Nitrosocyanamide derivatives:  (Y is C{sp3} or H) B. N-Nitrosamide derivatives:  (R is C{ar} or C{sp3} R1 is C{sp3} or H V. N-Nitrosourea and N-Nitrosoguanidine derivatives  (Y_1 is any of the following: $-CH_2CH_2Cl$, $-CH_2CH_2OH$, $-(CH_2)_nH$ (n = 1 – 6) or -CH{scy}- or $–CH_2-CH_2-$; Y_2, Y_3 are: -H (both); or -H and $-CH_2CH_2Cl$; or -H and -CH{scy}-; or -H and –C{scy}=O; or –H and  (bound via CH{sp3}) or –H and $–CH_3$; or $–CH_3$ (both)  (Y1 is –NO2 or H; Y2 is –(CH2)nH or –H)  (Y3 is –NO2 or H; Y4 is –(CH2)nH or –H) VI. N-Nitrosocarbamate devatives  (Y1 is C{ar}(benzoid) or –CnH2n+1 (n = 1 – 3); Y2 is –(CH2)mH (m = 1 – 3) or –CH2CH2OH or –CH2CH2-O-C(O)CH3)
Mechanism	Mechanistic Domain: SN1

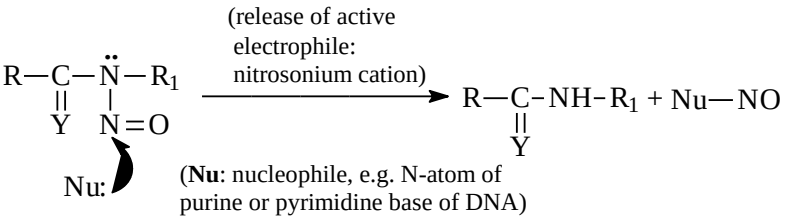
	Mechanistic Alert: Nucleophilic attack after nitrosonium cation formation

As parent chemicals, some N-nitroso compounds are capable of nitrosation of biological macromolecules and elicit in vitro bacterial (Ames) mutagenicity. They are potential NO+ and NO. donors through heterolytic or homolytic cleavage of the N─N=O bond, and their NO-releasing potential can be evaluated in terms of N─NO bond energy

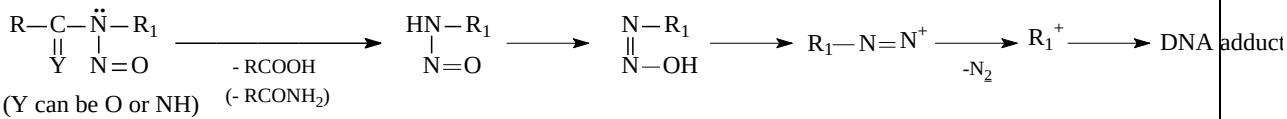




N-nitroso compounds with electron-withdrawing groups attached to the nitrogen, bearing the NO-group, e.g., N-nitrosamides, N-nitrosoureas, N-nitrosoguanidines, N-nitrosocarbamates, N-nitrosohydrazines, N-nitrosohydroxylamines, etc. show greater NO-releasing capability than the N-Nitrosamines mentioned above. For N-acyl-N-nitroso compounds, this could be explained by the electronic repulsion between the carbonyl and nitroso group oxygens or the attraction of the lone-pair electrons at nitrogen by the carbonyl group: both effects weaken the N─NO bond. In a number of cases regarding N-acyl-N-nitroso compounds or N-alkyl-N-nitrosoamidines, for instance, the more predominant mechanistic pathway is nucleophilic attack on the nitrogen of the nitroso group resulting in denitrosation (heterolytic NO release):

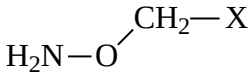
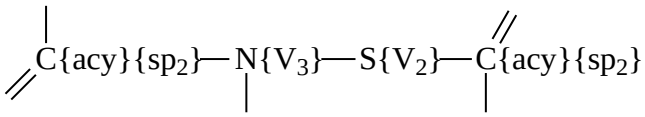
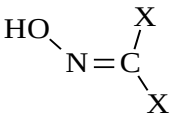
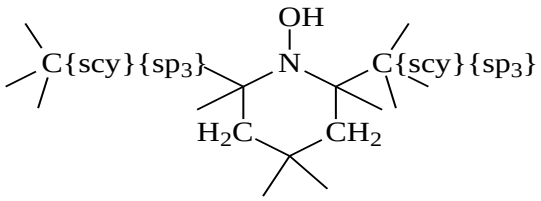


Another mechanistic pathway could be nucleophilic attack at the carbon of C=O or C=NH bonds by DNA base or water with breakage of the C-N bond, to generate intermediate, which decomposes to an active diazotate ion (R-N=N-O-)

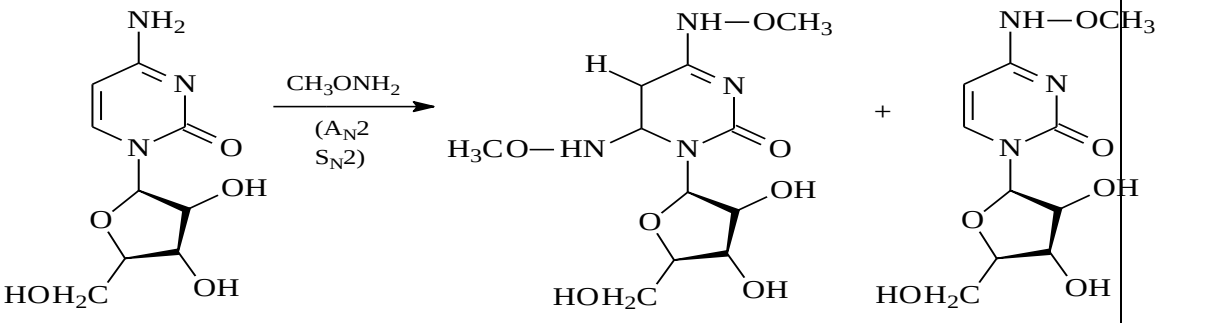


Set of chemicals used for profile development	N-Nitroso Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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. 2. Miura, M., S. Sakamoto, K. Yamaguchi, T. Ohwada, Influence of Structure on N-NO Bond Cleavage of Aliphatic N-Nitrosamines, Tetrahedron Lett. 41 (2000), 3637 – 3641. 3. Wang, P. G., M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai, A. Janczuk, Nitric Oxide Donors: Chemical Activities and Biological Applications, Chem. Rev. 102 (2002), 1091 – 1134. 4. Janczuk, Nitric Oxide Donors: Chemical Activities and Biological

Applications, Chem. Rev. 102 (2002), 1091 – 1134.
5. Guttenplan, J. B., N-Nitrosamines: Bacterial Mutagenesis and In Vitro Metabolism, Mutat. Res. 186 (1987), 81 – 134.
6. Kazius J, McGuire R., Bursi R., Journal of Medicinal Chemistry 48 (1), pp. 312-32, 25.
7. CCRIS database, Toxnet databases. https://pubchem.ncbi.nlm.nih.gov/bioassay/1259407#sid=363899992&section=Test-Results. Last visited: April, 2024.
8. CCRIS database, Toxnet databases. https://pubchem.ncbi.nlm.nih.gov/bioassay/1259407#sid=363902040&section=Test-Results. Last visited: April, 2024.
9. Hrabie, J. A., L. K. Keefer, Chemistry of Nitric Oxide Releasing Diazeniumdiolate ("Nitrosohydroxylamine") Functional Group and Its Oxygen-Substituted Derivatives, Chem. Rev. 102 (2002), 1135 – 1154.
10. Liang, Nacharaju, Friedman & Friedman (Special Report), Nitric oxide generating/releasing materials, JM Friedman Future Sci. OA (2015) 1(1), FSO54 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4739797/pdf/fso-01-54.pdf.
11. Stopper, H., M. Moller, H. M. Bommel, H. H. H. W. Smidt, Cytotoxic versus genotoxic effects of nitric oxide (NO), Toxicol. Lett. 106 (1999), 59 – 67.
12. Chemical Carcinogenesis Research Information System (CCRIS); https://chem.nlm.nih.gov/chemidplus/ ; (for bacterial mutagenicity data for chemicals such as Dinitrosopentamethylenetetramine, N-nitroso phenylhydroxylamine and N-Nitrosodiethanolamine).
13. 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: April, 2024.
14. Lee, K., B. Gold, S. S. Mirvish, Mutagenicity of 22 N-Nitrosamides and Related Compounds for Salmonella typhimurium TA1535, Mutat. Res. 48 (1977), 131 – 138.
15. Nakamura, S.-i., Y. Oda, Ts. Shimada, I. Oki, K. Sugimoto, SOS-Inducing Activity of Chemical Carcinogens and Mutagens in Salmonella typhimurium TA1535/pSK 1002: Examination with 151 Chemicals, Mutat. Res. 192 (1987), 239 – 246.
16. Naga, M., T. Yahagi, M. Nakadate, T. Kawachi, T. Sugimra, Mutagenic or DNA-Modifying Activities of N-Alkyl-N'-Nitro-N-Nitrosoganidines, Jap. J. Genetics 50(5) (1975), 403 – 408.
17. Danno, Gen-ichi, K. Kanazawa, M. Toda, M. Mizuno, H. Ashida, M. Natake, A Mutagen from Histidine Reacted with Nitrite, J. Agric. Food Chem. 41 (1983), 1090 – 1093.
18. Umetsu, N., E. Kuwano, T. R. Fukuto, Nature of N-S Bond Cleavage of 2,3-Dihydro-2,2-Dimethyl-7-Benzofuranyl (Di-(n-butylaminosulfenyl) (methyl)carbamate, J. Environ. Sci. Health B15(1) (1980), 1 – 23.
19. DeBaun, J. R., J. B. Miaullis, J. Knarr, A. MIhailovski, J. J. Menn, The Fate of N-Trichloro[14C] Methylthio-4-Cyclohexene-1,2-Dicarboximide ([14C]Captan) in the Rat, Xenobiotica 4(2) (1974), 101 – 119..

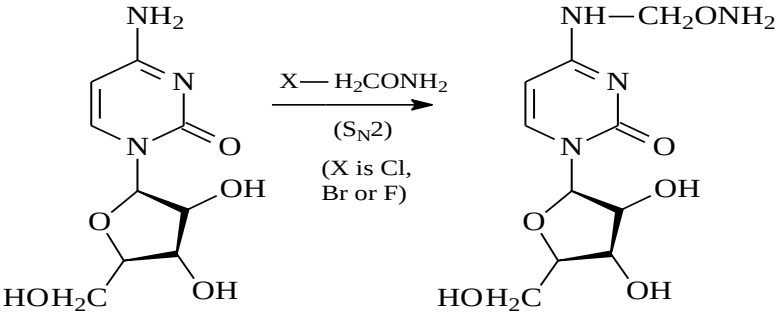
Individual profile/alert	
Name	Non-Aromatic Hydroxylamine Derivatives
Type of profile	Structural alert
Description/applicability domain	 (X is F, Cl, Br, H)   (X can be F, Cl, Br) 
Mechanism	AN2 Nucleophilic addition to activated C=C bond SN2 Nucleophilic substitution on activated primary amino group 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:



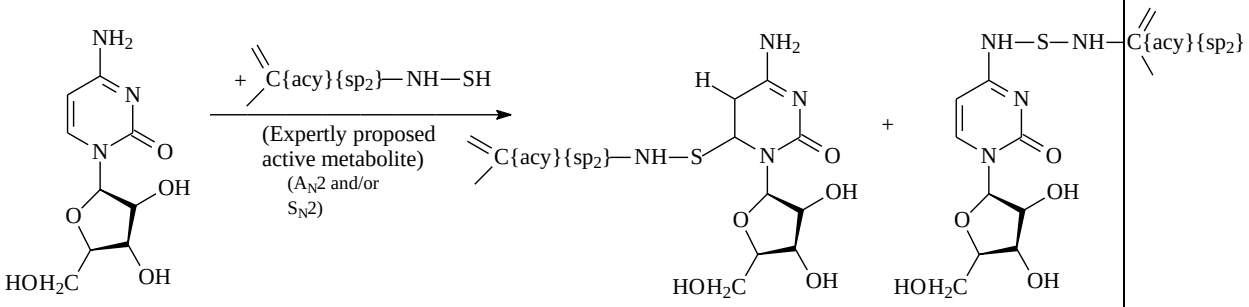
(Cytidine) (6-Methoxyamino-5,6-dihydro-N-4-methoxycytidine) (N-4-Methoxycytidine)

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):

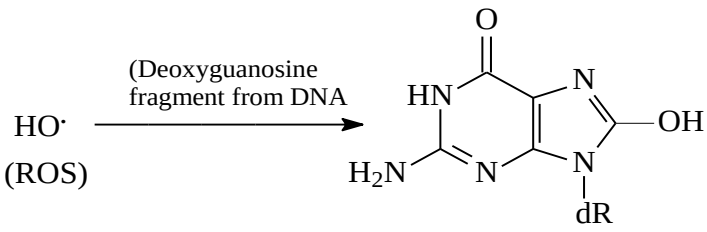
$$NH_2OH + Me^n \rightarrow NH_2O^{\cdot} + H^+ + Me^{n-1}$$

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

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

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

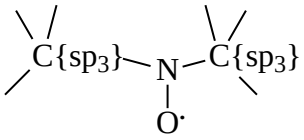
$$H_2O_2 \rightarrow 2HO^{\cdot}$$



DNA adduct

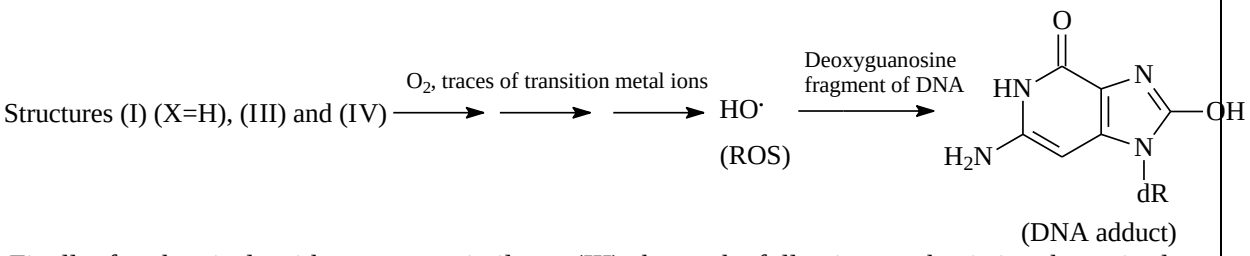
The oxidative potency of hydroxylamine and its O-derivatives such as O-methyl- and O-ethyl hydroxylamine was reported to be generally higher than the corresponding effects of the N-derivatives (N-methyl-, N-dimethyl-, and N,O-dimethyl hydroxylamine). The occurrence of cell-damaging products like superoxide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

Nitroxyl radicals of the type:

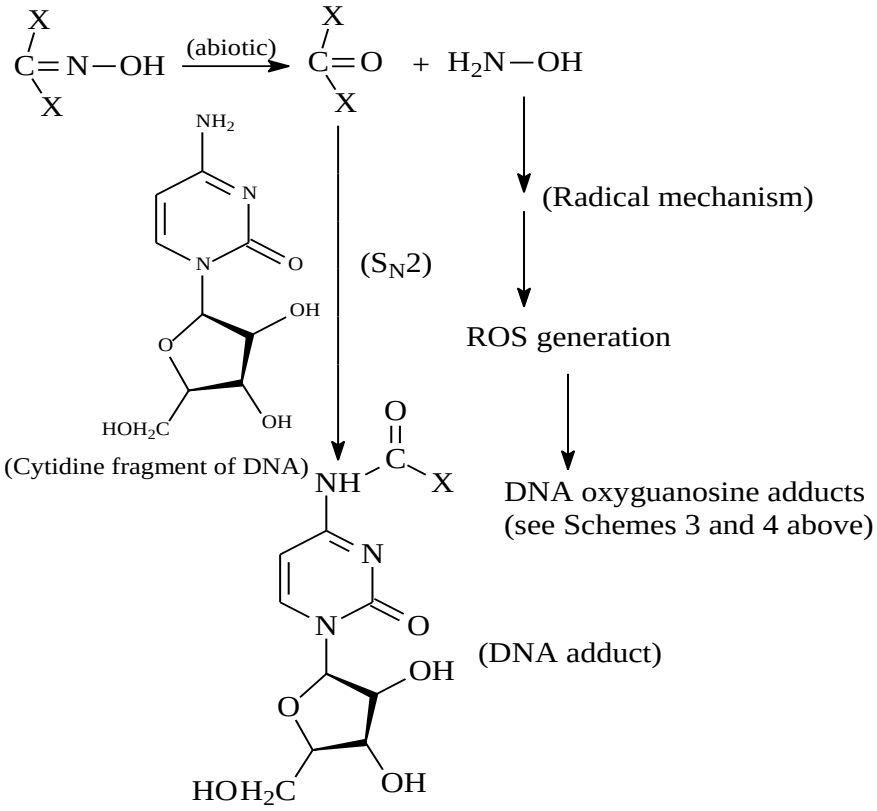


formed from N,N-dialkylhydroxylamines by mechanism, similar to that shown in Scheme 3 above (see the formation of the hydroxylamine-derived radical NH2O.) enhanced the formation of H2O2 and ROS, and were reported as mutagenic in Salmonella typhimurium strains TA104 and TA100 [5].

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

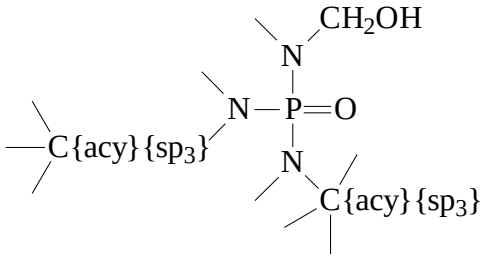


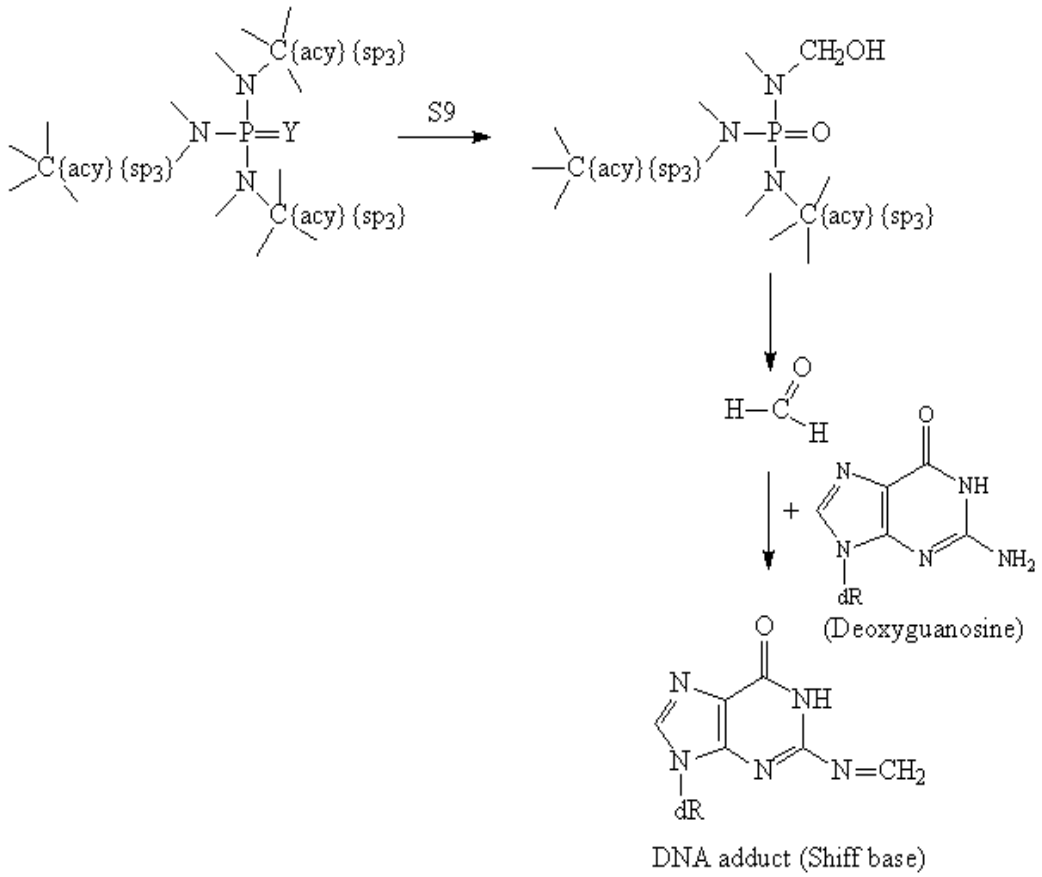
Finally, for chemicals with structures similar to (III) above, the following mechanistic scheme is also possible:



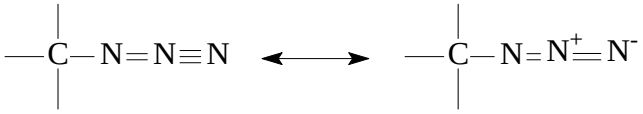
Set of chemicals used for profile development	Non-Aromatic Hydroxylamine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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,

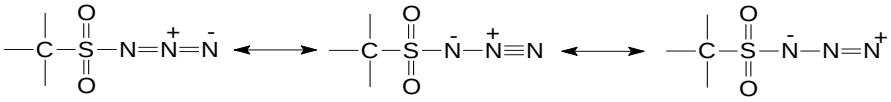
	Hydroxylamines, Carbamates, Acetamides, Thioacetamides and Thioureas, 1979, 307 – 330; DOI: http://anonym.to/?http://doi.org/10.1016/S0166-1116%2808%2971327-X Last visited: June, 2021. 3. Kono, Y., Generation of Superoxide Radical during Autoxidation of Hydroxylamine and an Assay for Superoxide Dismutase, 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)

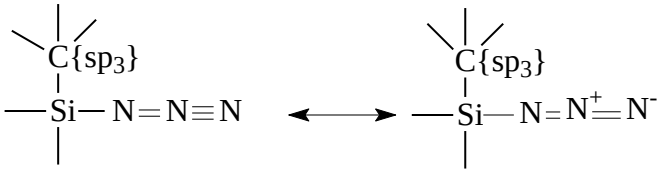


Set of chemicals used for profile development	Non-Cyclic Alkyl Phosphoramides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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=680-31-9. 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)



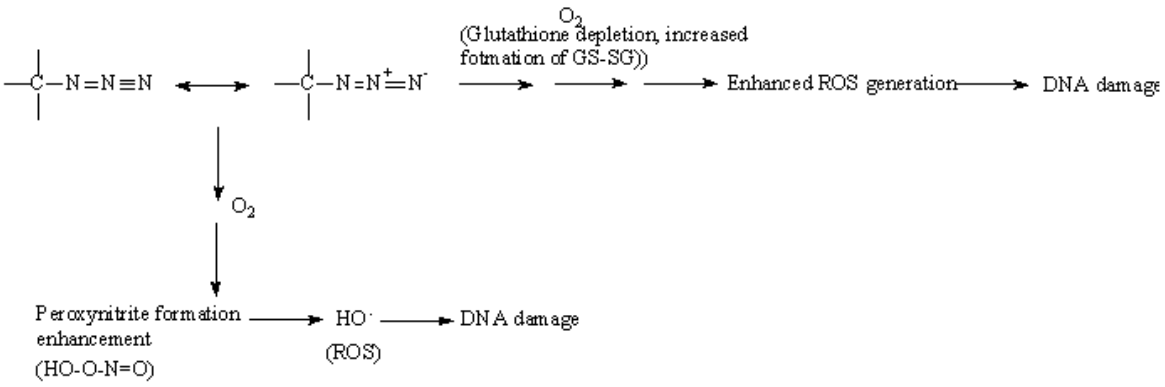
(Organic Sulfonyl Azides: resonance structures)



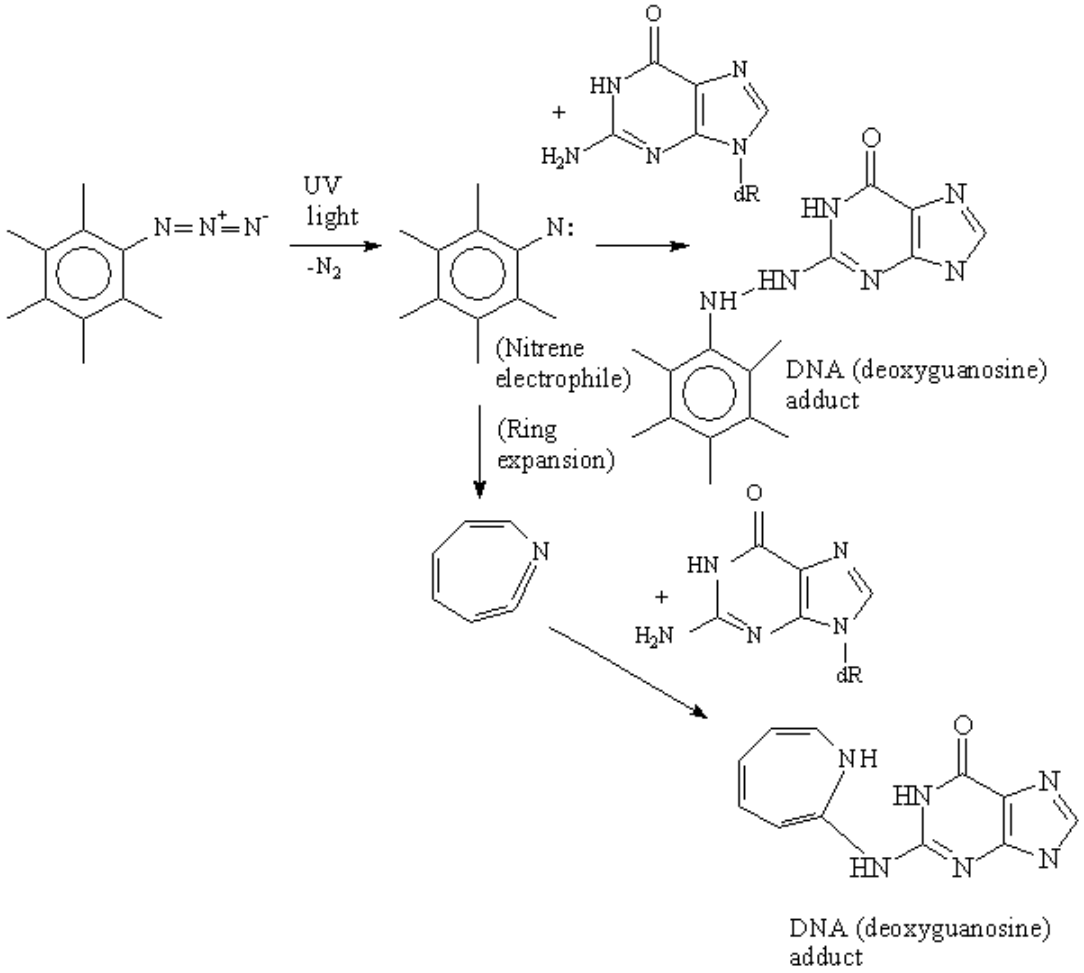
(Organic Organosilicon Azides: resonance structures)

Mechanism	Radical ROS generation, S_N1 Nucleophilic attack after nitrene formation and Non-covalent interactions DNA intercalation; SN2 Nucleophilic attack on sulfonyl azide

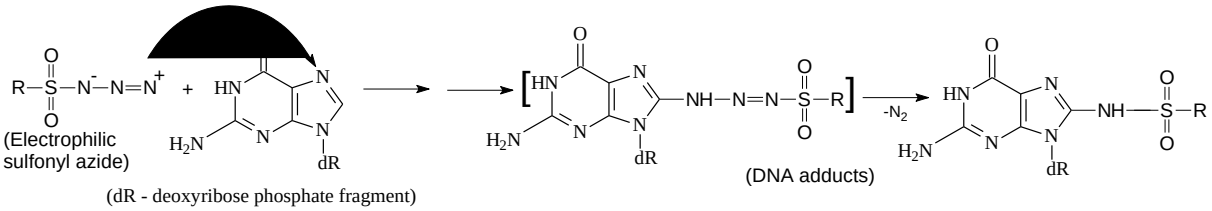
Two principal mechanisms of DNA damage, eliciting bacterial mutagenicity can be suggested. The first one is associated with the pro-oxidant properties of organic azides such as AZT, resulting in endogenous glutathione depletion and enhanced peroxynitrite and reactive oxygen species (ROS) formation [9,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

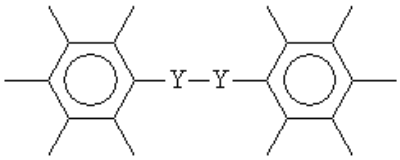
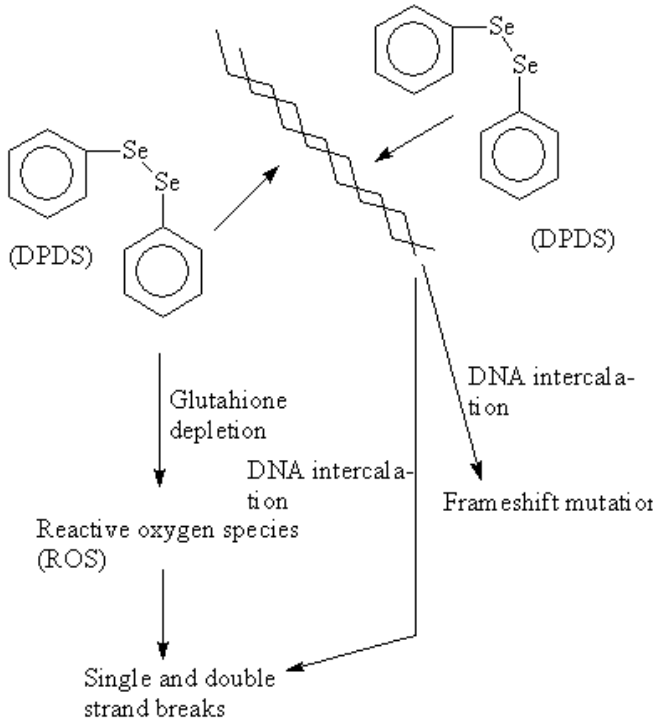


One additional mechanistic scheme is associated with eliciting cross-linking effects on biopolymer molecules or by forming DNA adducts. The mechanism below is only expertly assumed and is based on the electrophilicity of sulfonyl azides

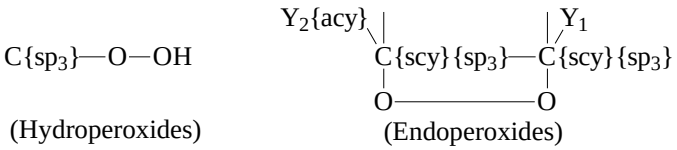
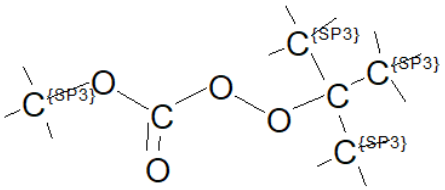


Set of chemicals used for profile development	Organic Azides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

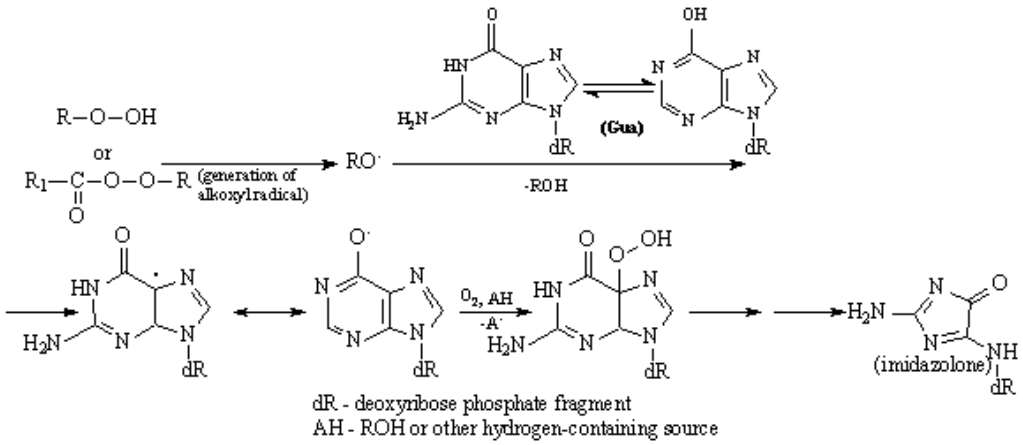
	7. Owais, Mutat. Res. **118** (1983), 229 – 239. 8. Owais, Mutat. Res. **197** (1988), 313 – 323. 9. Osborne, J. AIDS Clin. Res. 6(4) (2015); DOI: 10.4172/2155-6113.1000441. 10. Mak, Cardiovasc. Toxicol**. 04** (2004), 109 – 115). 11. Photoreactive Crosslinker Chemistry, Transfection & Genome Engineering 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	Organic Diselenides and Ditellurides
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.

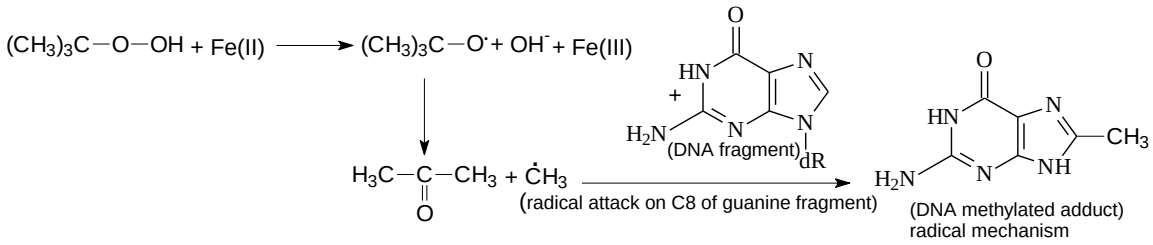
	3. Rosa, Braz. J. Med. Biol. Research **40** (2007), 1287 – 1304. 4. Brito, Acta Biochim. Pol. **56**(1) (2009), 125 – 134. 5. Prigol, Chem. Biol. Interact. **200** (2012) 65 – 72.

Individual profile/alert	
Name	Organic Peroxy Compounds
Type of profile	Structural alert
Description/applicability domain	 (Hydroperoxides) (Endoperoxides) (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]:

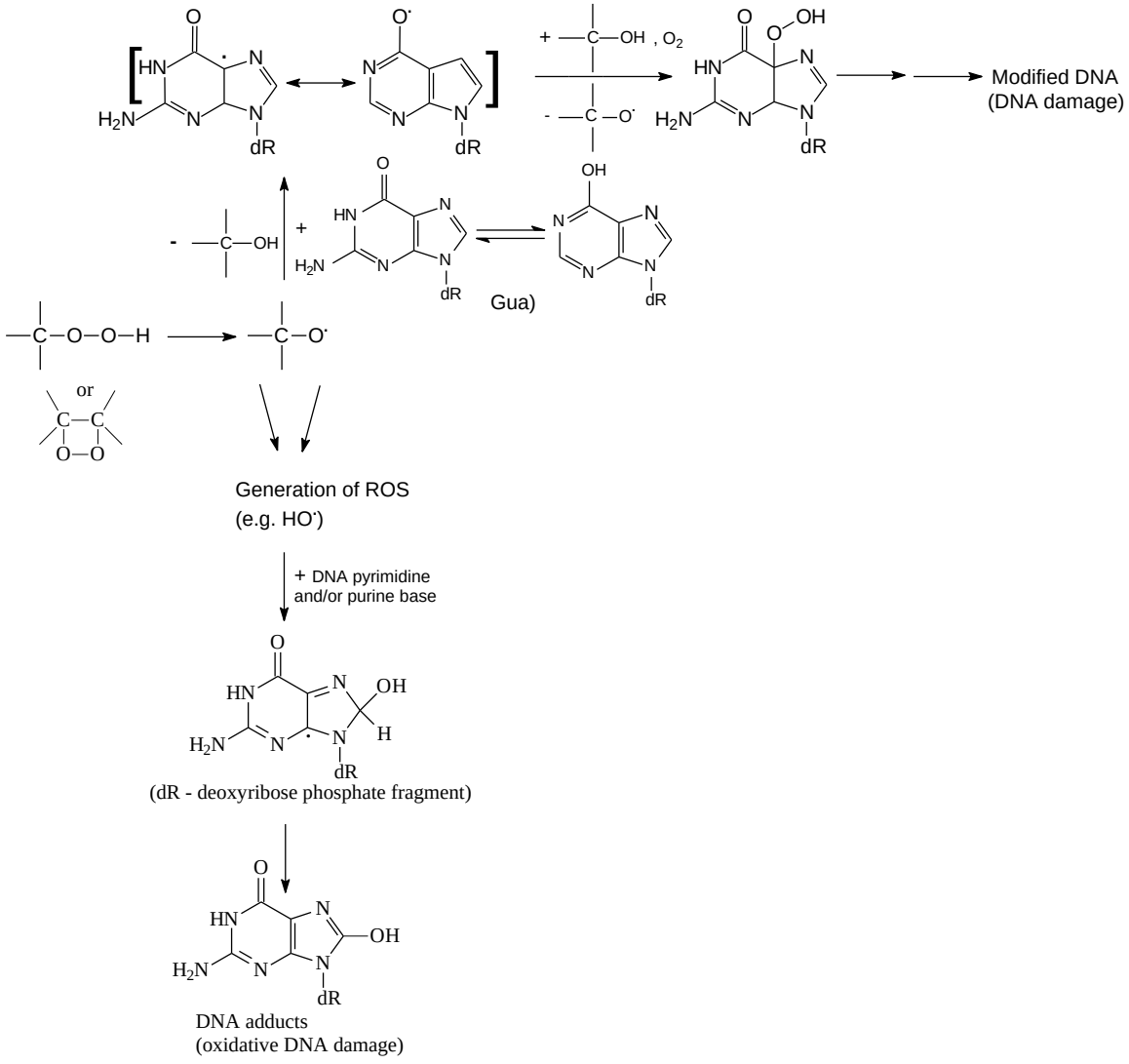


Such radicals, similarly to the hydroxyl ones are also involved in the oxidative stress [2].
Mutagenicity of various organic peroxy compounds, including TBHP, cumene hydroperoxide, 1,2,3,4-tetrahydronaphtyhalene hydroperoxide, etc. has been observed.
Based on the above assumptions and other considerations 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]:



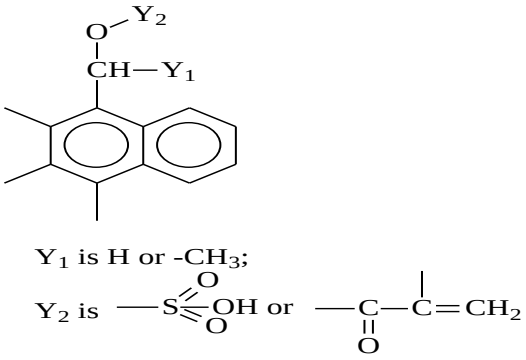
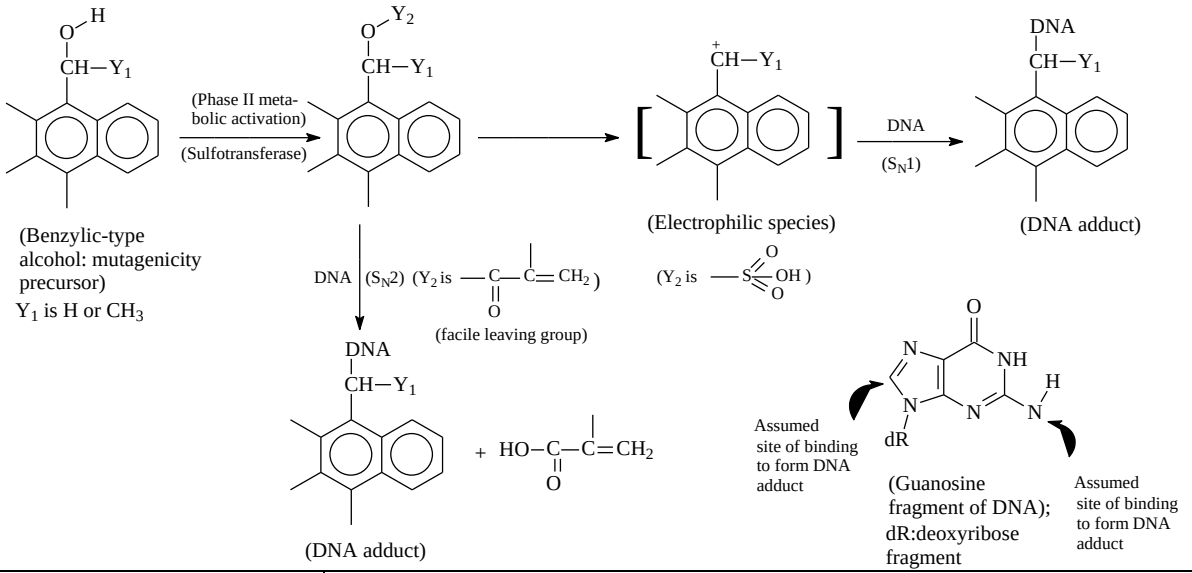
Endoperoxides have also been suggested to induce generation of reactive oxygen species [11]. However, with endoperoxides, it is assumed that hydrophobicity and the lack of bulky substitients on the carbon atoms, which could cause steric hindrance are important factors contributing to mutagenicity.

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:

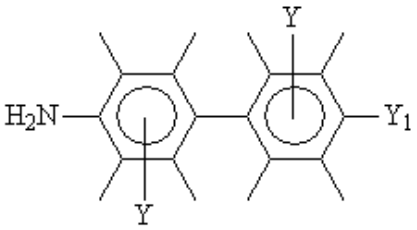
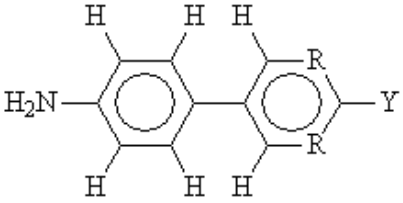


Set of chemicals used for profile development	Organic Peroxy Compounds
Data/Knowledge used for	An extensive review of the literature was performed enabling the

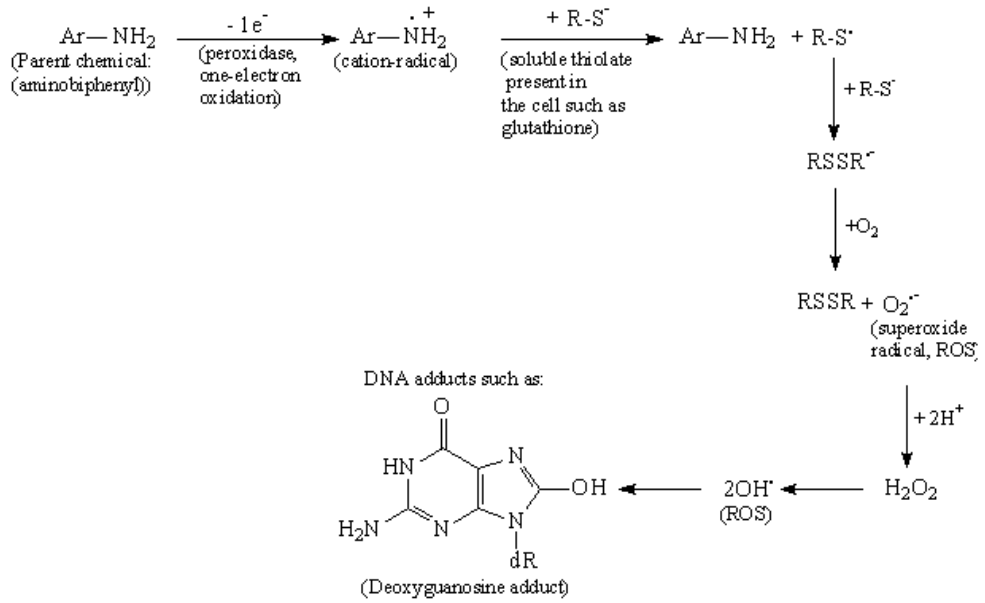
profile development	chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.O' Donnel, Biochem. J. **304** (1994), 707 - 713. 2. Adam, Chem. Res. Toxicol. **11** (1998), 1089 - 1097. 3. Stock, S., Arch. Toxicol. **72**(6) (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	
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	PAH Benzylic Alcohol 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

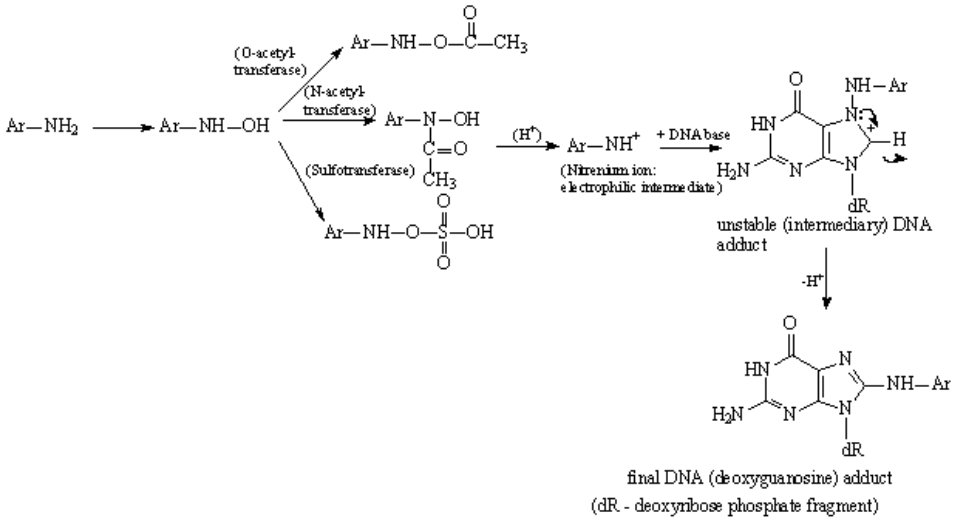
	profiler according to the references listed below.
References	1. Glatt, H., Bioactivation of Mutagens via Sulfation, The FASEB Journal 11 (1997), 314 – 321; https://faseb.onlinelibrary.wiley.com/doi/pdf/10.1096/fasebj.11.5.9141497. Last visited: June, 2021. 2. Ravi Kumar, M. N. V., M. V. Vadhanam, J. 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)**

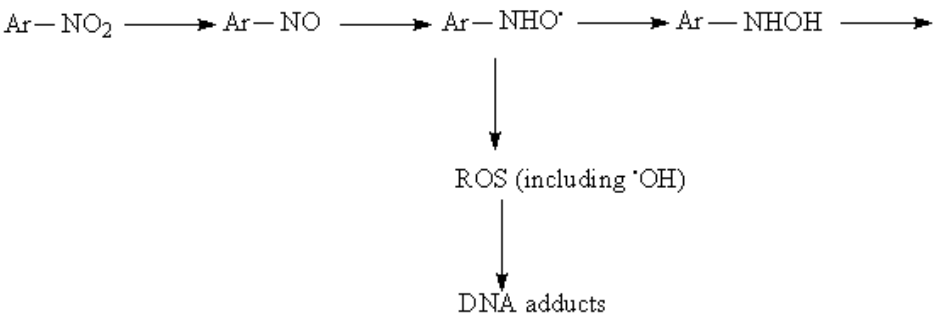
If the presence of endogenous peroxidase enzymes in the "classical" *Salmonella typhimurium* strains is assumed, the following mechanistic scheme involving the formation of reactive oxygen species (ROS) could explain the observed positive *in vitro* bacterial mutagenicity results for aminobiphenyls as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



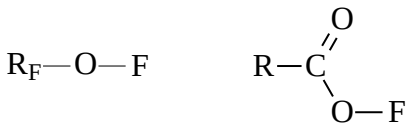
Reduction of the nitro group to nitroso intermediate is followed by formation of N-hydroxylamine species, and may occur endogenously by the bacterial nitroreductase in the prokaryotic *Salmonella typhimurium* cell. As a result, from the generation of reactive radical species such as $ArNHO^{\cdot}$, 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]:



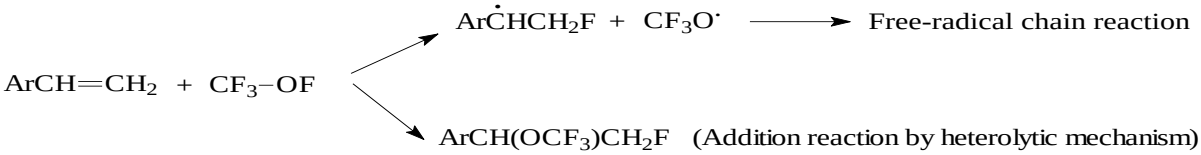
Scheme 3

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.

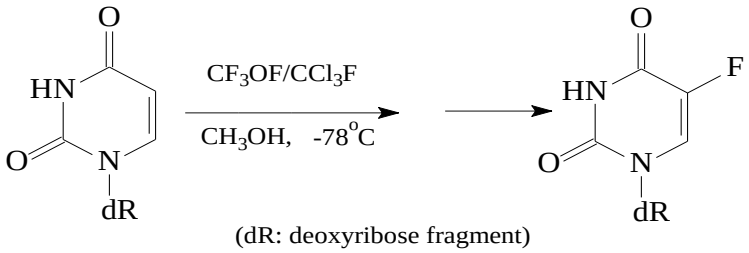
	11. El-Bayoumy, Mutat. Res. 90 (1981), 345 – 354. 12. Sinsheimer, Mutat. Res. **268** (1992), 255 – 264. 13. Chung, Toxicol. Sci **56** (2000), 351 – 356. 14. Ioannides, Carcinog. **10**(8) (1989), 1403 – 1407 (Abstract); http://www.ncbi.nlm.nih.gov/pubmed/2665965. Last visited: June, 2021. 15. Witherell, 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]:

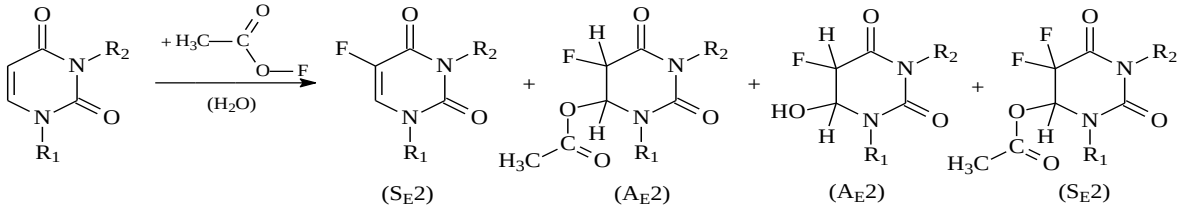


(dR: deoxyribose fragment)

(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]:

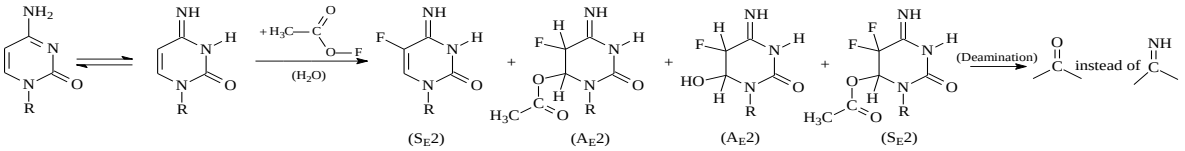
Uracil and Its Derivatives (Scheme 2):



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

(Scheme 2)

Cytosine and Its Derivatives (Scheme 3):

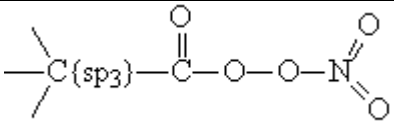


(R is H or -CH_3)

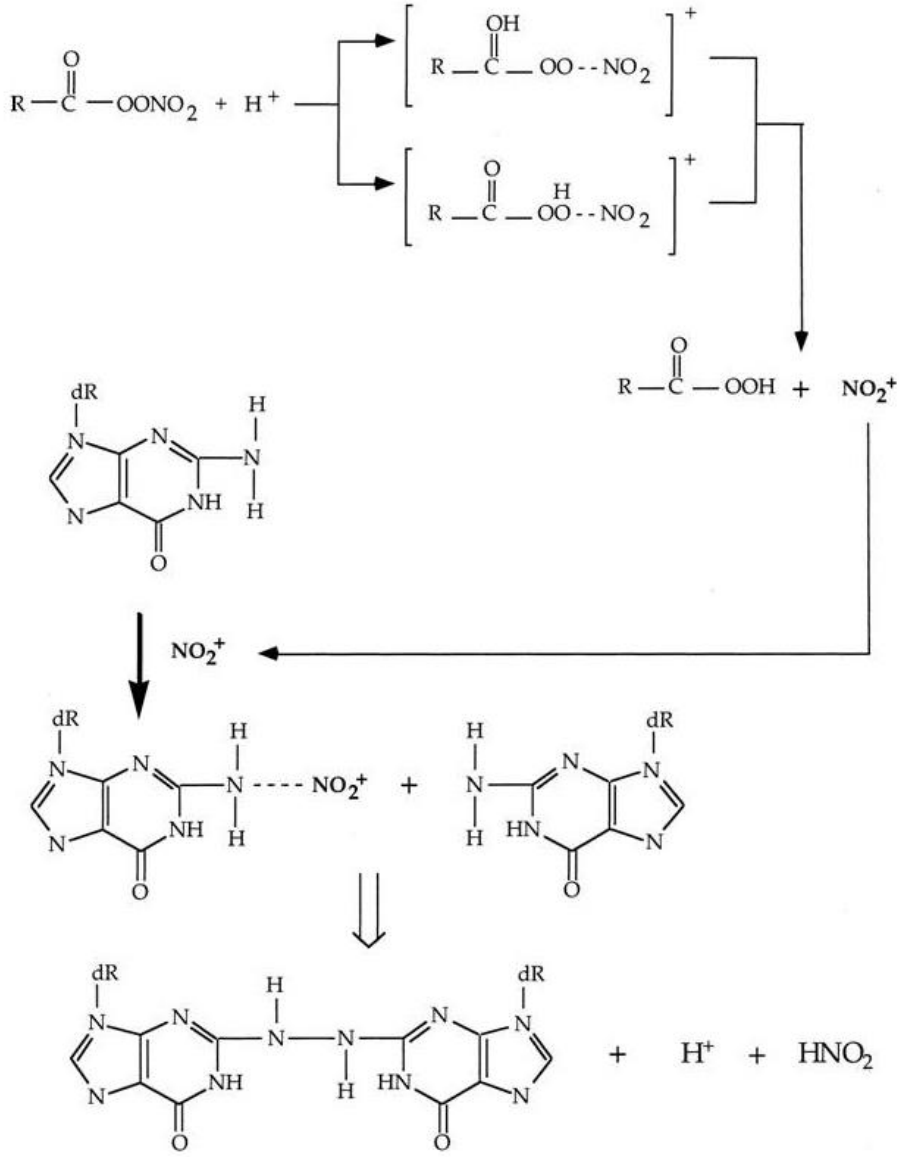
(Scheme 3)

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	Perfluorinated Hypofluorites
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.

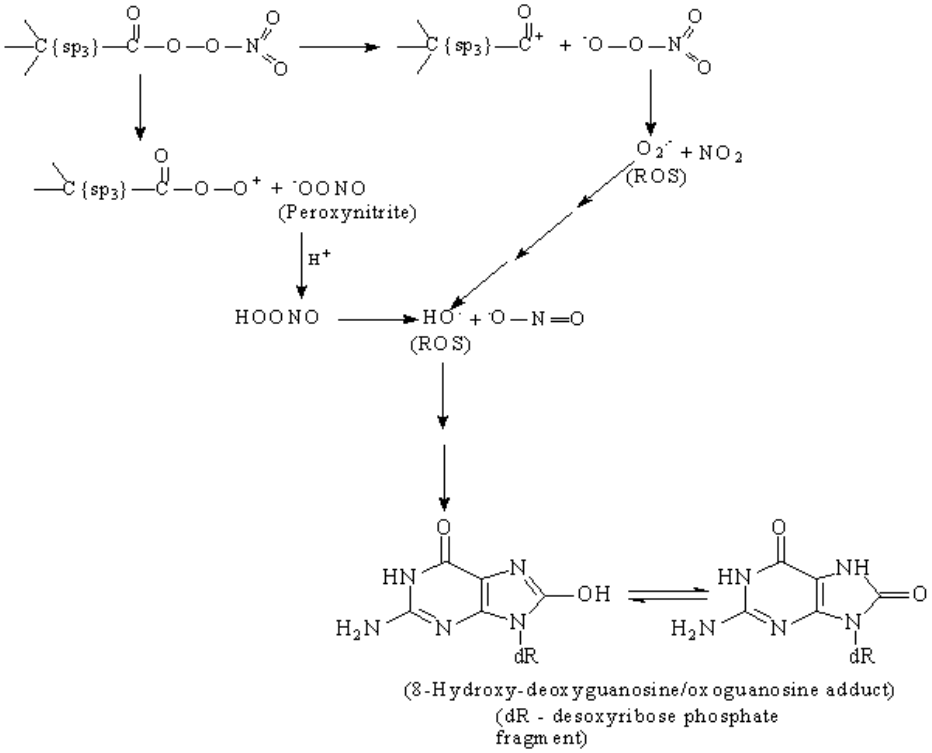
Individual profile/alert	
Name	Peroxyacyl Nitrates
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS generation and S_N1 or S_N2 Nitrosation

The following mechanistic scheme for the generation of active electrophilic species and interaction with DNA (deoxyguanosine fragment) has been suggested [3]:

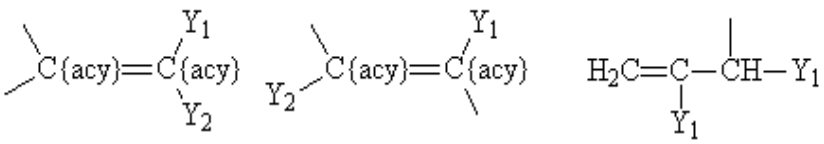


According to another mechanistic hypothesis, PAN may release peroxyacetyl nitrite and other reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxide radical, which may cause mutagenicity and cell apoptosis. The corresponding scheme of ROS generation and formation

of DNA adduct can be outlined as follows [5]:

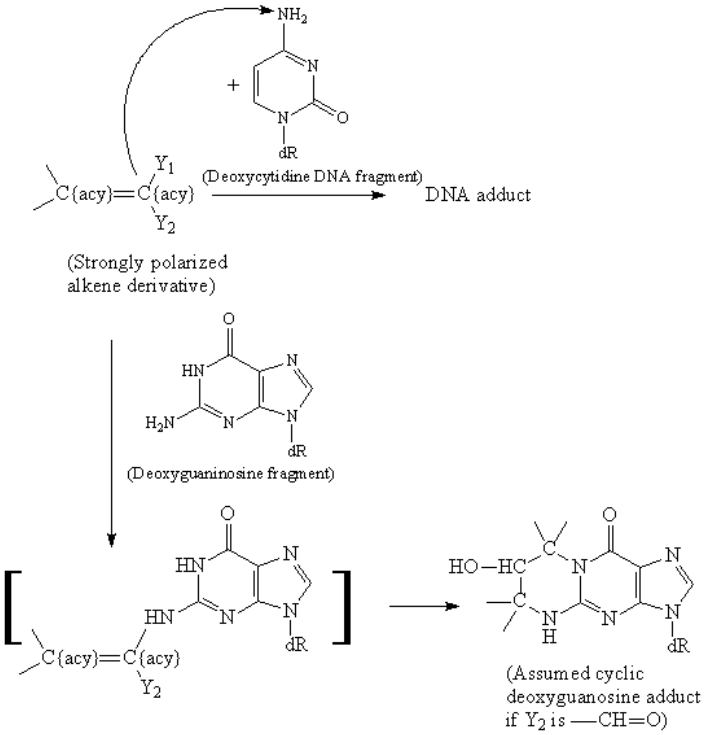


Set of chemicals used for profile development	Peroxyacyl Nitrates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

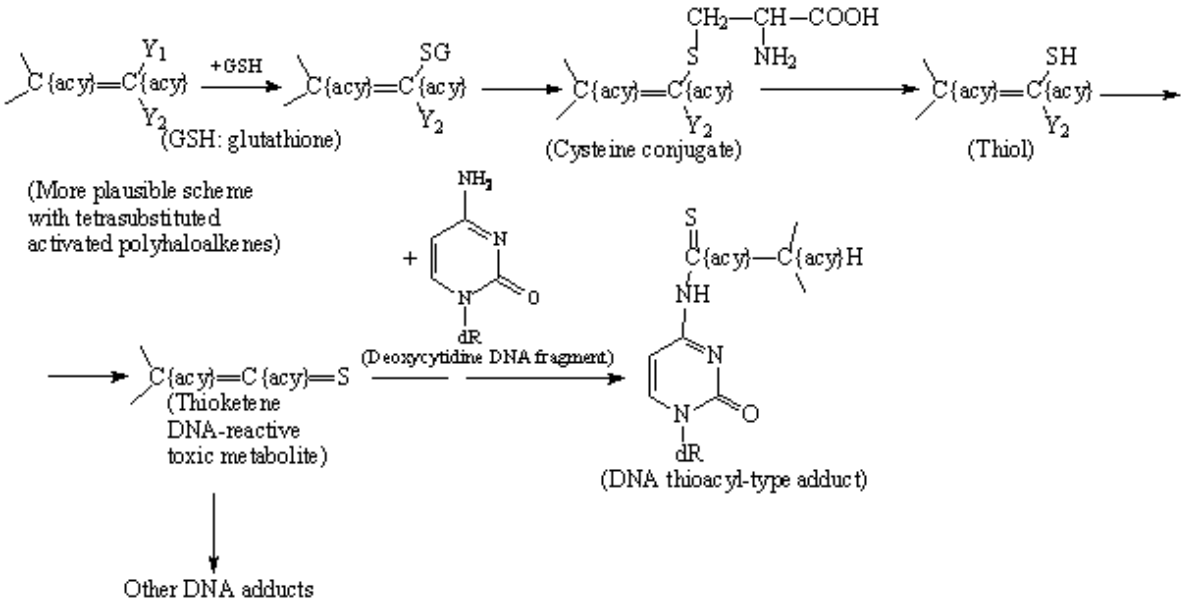
Individual profile/alert	
Name	Polarized Haloalkene Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y_1 is -Cl, -Br, -I; Y_2 is C(O) (carbonyl), -CN, -C-Cl, -C-Br, -C-I -OP(O)O- (phosphate group), -NO_2)
Mechanism	S_N2 Alkylation, direct acting epoxides and related after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

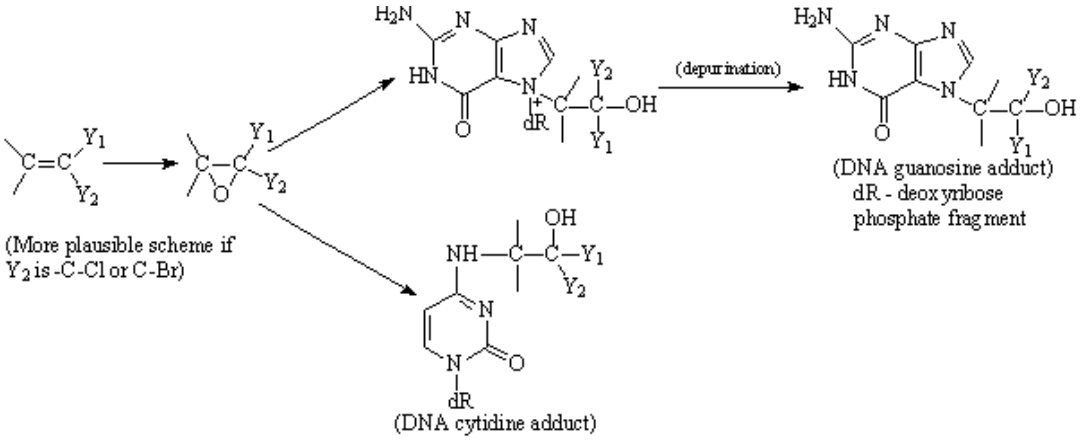
Direct alkylation (expertly assumed) – geminally bound halogen (Y_1) and strong electron-withdrawing substituent (Y_2) could make the former more labile, eliciting alkylating capability towards DNA pyrimidine and/or purine bases shown in Scheme 1:



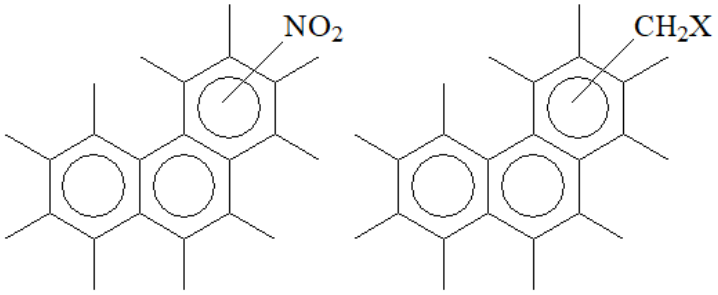
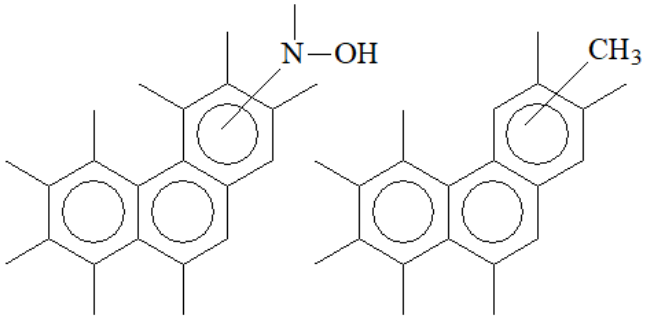
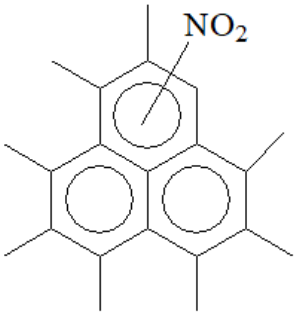
Bearing in mind the structural similarity of compounds such as trichloropropenenitrile and 2-chloropentene-2-nitrile with other haloalkenes such as trichloroethylene, terachloroethylene, trichlorotrifluoropropene, etc., glutathione-dependent enzymatic metabolic bioactivation with the formation of active 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:

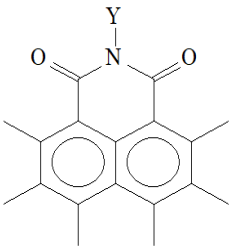
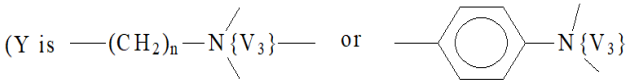
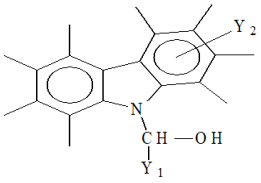


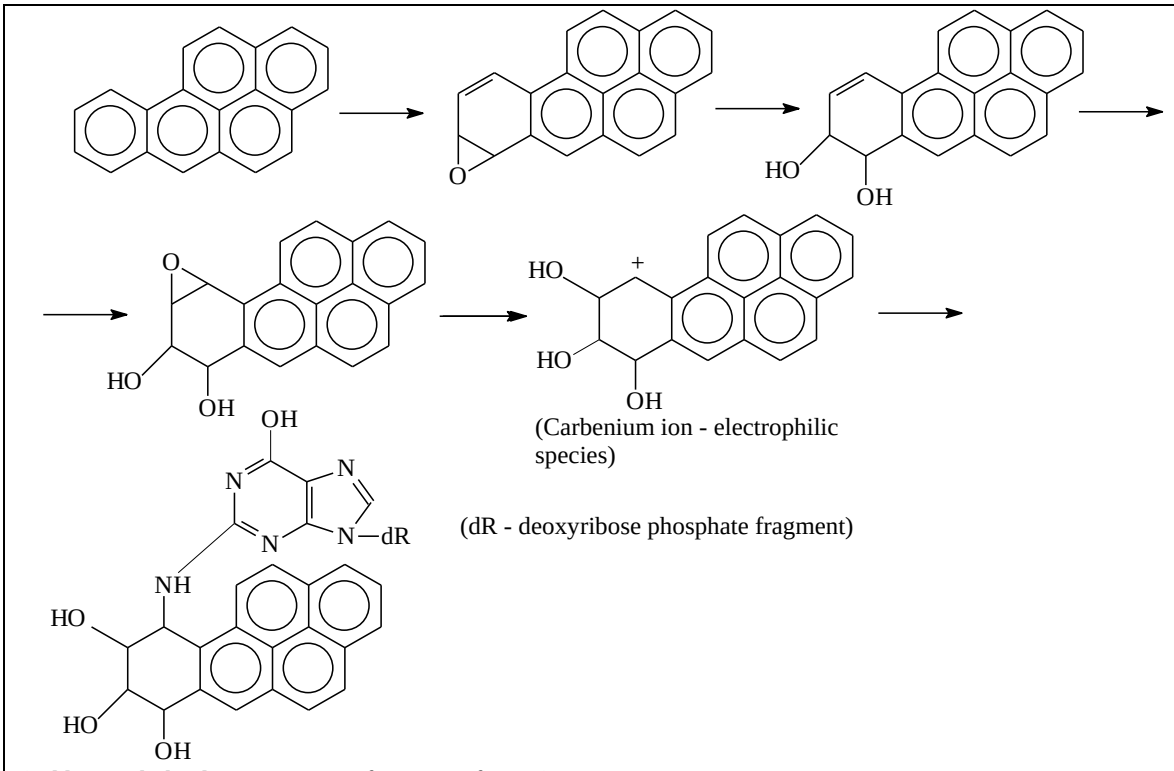
Scheme III: Metabolic activation *via* epoxidation shown in Scheme 3:

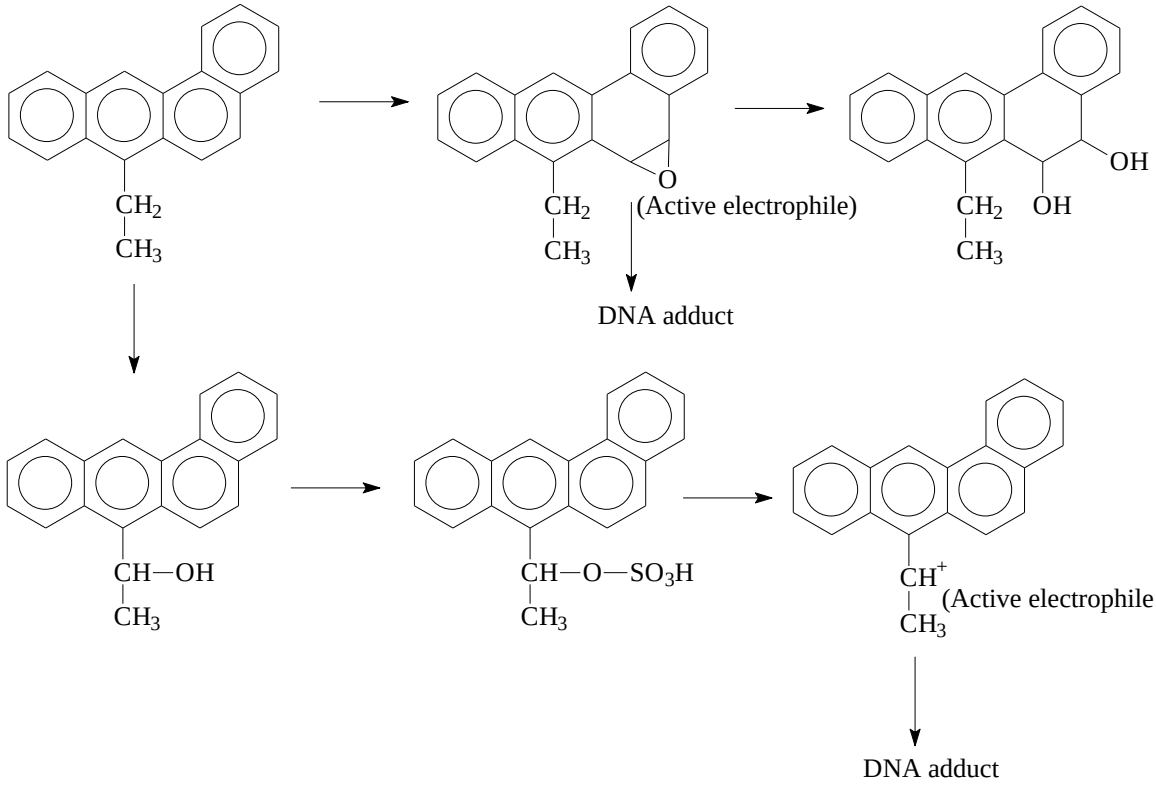


Set of chemicals used for profile development	Polarized Haloalkene Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

	  (n = 2 or 3) No more than two fused benzene rings; No -C(O)O-, -C(O)NH- or -SO_3H 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

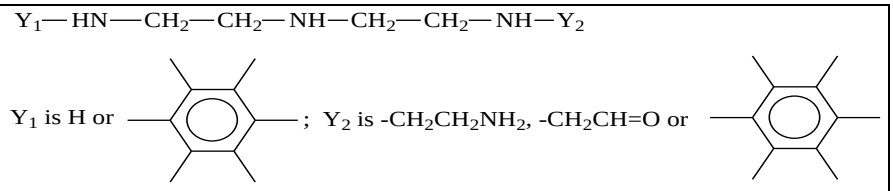




Set of chemicals used for profile development	Polycyclic Aromatic Hydrocarbon, Naphthaleneimide and Carbazole Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this 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.

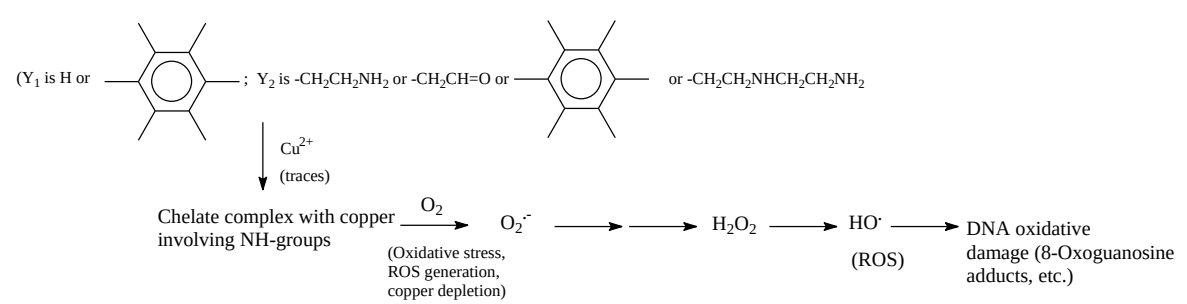
Individual profile/alert	
Name	Polyethylene Polyamines
Type of profile	Structural alert

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].

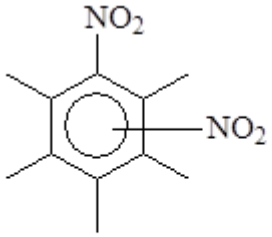
According to another report, trientine possesses strong chelating properties with respect to copper, due to the specific structure of linear tetramine with nitrogen-containing moieties as ligands. Trientine showed mutagenicity in bacterial cells with and without S9 metabolic activation in a broad range 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:

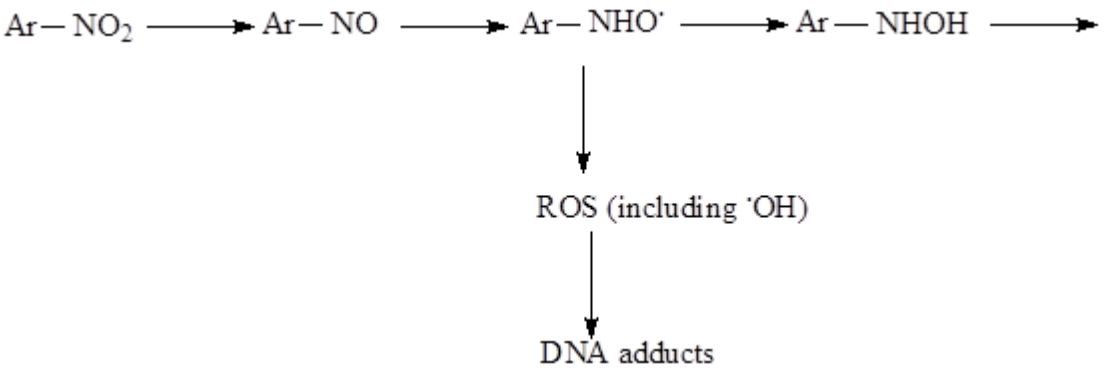


Set of chemicals used for profile development	Polyethylene Polyamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

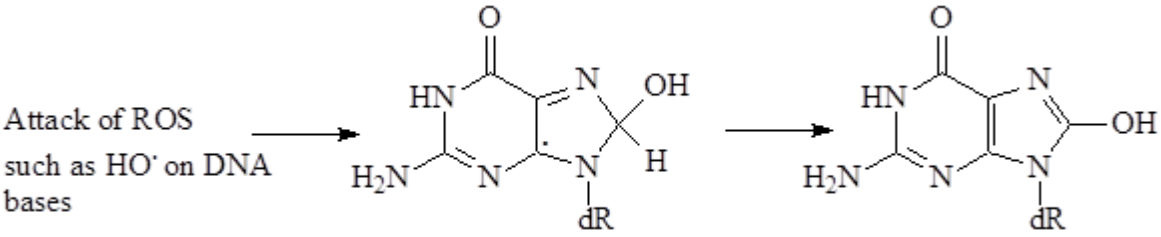
Individual profile/alert	
Name	Polynitroarenes
Type of profile	Structural alert

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)**:**

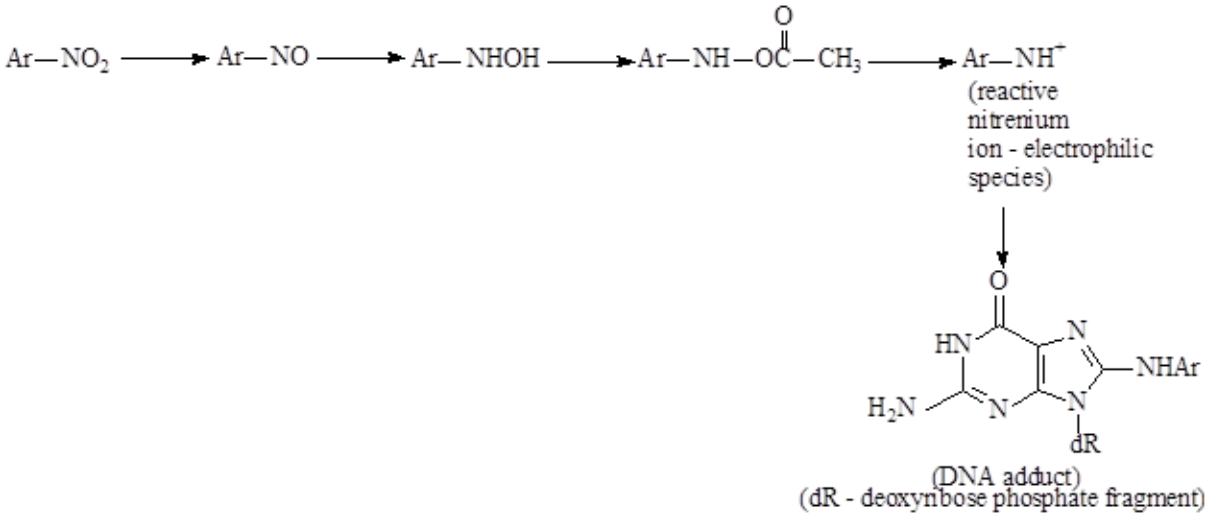


As a result, from the generation of reactive radical species such as $ArNHO^{\cdot}$, an additional formation of ROS such as $O_2^{\cdot-}$ and/or $HO^{\cdot}$ occurs. The hydroxyl radical, for example, is DNA-reactive and adducts, involving pyrimidine and purine nucleoside bases can be formed. The 8-hydroxyguanine 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]:



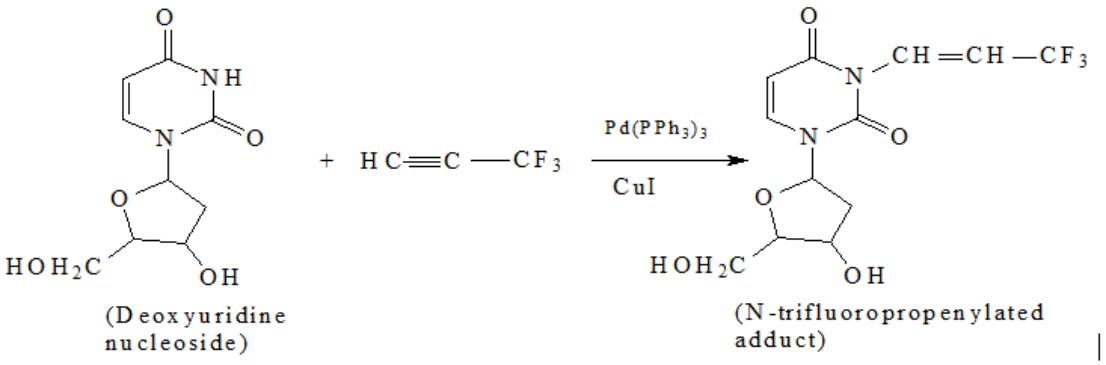
Chemicals such as 2,6-dinitrotoluene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene, etc., containing more than one nitro group were found to be bacterial mutagens both in the presence and the absence of S9 mix [4].

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:

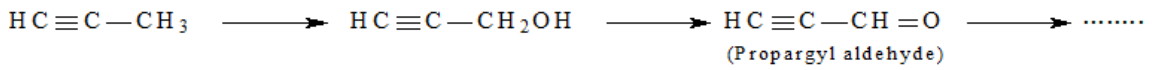


(Scheme 1)

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.

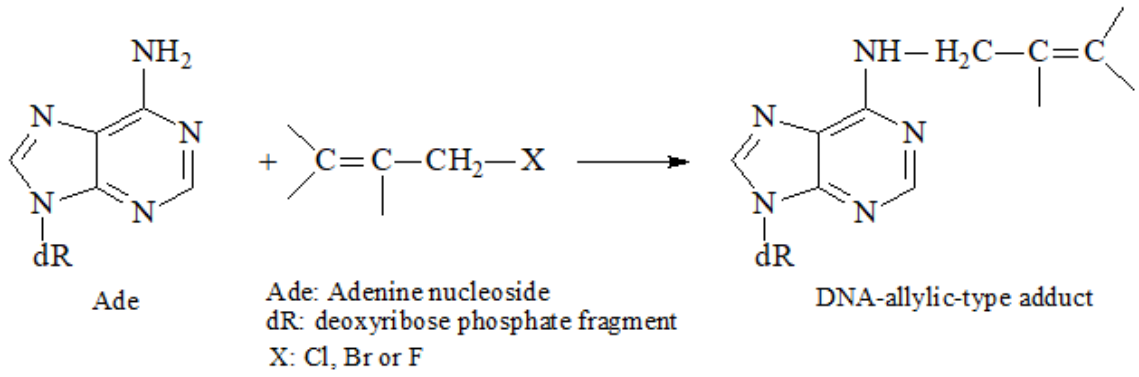
After microsomal/S9 metabolic activation, propyne may be converted into propargyl aldehyde by the following scheme:



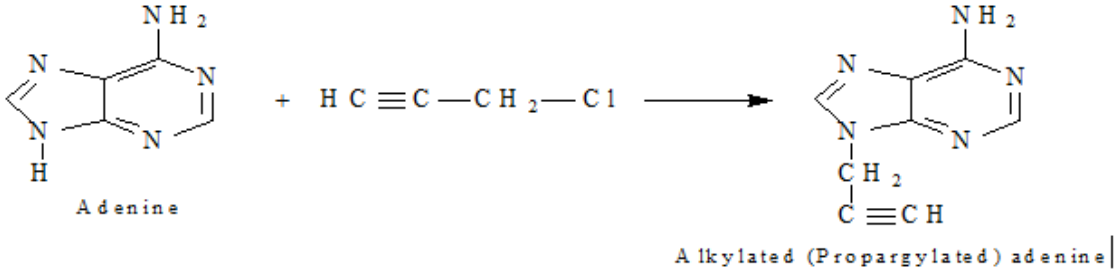
(Scheme 2)

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)

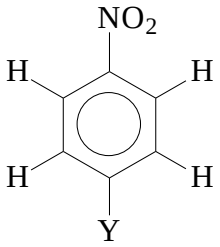


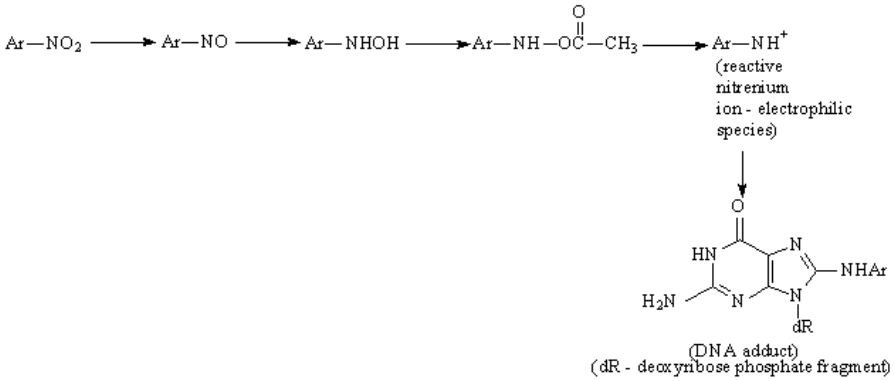
(Scheme 4)

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

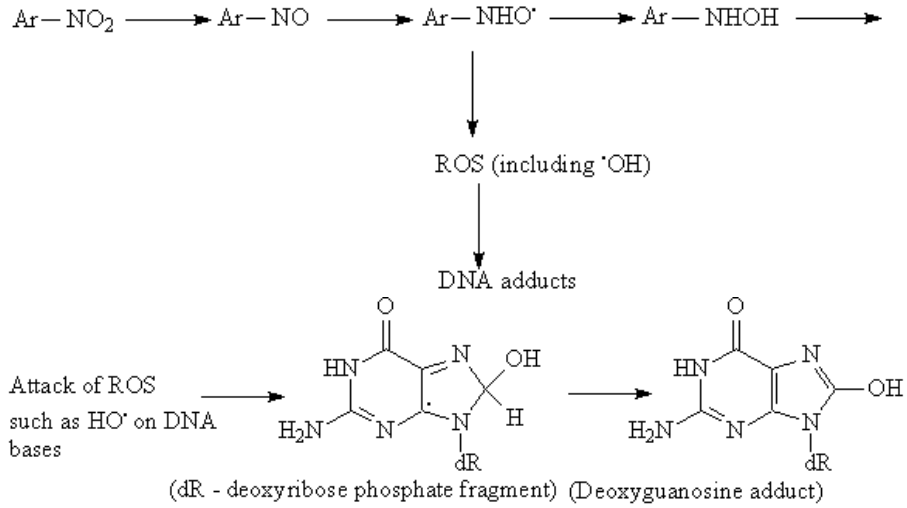
Set of chemicals used for profile development	Propyne Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

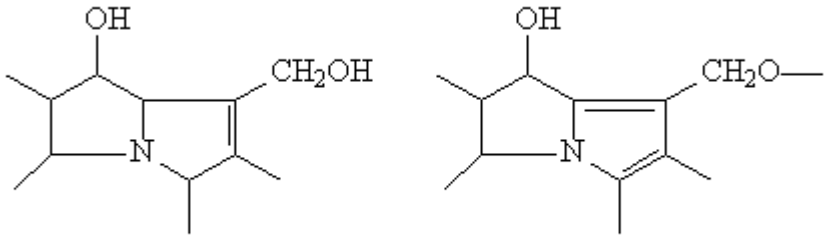


Homolytic

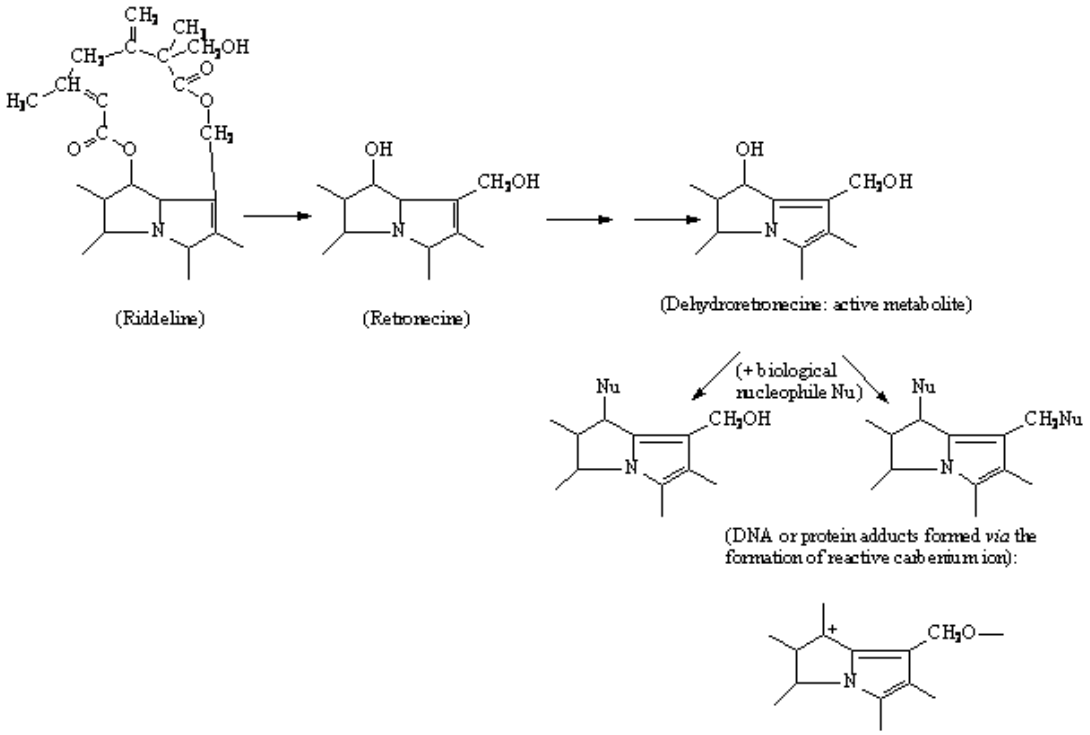


Set of chemicals used for profile development	p-Substituted Mononitrobenzenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

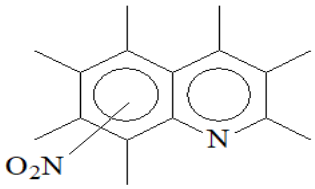
Description/applicability domain	
Mechanism	S_N1 Nucleophilic attack after carbenium ion formation

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

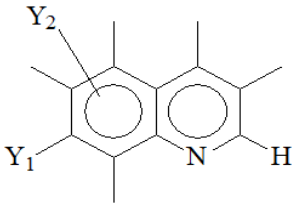


Set of chemicals used for profile development	Pyrrolizidine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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



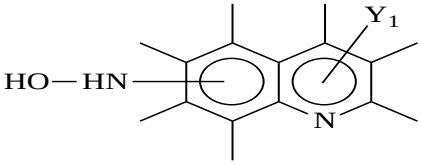
Aminoquinoline Derivatives



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

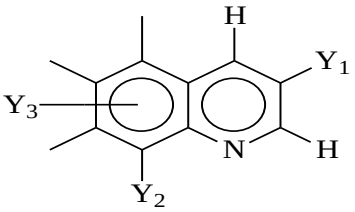
(-NH_2 can be attached to phenyl ring only: one substituent only;
Y_1 is –CH_3 or –C_2H_5 (one substituent only) or –H (all):
can be attached to *any* ring;

N-Hydroxylaminoquinoline Derivatives



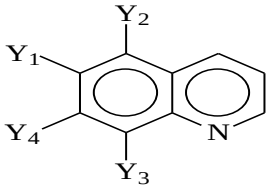
(-NHOH can be attached to any ring: one substituent only;
Y1 is -CH3 or –C2H5 (one substituent only) or –H (all):
can be attached to any ring);

Halogenated Quinolinecarboxylic Acids

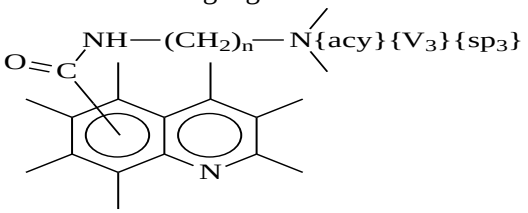
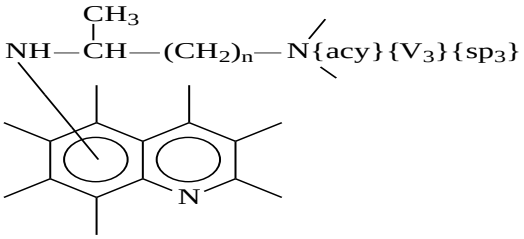
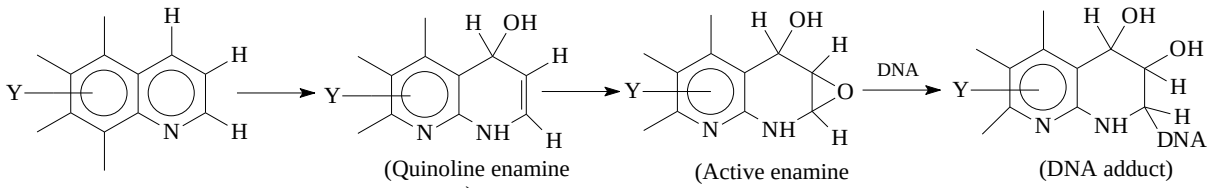
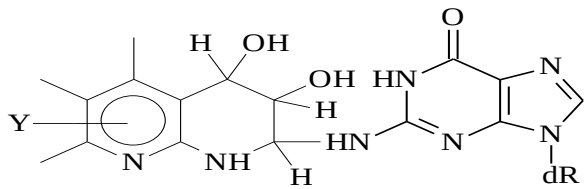


(Y1 is Cl, Br or –COOH;
Y2 is –COOH or –CH3 or -C2H5;
Y3 is –Cl or –Br (number of halogens 1 or 2);
No more than totally four substituents)

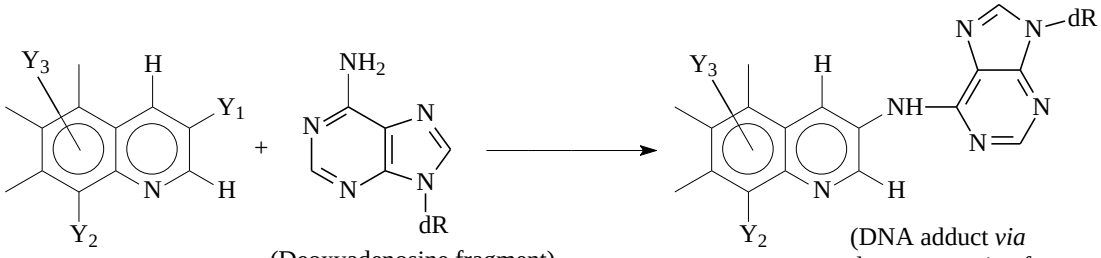
Other Quinoline Derivatives



(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)

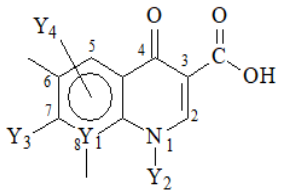
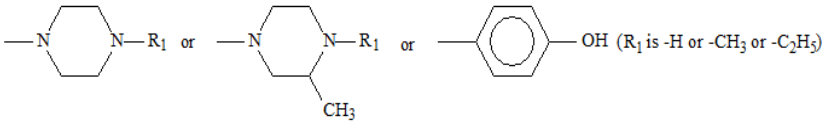
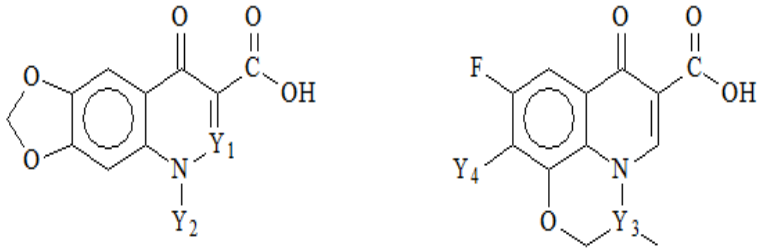
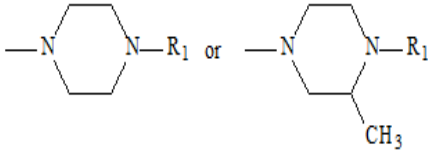
	No more than one substituent (-F or -Cl or –OH) DNA Intercalating Agents  (Carboxamide side chain attached to phenyl ring; n = 1 – 3; No more than totally two substituents)  (Aminoalkylamine side chain can be attached to any of the rings; n = 1 – 3; No more than totally two substituents) (see also DNA 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):

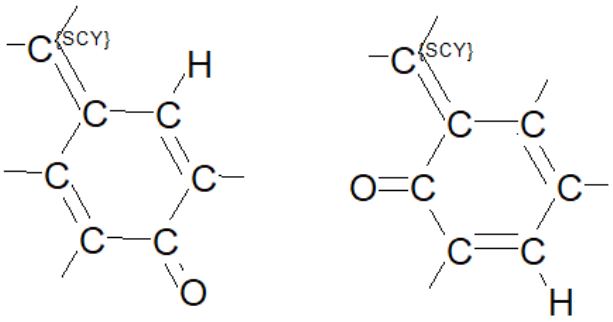


(Y_1 is Cl, Br or -COOH;
Y_2 is -COOH or $-CH_3$ or $-C_2H_5$;
Y_3 is -Cl or -Br (number of halogens 1 or 2);
No more than totally four substituents)

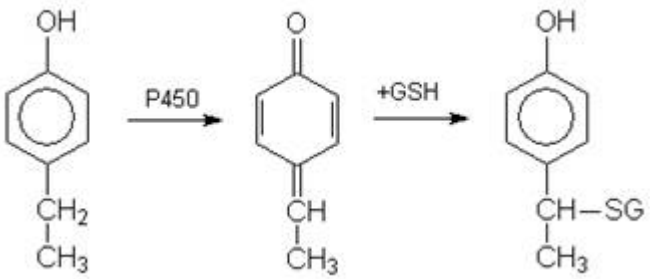
Set of chemicals used for profile development	Quinoline Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

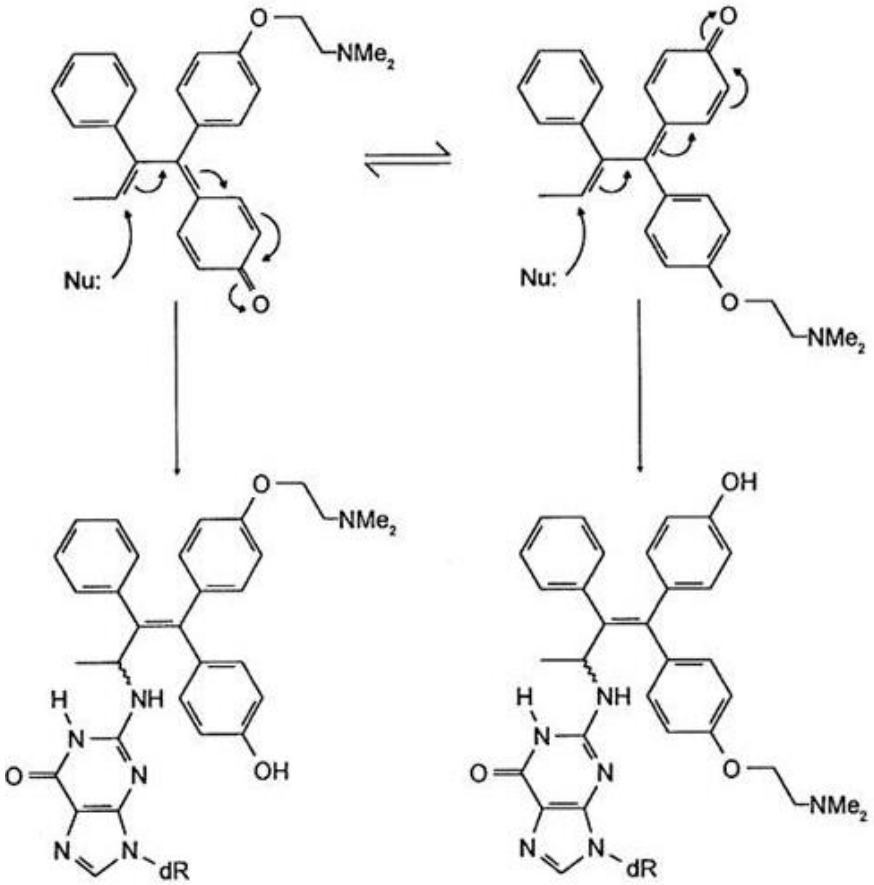
Individual profile/alert	
Name	Quinone methides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	Radical ROS formation after GSH depletion & AN2 Michael addition Quinone type compounds

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]:

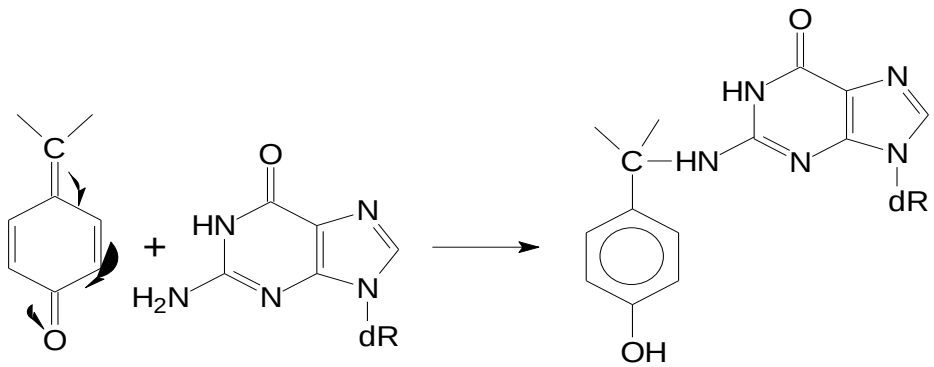


Tamoxifen is a liver carcinogen in rats and has been shown to increase the risk of specific cancer in women. One of the proposed pathways for the metabolic activation of tamoxifen involves oxidation to

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]:



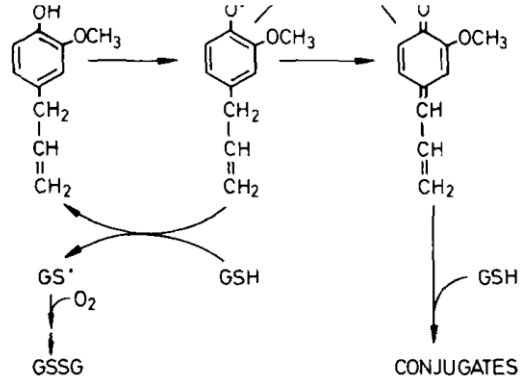
Therefore, based on the above data, the following general scheme of DNA reactivity, and the resulting mutagenicity effects of quinone methide structural fragments can be assumed:



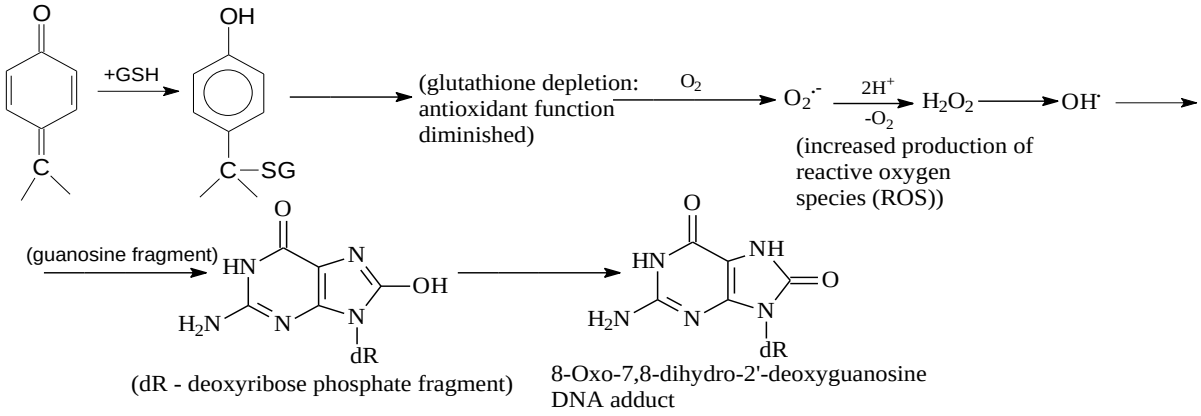
where dR represents desoxyribose fragment.

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

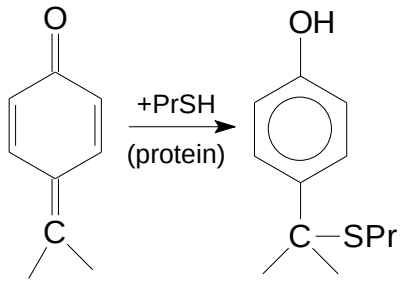
basil, cinnamon and nutmeg. The genotoxicity of eugenol in V79 cells was evaluated with respect to chromosomal aberration effects. Eugenol was found to induce chromosomal aberration to a significant degree, and S9 liver fraction increased this effect in a dose-dependent manner. The results demonstrated 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:



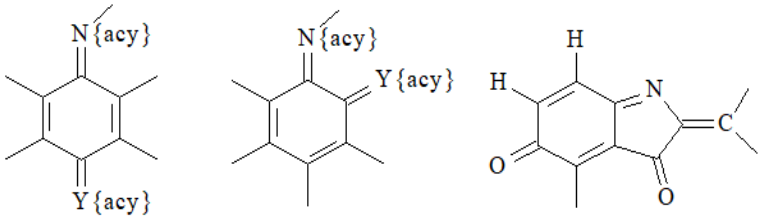
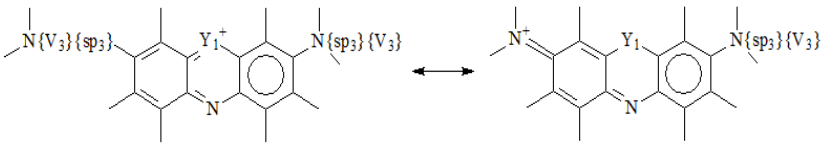
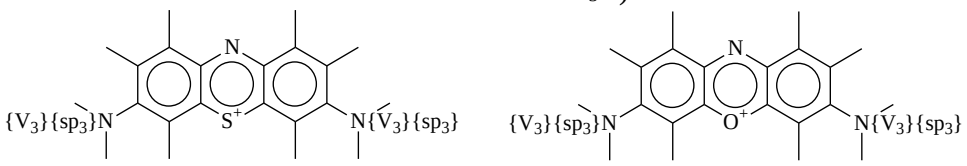
Formation of topoisomerase II inhibition complex, contributing to the chromosomal aberration *via* attack of quinone methide metabolite on the thiol functional groups of cysteine fragments in a protein (enzyme) in a similar mode as that of glutathione conjugation showed above cannot be excluded [7]:

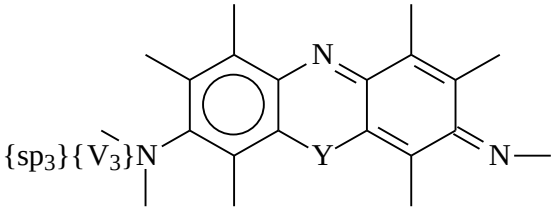


Consequently, it can be assumed that quinone methide intermediates formed during the metabolism of various chemicals can cause both the mutagenicity and chromosome aberration effects.

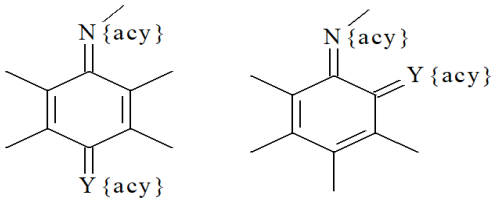
Set of chemicals used for	Quinone Methides

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. 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.

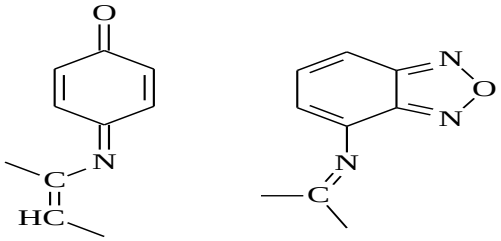
Individual profile/alert	
Name	Quinoneimine, Thione and Phenoxazinium Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is O or N{V3}); {acy}: acyclic atom (No more than one *additional* substituent on the six-membered ring; (in case of $–CH_3$ and/or $–C_2H_5$ the number of *additional* substituents should be no more than two); No halogens (F, Cl, Br, I) or –OC{sp3} substituent(s) attached; General “mask”: $-SO_3H$)  (Thionine and phenoxazinium derivatives) (Y_1 is S or O) (No more than one *additional* substituent attached; General “mask”: $-SO_3H$) 



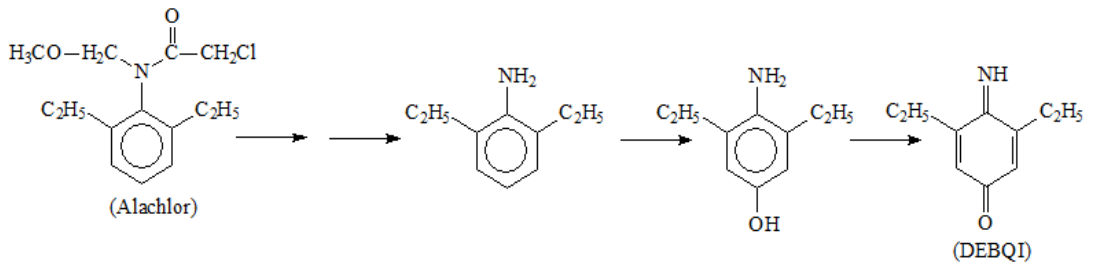
(Thionine and Phenoxazine Derivatives)
(Y is S or O)
(No more than one additional substituent attached;
General “mask”: -SO3H)

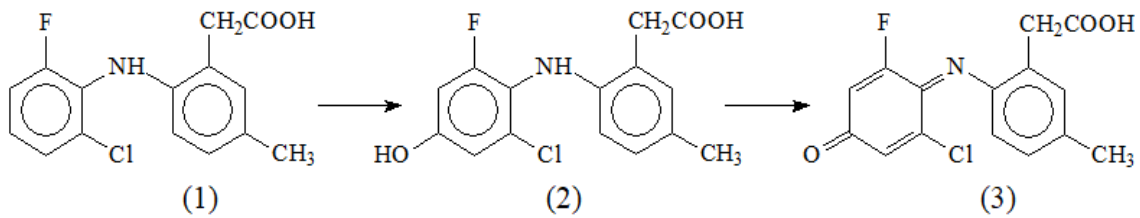


Quinoneimine Derivatives)
(Y is O or N{V3});
{acy}: acyclic atom
(No more than one additional substituent on the six-membered ring;
(in case of –CH3 and/or –C2H5 the number of additional substituents should be no more than two);
No halogens (F, Cl, Br, I) or –OC{sp3} substituent(s) attached;
General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



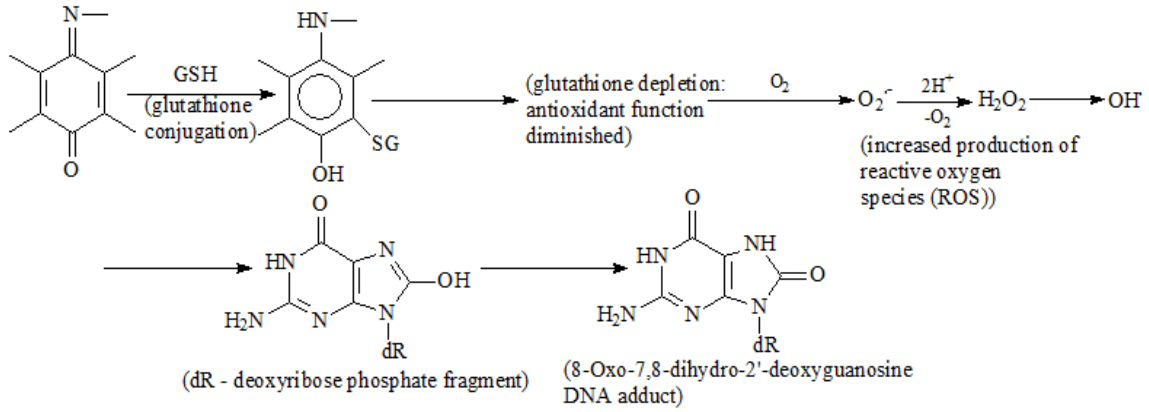
Mechanism	Radical ROS formation after GSH depletion (indirect), A_N2 Michael-type addition, quinoid structures & Non-covalent interactions DNA intercalation



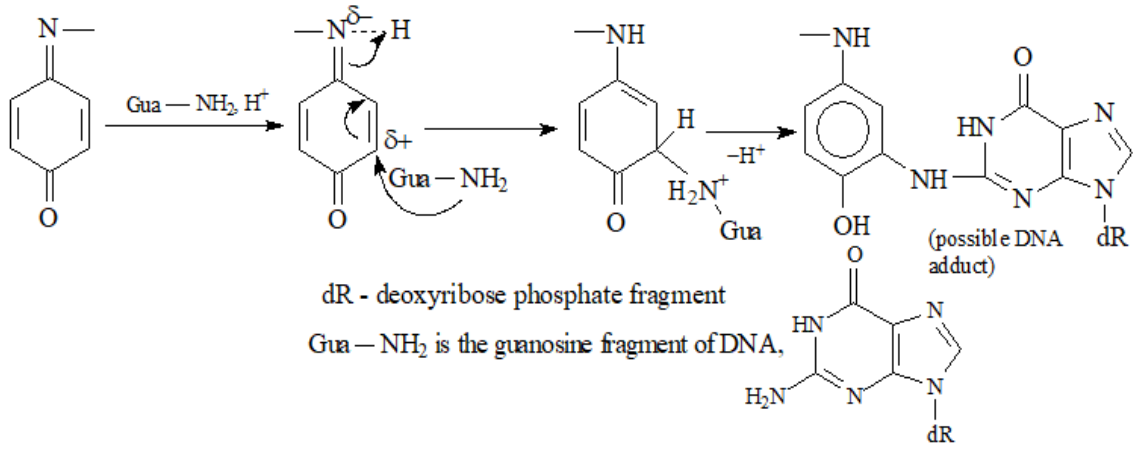


I. **<u>Generation of reactive oxygen species (ROS).</u>** It may be caused by an interaction with protein (enzyme) thiols or glutathione in the microsomal metabolic activation system. This mechanistic

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:



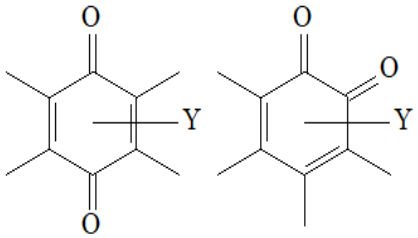
II. **Michael-type addition mechanism.** Such a scheme is regarded as less plausible, since it is based on the direct interaction of "soft" electrophile (quinoneimine derivative) with "hard" nucleophile (DNA base):

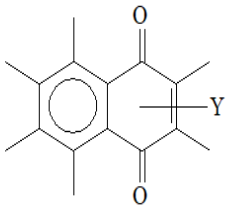


III. **DNA intercalation between DNA base pairs:** This mode of action could be associated with non-covalent interactions, due to the polycyclic planar structure of thionine and phenoxazinium derivatives, and their positively-charged resonance structures.

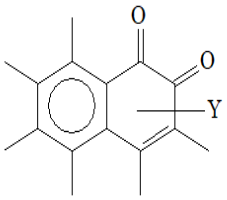
Set of chemicals used for profile development	Quinoneimine, Thionine and Phenoxazinium Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to 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:



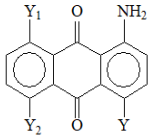
1,4-Naphthoquinones



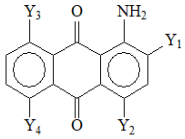
1,2-Naphthoquinones

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

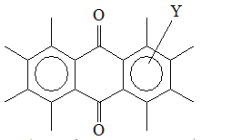
Anthraquinone Derivatives:



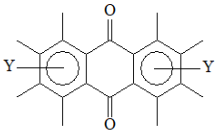
(Y can be -OH or -NH; Y_1, Y_2 can be -OH, -NH_2 or -H)



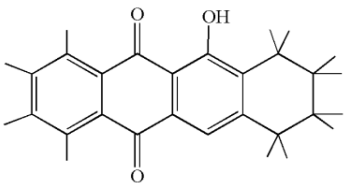
(Y_1 can be -Cl, -Br, -COOH, -OH, -OCH_2 or -NH_2); Y_2 can be Cl or Br or -H; Y_3, Y_4 can be -OH, -NH_2 or -H)

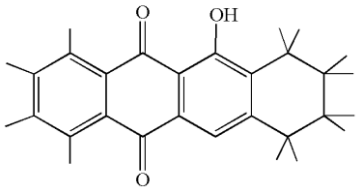


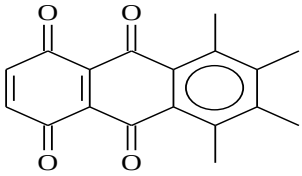
(Y can be —NO_2 —$N^+{\equiv}N$ —N=NH N{V_3}—N{V_3}; could be anywhere)

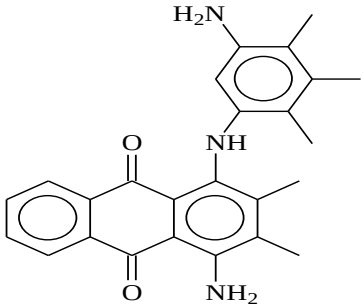


(Y is -OH or -CH_2OH or -H or -O-C{sp3} or -C(C{sp3})$_3$ or C{scy}{sp3} or -$NHCH_3$ or -$NHCH_2OH$ or -NHC_2H_5 or -$NHCH_2CH_2OH$ or -NH-$C(O)$-C_6H_5 or combinations)

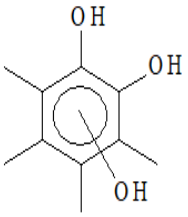
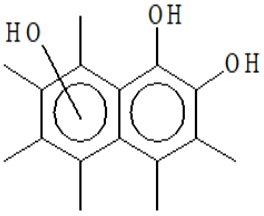




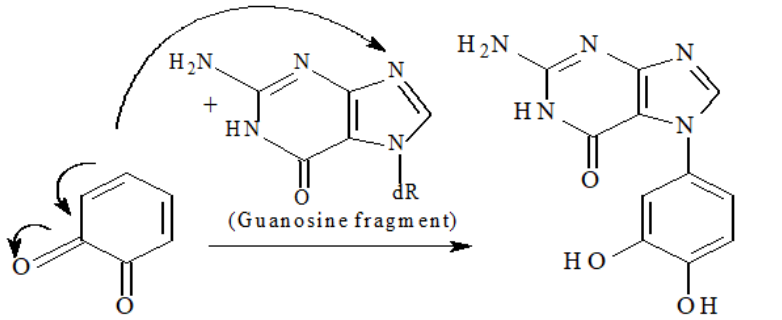


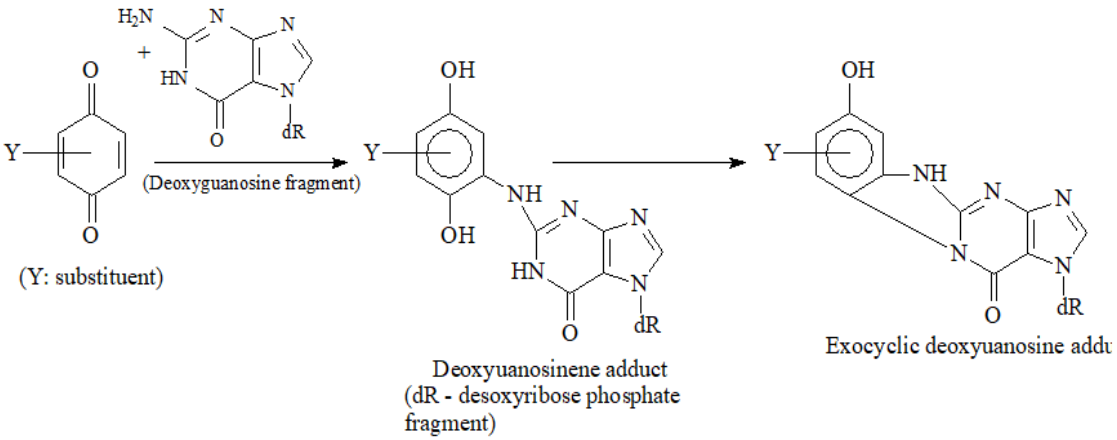


Trihydroxybenzenes:

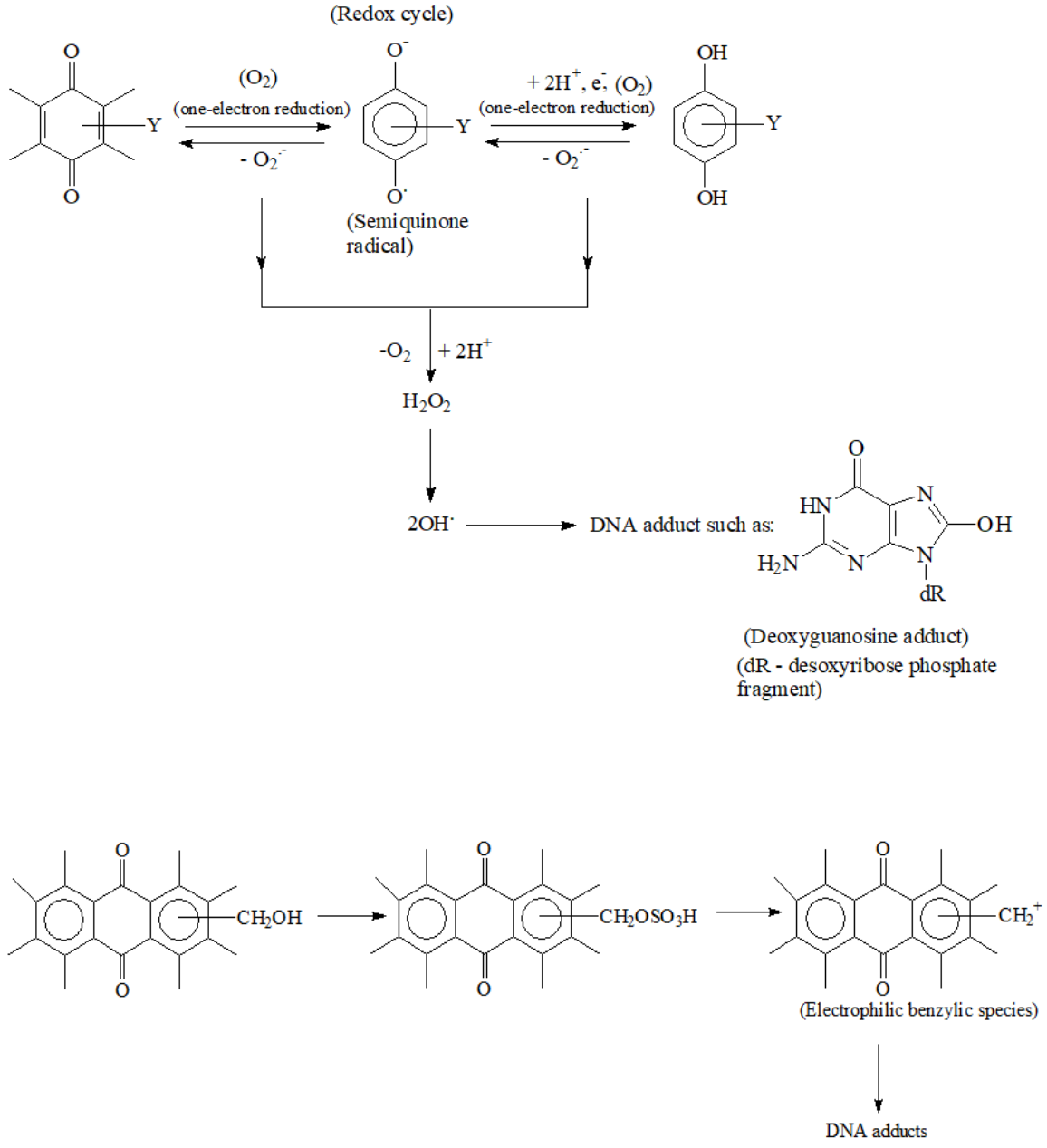
	  (Other possible substituents: -H, -CH_3,-OCH_3, -NH_2; No substituents other than these)
Mechanism	A_N2 Michael-type addition, quinoid structures , Radical ROS generation (indirect) & Non-covalent interactions DNA intercalation SN1 Nucleophilic attack after carbenium ion formation

1. Electrophilic mechanism for simple quinones and naphthoquinones:





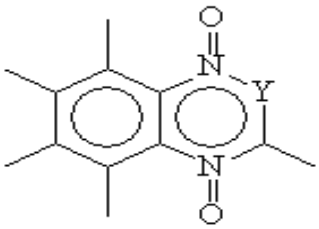
2. Radical mechanism for simple quinones, naphthoquinones, anthraquinone derivatives and trihydroxybenzenes



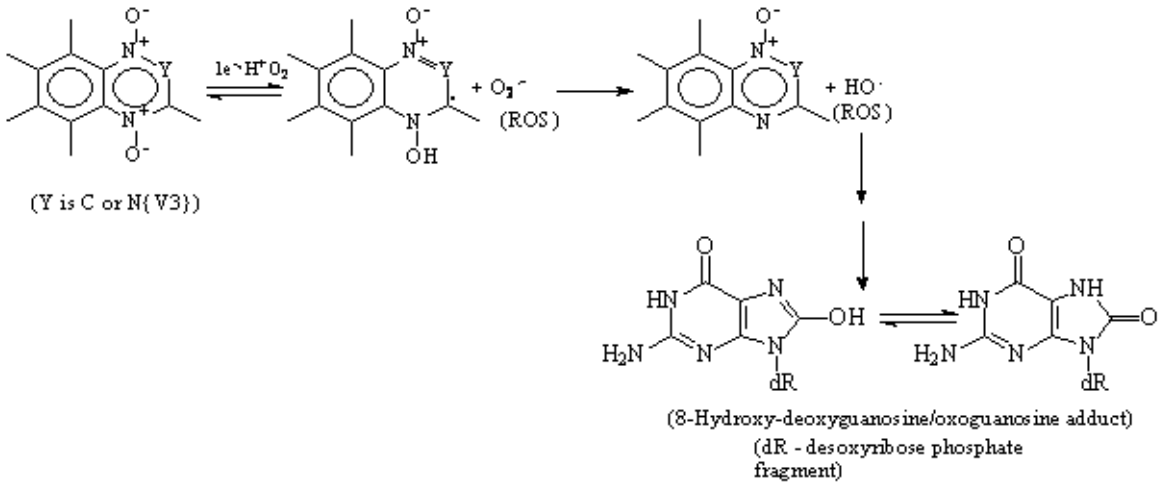
Set of chemicals used for profile development	Quinones and Trihydroxybenzenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

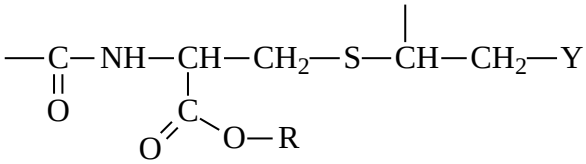
Individual profile/alert	
Name	Quinoxaline-Type 1,4-Dioxides
Type of profile	Structural alert

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]:



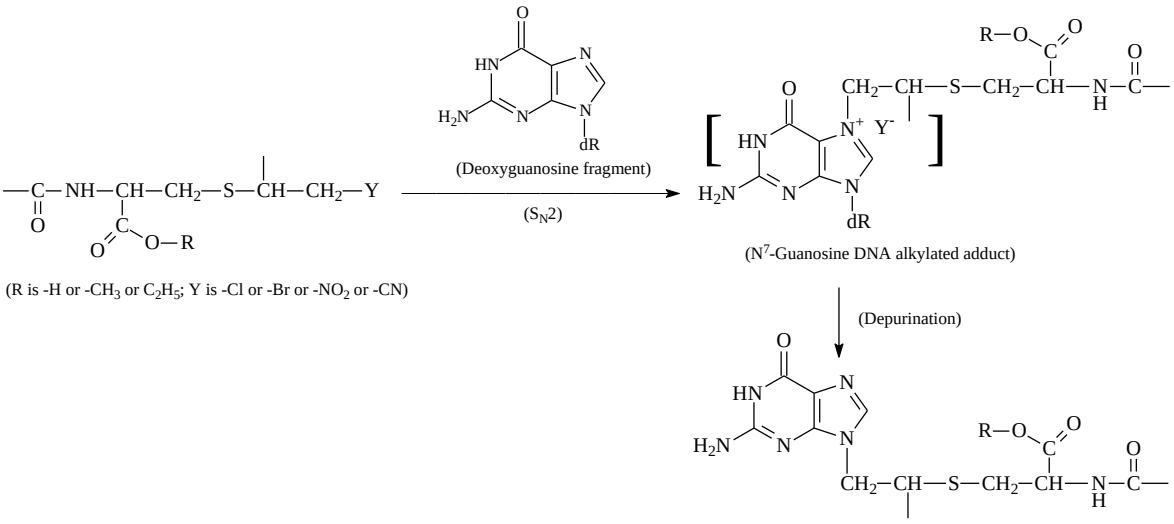
Set of chemicals used for profile development	Quinoxaline-Type 1,4-Dioxides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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

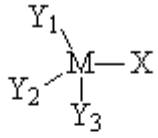
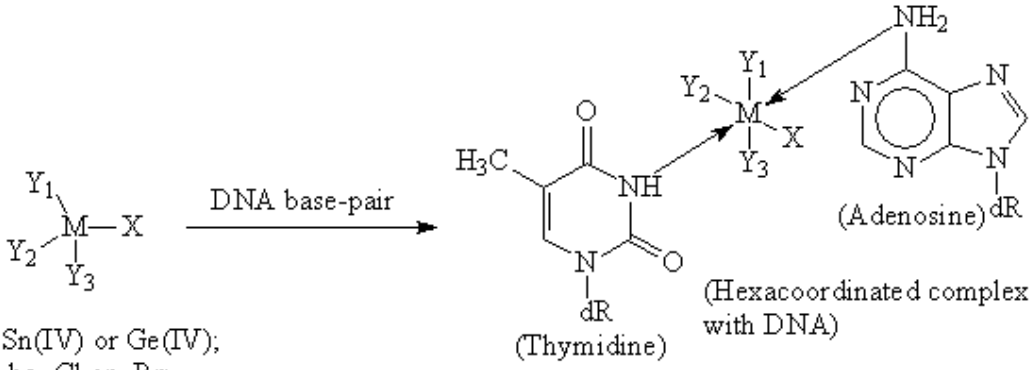
Newly synthesized S-haloethyl conjugates of cysteine and glutathione, as well as selected methyl ester and N-acetyl derivatives were tested for their ability to act as direct mutagens in Salmonella typhimurium TA98 and TAl00. In the strain TA100, where mutation of a specific guanine by base-pair substitution 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:

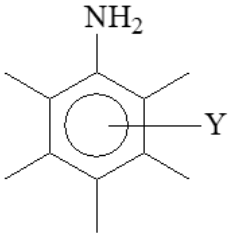
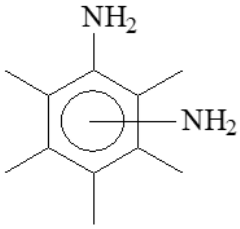
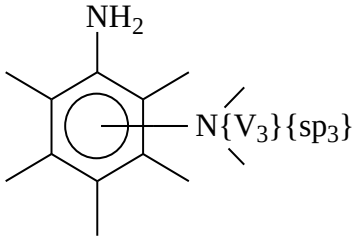
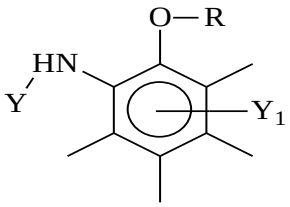
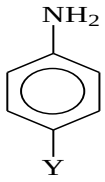
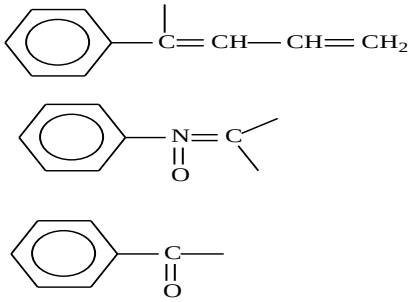
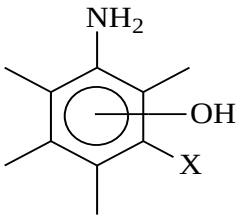


Set of chemicals used for profile development	S-Activated Cysteine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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.

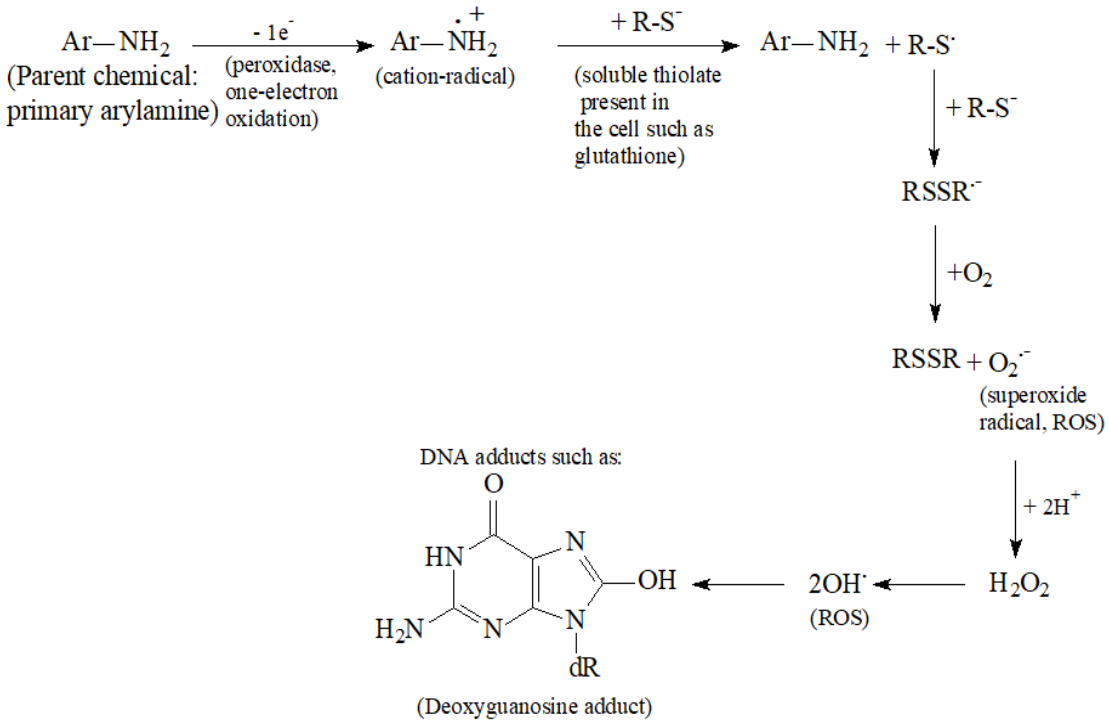
Individual profile/alert	
Name	Short-Chain Alkytin and Alkylgermanium Halides

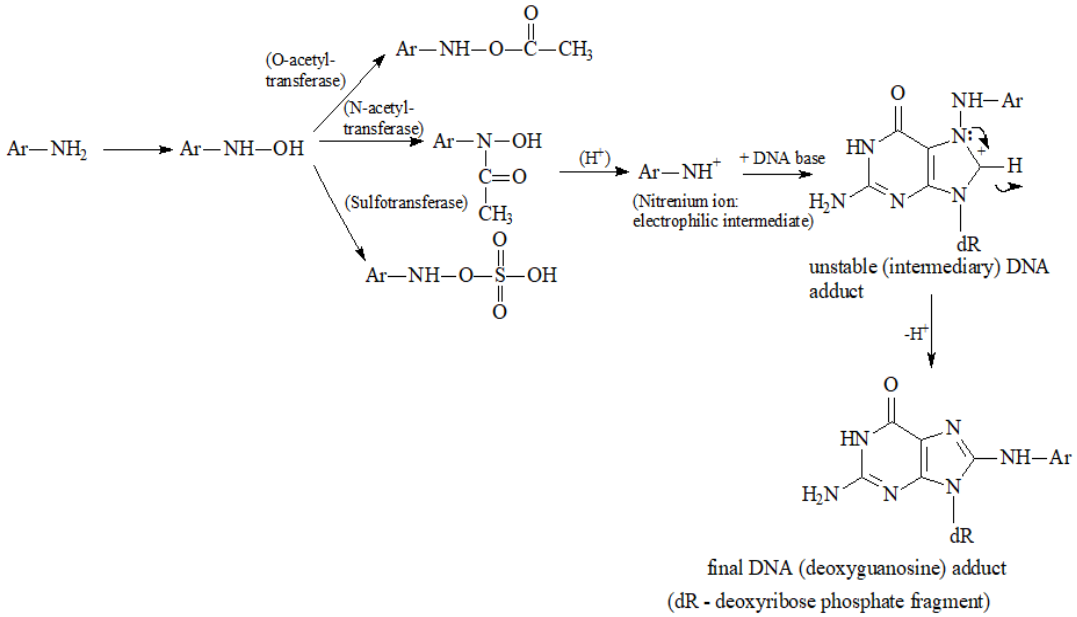
Type of profile	Structural alert
Description/applicability domain	 (M is Sn(IV) or Ge(IV); X can be -Cl or -Br; Y_1, Y_2 can be -Cl or -Br or $-(CH_2)_nH$ (n = 1 - 4) Y_3 can be $-(CH_2)_nH$ (n = 1 - 4))
Mechanism	S_N2 Coordination with nucleoside bases
	 (M is Sn(IV) or Ge(IV); X can be -Cl or -Br; 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	Short-Chain Alkyltin and Alkylgermanium 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. 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.

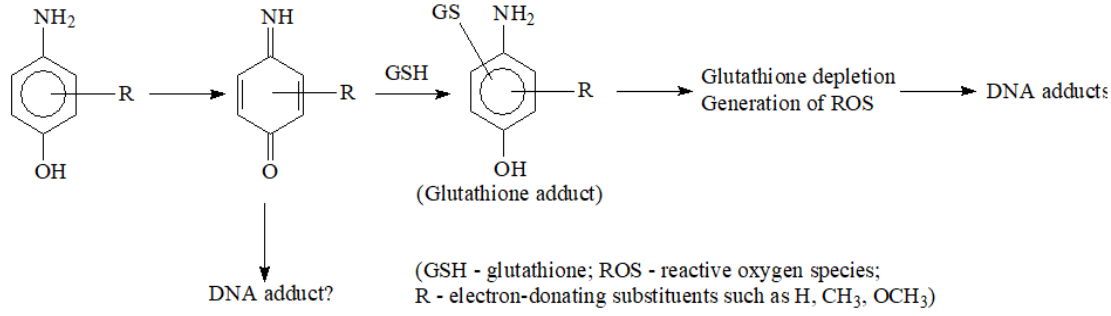
Individual profile/alert	
Name	Single-Ring Substituted Primary Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	

	 (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

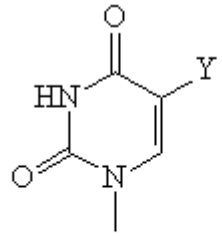


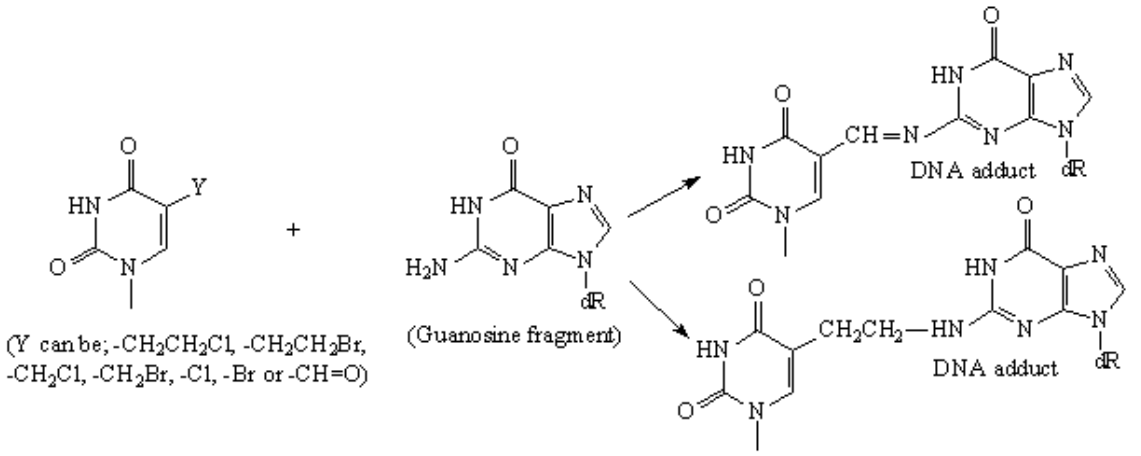




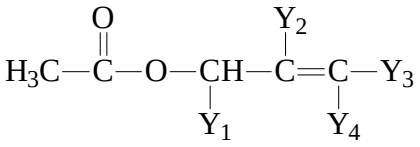
Set of chemicals used for profile development	Single-Ring Substituted Primary Aromatic Amines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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.

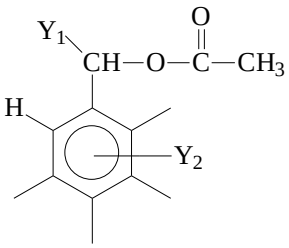
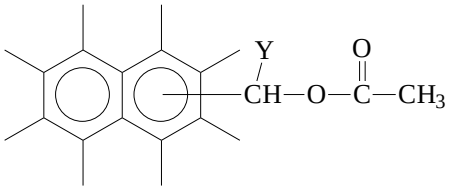
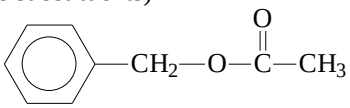
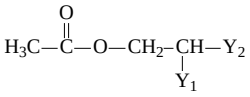
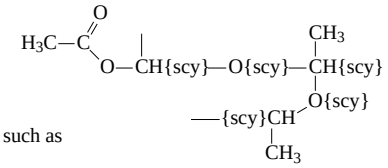
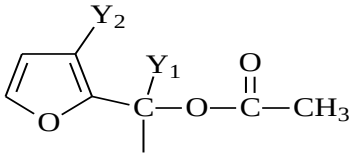
	15. Nitrenium Ion; https://www.wikidoc.org/index.php/Nitrenium_ion. Last visited: June, 2021. 16. Guengerich, F. P., A. Parikh, E. F. Johnson, T. H. Richardson, C. von Wachenfeldt, J. Cosme, Fr. Jung, C. P. Strassburg, M. P. Mannis, R. H. Tukey, M. Prichard, S. Fournel-Gigleux, Br. Burchell, *Heterologous 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.

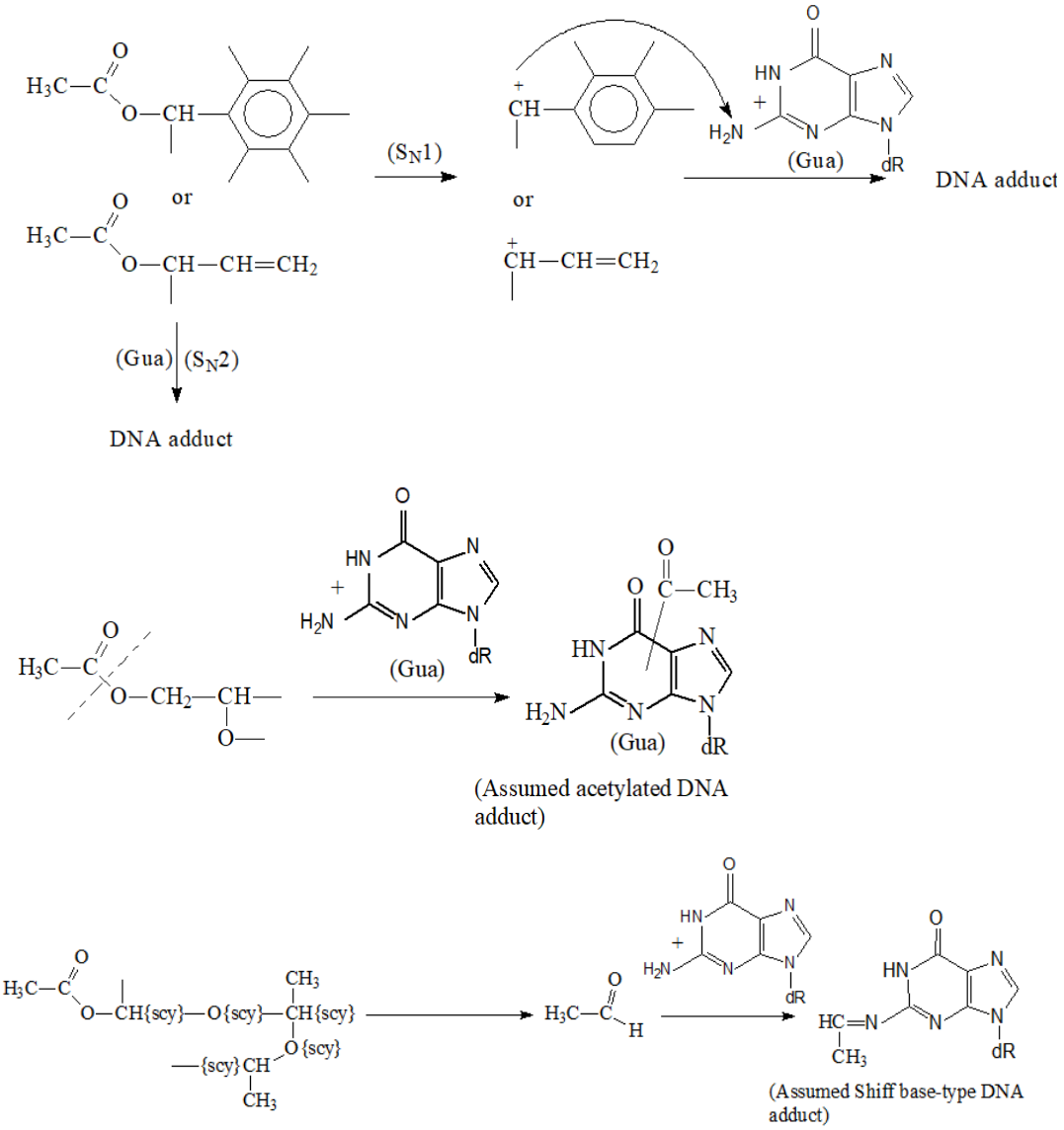
Individual profile/alert	
Name	Specific 5-Substituted Uracil Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y can be; -CH_2CH_2Cl, -CH_2CH_2Br, -CH_2Cl, -CH_2Br, -Cl, -Br or -CH=O)
Mechanism	A_N2 Shiff base formation, S_N2 Alkylation, nucleophilic substitution at sp3-carbon 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.

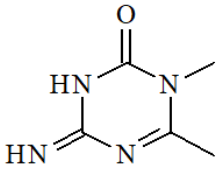
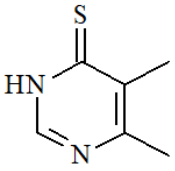
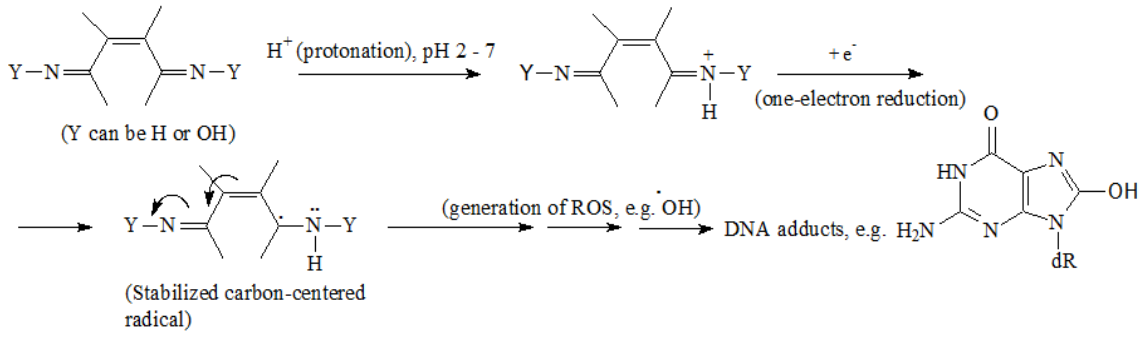
Individual profile/alert	
Name	Specific Acetate Esters
Type of profile	Structural alert
Description/applicability domain	Allyl acetate derivatives  (Y_1: -H or C{ar}; Y_2, Y_3: -H or electron-withdrawing substituents such as -O-, -NO_2, -CN, -C(O)-, -CHO capable of conjugation); Y_4: -H or -C: number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

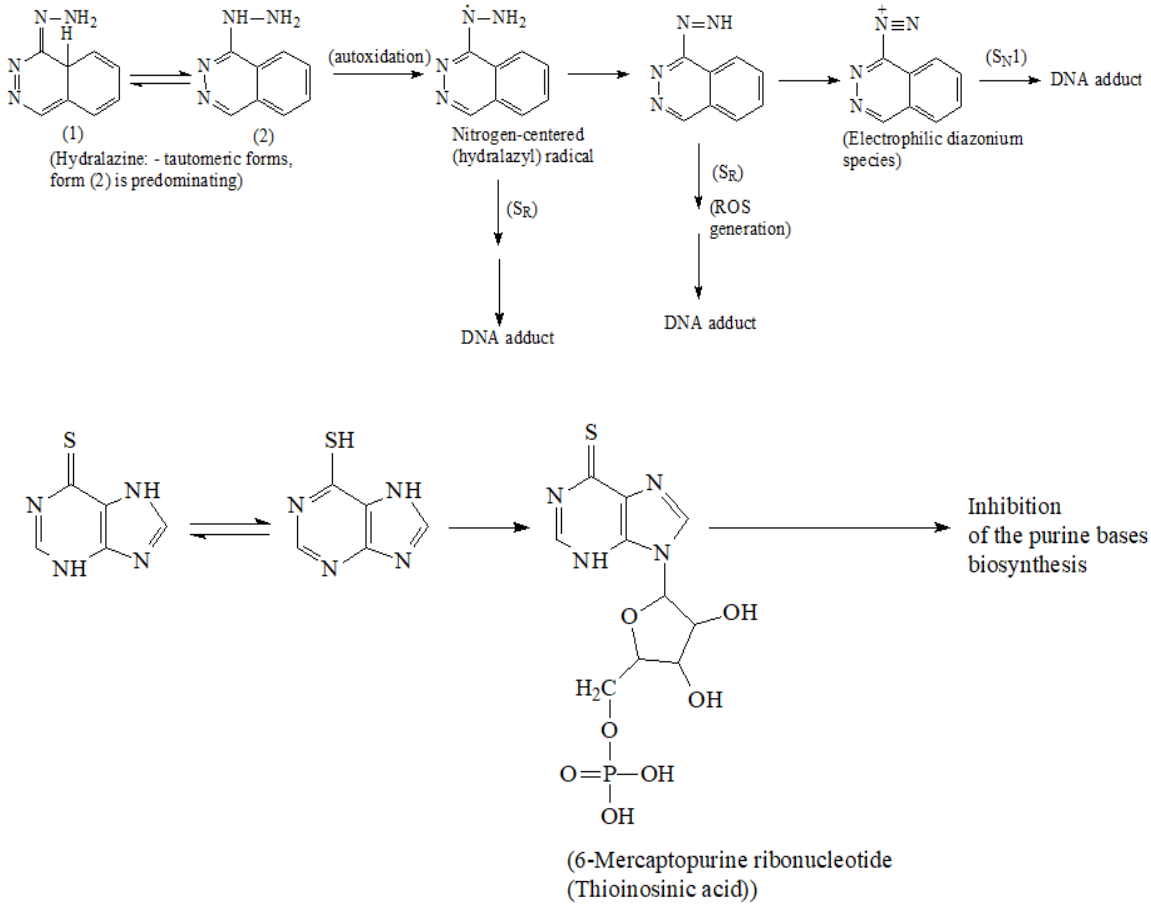
	 (Single-ring, Y_1: -H or C=C; Y_2: electron-withdrawing substituents such as -O-, $-NO_2$, -CN, -C=O, -CHO, -OC=O); no more than three substituents)  (Fused-ring polycyclic derivative; Y can be -H or $-CH_3$) "Masks": $-SO_3H$ (general);  Other specific acetate esters  (Y_1 and Y_2 can be OH and $-CH_2OH$ 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*

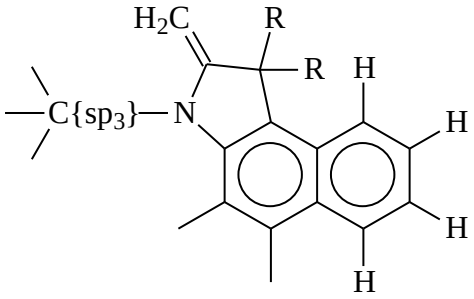
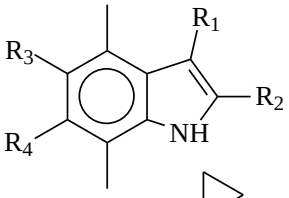
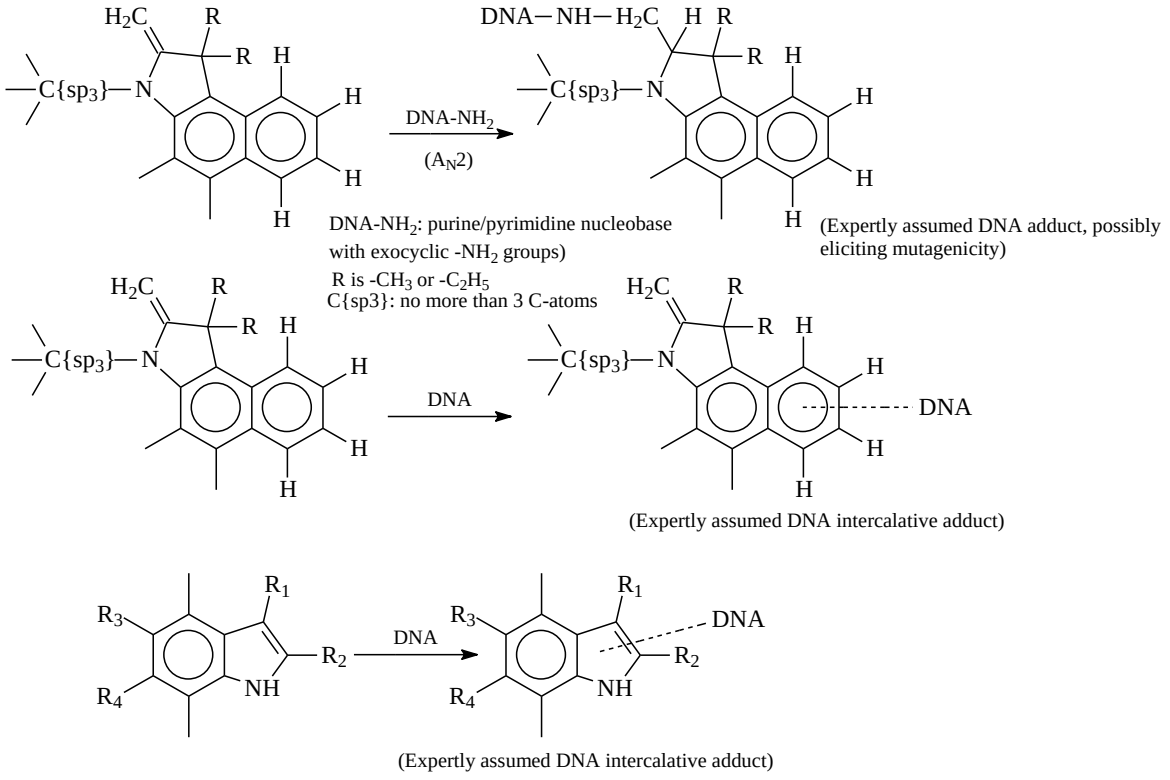
	(CCRIS); https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=26446-35-5. Last visited: June, 2021.

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
	

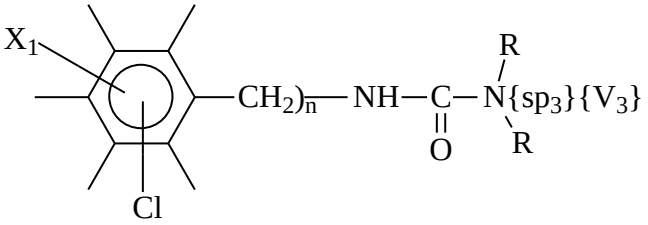


Set of chemicals used for profile development	Specific Imine and Thione Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references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

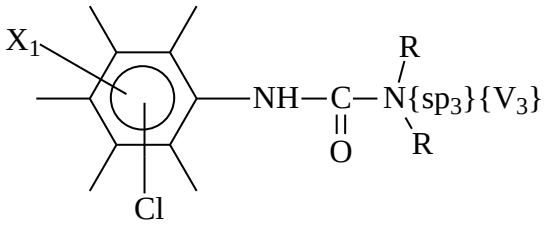
Individual profile/alert	
Name	Substituted Benzoindoline and Indole Derivatives
Type of profile	Structural alert

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) R is $-CH_3$ or $-C_2H_5$ C{sp3}: no more than 3 C-atoms (Expertly assumed DNA adduct, possibly eliciting mutagenicity) (Expertly assumed DNA intercalative adduct) (Expertly assumed DNA intercalative adduct)
Set of chemicals used for profile development	Substituted Benzoindoline and Indole 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. 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.

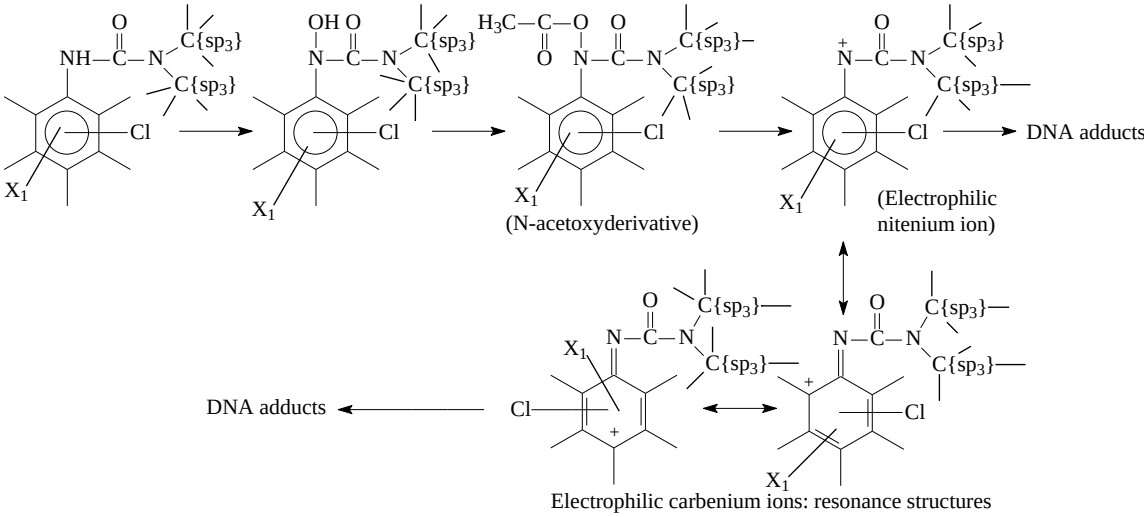
Individual profile/alert	
Name	Substituted Chlorophenylalkylurea Derivatives
Type of profile	Structural alert
Description/applicability domain	 (n = 0 - 1); R is CH_3 (both) or combination of H and C{sp3}; X_1 is Cl or -OCH_3 or -CH_3 or H; No more than two substituents (Cl+X_1)
Mechanism	SN1 Nucleophilic 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:



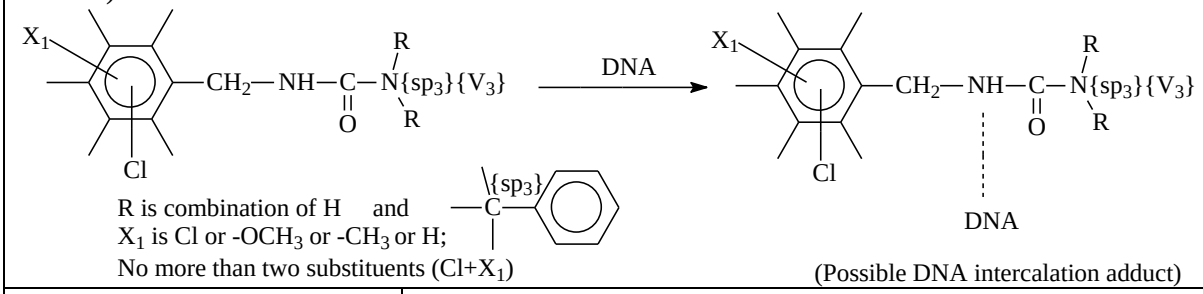
R is CH_3 (both)

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):



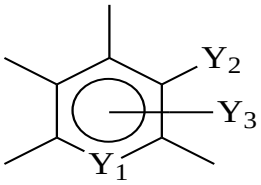
R is combination of H and

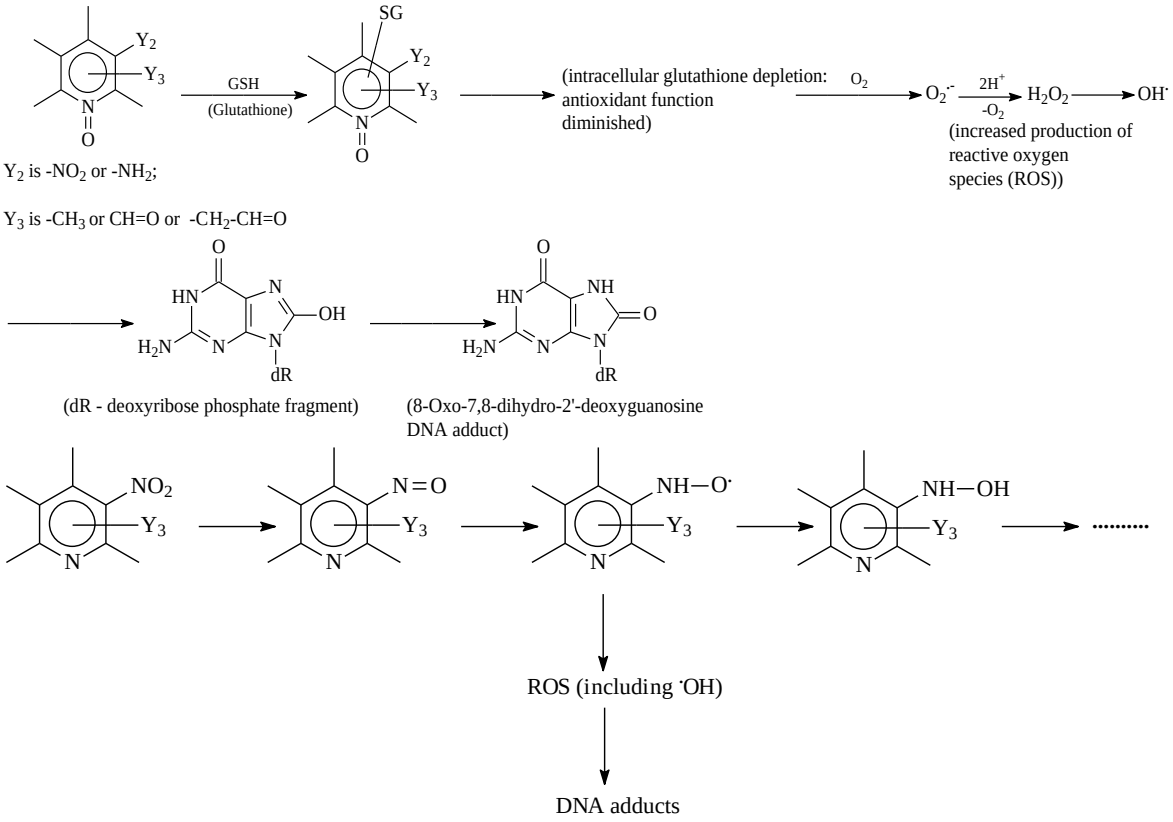
X_1 is Cl or $-OCH_3$ or $-CH_3$ or H;

No more than two substituents (Cl+X_1)

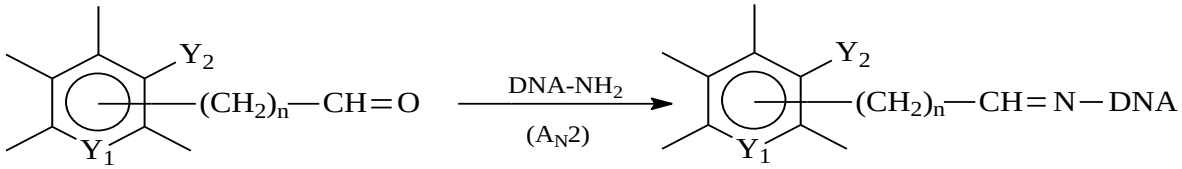
(Possible DNA intercalation adduct)

Set of chemicals used for profile development	Substituted Chlorophenylalkylurea 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. 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:	



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



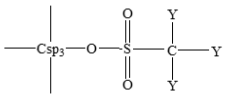
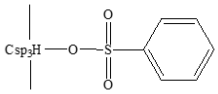
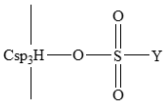
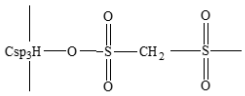
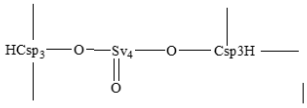
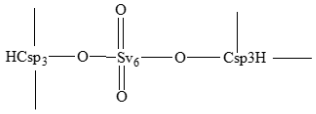
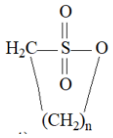
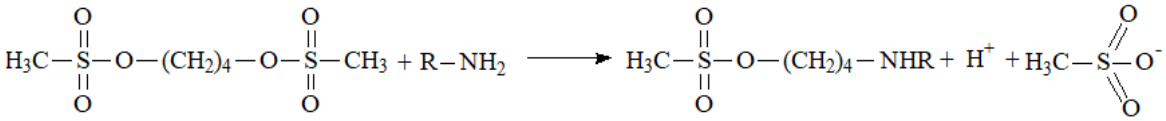
(n = 0- 1)

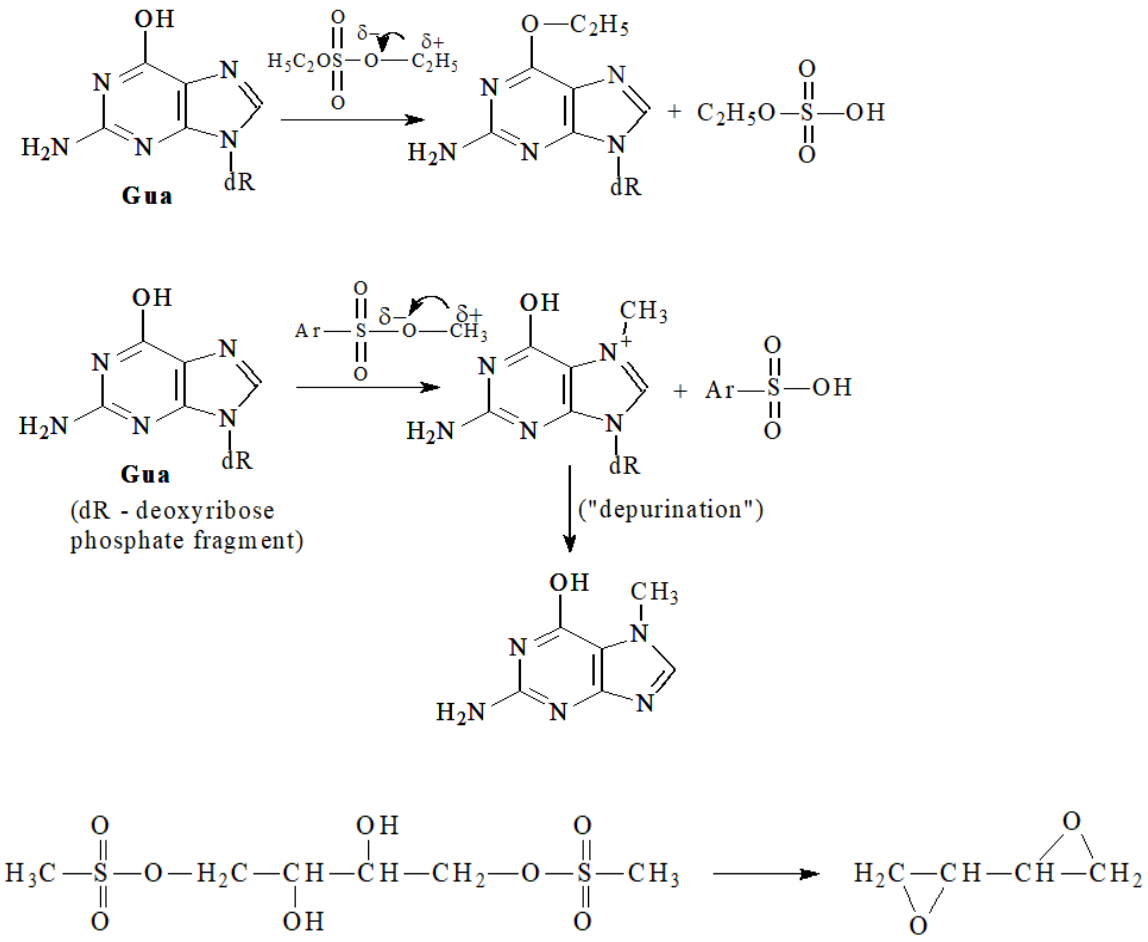
Y_1 is N{ar} or N{ar}=O;

Y_2 is -NO_2 or -NH_2

Set of chemicals used for profile development	Substituted Nitropyridines, Aminopyridines 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. 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.

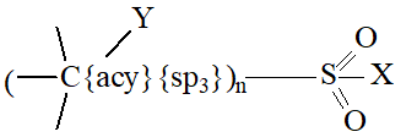
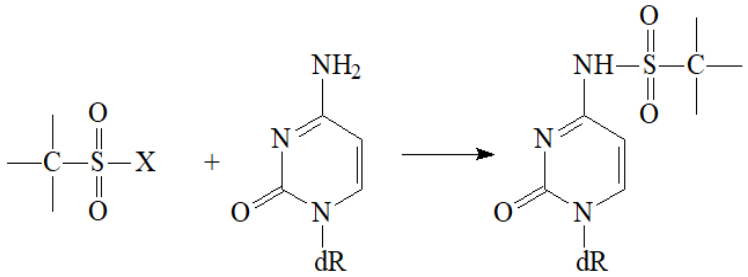
	4. Plošnik A, Vračko M, Sollner Dolenc M. Mutagenic and carcinogenic structural alerts and their mechanisms of action, Arh. Hig. Rada Toksikol 67 (2016), 169 - 182 5. Arima, Y., Ch. Nishigori, T. Takeuchi, Sh. Oka, K. Morimoto, A. Utani, Y. Miyachi, 4-Nitroquinoline 1-Oxide Forms 8-Hydroxydeoxyguanosine 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.

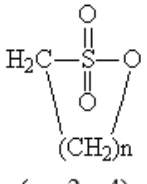
Individual profile/alert	
Name	Sulfonates and Sulfates
Type of profile	Structural alert
Description/applicability domain	 Structure (I) - Y= H, F;  Structure (II) – single aromatic ring only  Structure (III) – Y= -CH_3, -CH_2CH_3;  Structure (IV)  Structure (V)  Structure (VI)  Structure (VII) – n=2-4
Mechanism	S_N2 at sp3-carbon atom (alkylation)
	 (R-NH_2: biological macromolecule (e.g., adenine or guanine fragment in DNA)

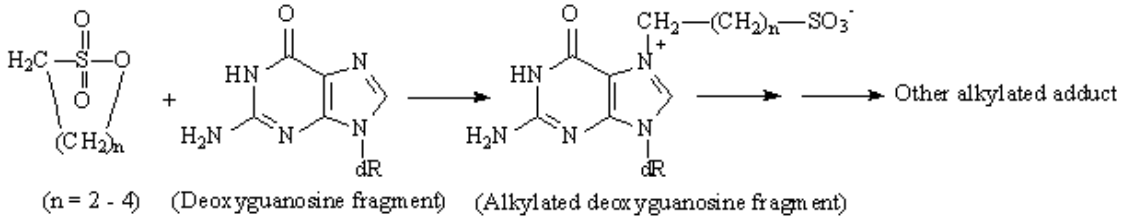


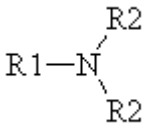
Set of chemicals used for profile development	Sulfonates and Sulfates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

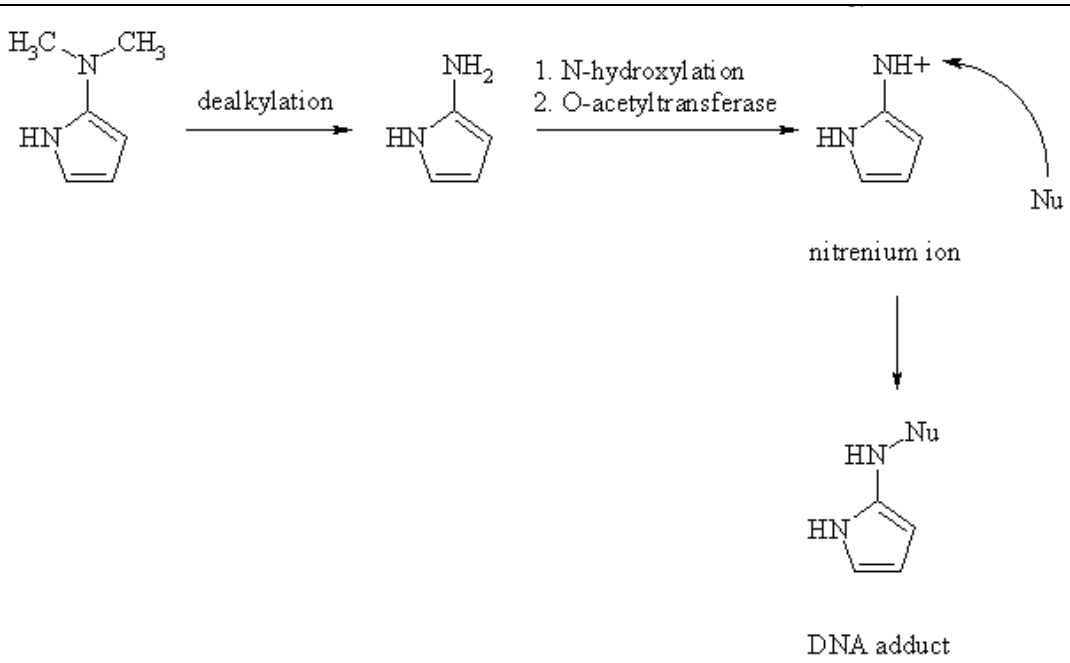
Individual profile/alert

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**
 (Deoxycytidine fragment) (DNA adduct)	
Set of chemicals used for profile development	Sulfonyl 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. 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	 (n = 2 – 4)

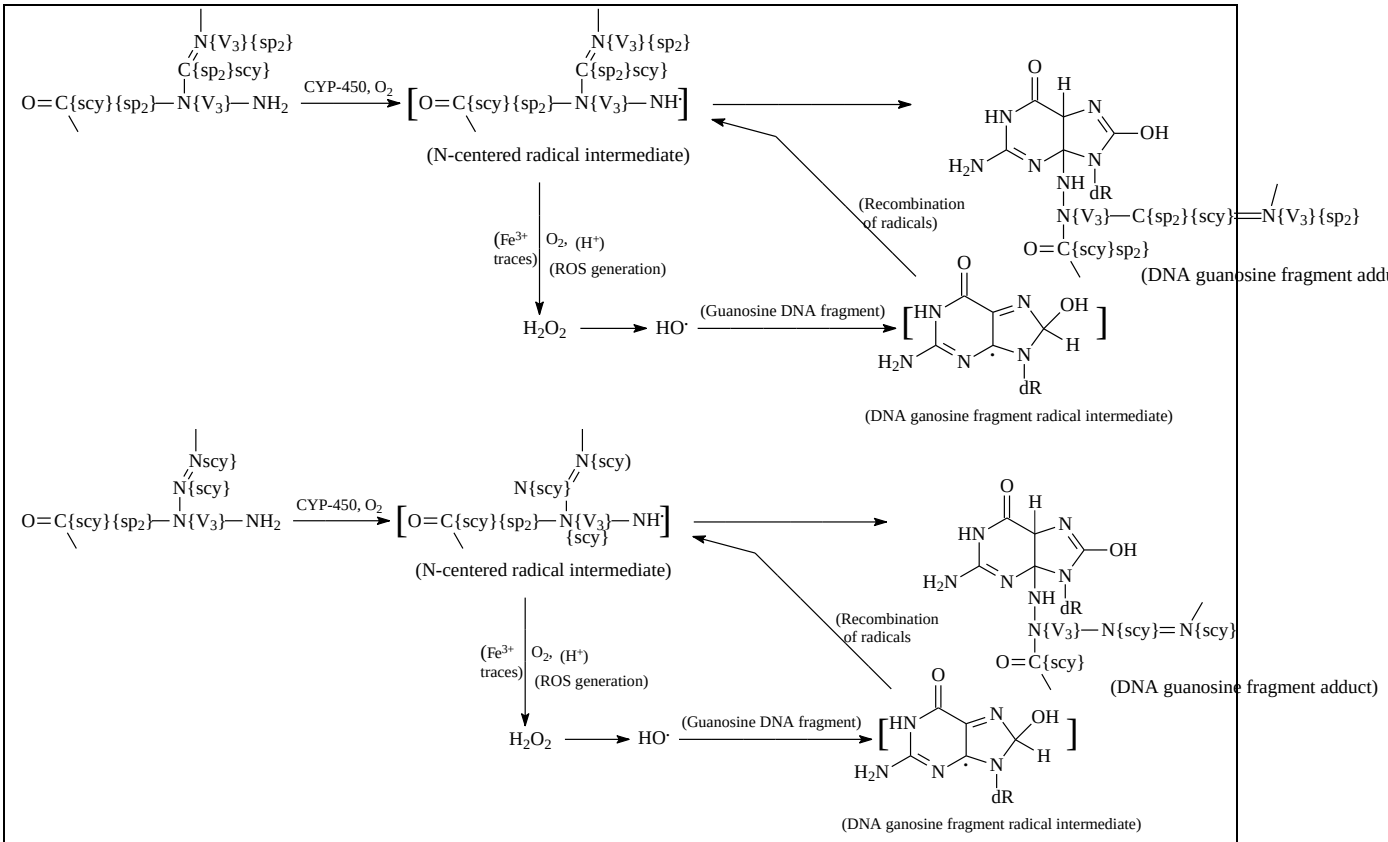
Mechanism	Ring opening S_N2 (alkylation)
DNA-alkylating capability and the *in vitro* genotoxicity of sultones can be expertly suggested:  (n = 2 - 4) (Deoxyguanosine fragment) (Alkylated deoxyguanosine fragment)	
Set of chemicals used for profile development	Sultones
Data/Knowledge used for profile 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).	

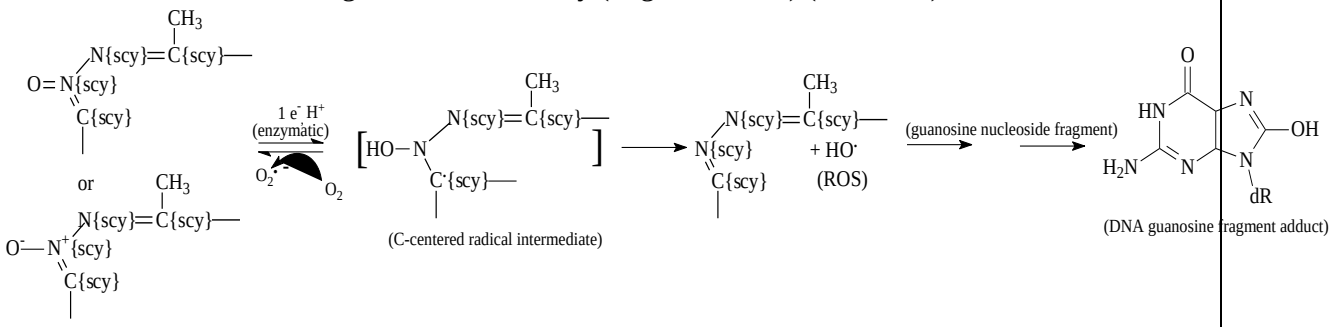


Set of chemicals used for profile development	Not appliciable – all chemicals are private and can`t be disclosed.
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225

Individual profile/alert	
Name	Triazinone N-Oxide and Triazinone Derivatives
Type of profile	Structural alert
Description/applicability domain	(Y is N{V_5}=O or N^+–O^- or —N{V_3}{sp_2}) (Target chemical) (Chemical A)
Mechanism	Mechanistic domain: Radical Mechanistic alert: ROS generation
• Interactions involving the hydrazine moiety:	

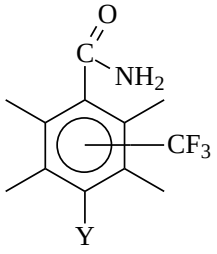
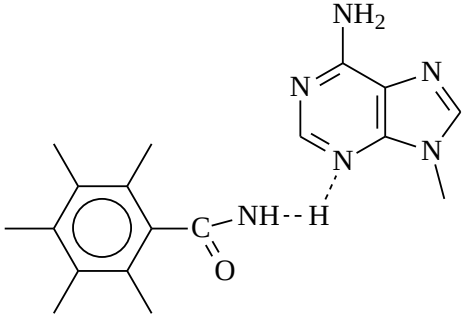


- Interactions involving the N-oxide moiety (target chemical) (Scheme 3):

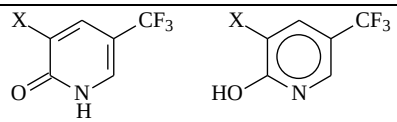


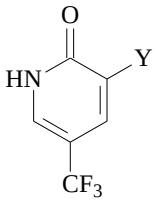
Set of chemicals used for profile development	Triazinone N-Oxide and Triazinone Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Draft Assessment Report (DAR)-public version, Initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance Metamitron of the third stage (part B) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC Volume 3, Annex B, part 2/B, B.6 November 2007; 2. Conclusion regarding the peer review of the pesticide risk assessment of the active substance metamitron, Issued on 29 September 2008, EFSA Scientific Report (2008) 185, 1-95; https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2008.185r. Last visited: April, 2024. 3. Lopez-de-Gerain, E. Garcia, A. Gullon, Mutagenesis 7

	(1992), 37 – 39. 4. Hirao, H., Pr. Chuanprasit, Y. Y. Cheon, X. Wang, Chem. Eur. J. 19 (2013), 7361 – 7369; DOI: 10.1002/chem.201300689. Last visited: April, 2024. 5. 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, Current Drug Metabol. 6 (2005), 161 – 225. 6. Kovacic, P., J. D. Jacinto, Current Med. Chem. 8 (2001), 773 – 796. 7. Rumyantseva, G., Chr. H. Kennedy, R. P. Mason, J. Biol. Chem. 266(32) (1991), 21422 – 21427. 8. Quintero, B., M. A. Miranda, Ars Pharmaceutica 41(1) (2000), 27 – 46. 9. Gannet, P. M., N. S. Dalai, X. L. Shi, B. Toth, Chem. Biol. Interact. 80(1) (1991), 57 – 72. 10. Chowdhury, C., D. Kotandeniya, J. Sc. Daniels, Ch. L. Barnes, K. S. Ganes, Chem. Res. Toxicol. 17(11) (2004), 1399 – 1405. 11. Public Release Summary on the Evaluation of the New Active Metamitron in the Product Brevis Fruit Thinner, APVWA Product Number 84928, Australian Government, Australian Pesticides and Veterinary Medicines Authority, Commonwealth of Australia, 2018; https://www.apvma.gov.au/sites/default/files/publication/31506-prs-84928-brevis-fruit-thinner.pdf. Last visited: April, 2024.

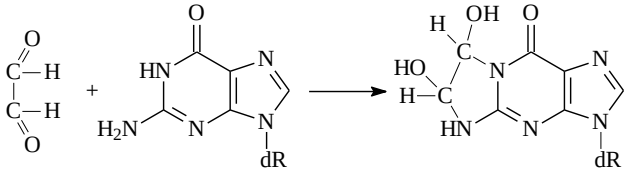
Individual profile/alert	
Name	Trifluoromethyl Benzamide Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is H or –NO2 or -C#N; -CF3: only one substituent; the rest are H)
Mechanism	Mechanistic domain: Non-covalent interactions Mechanistic alert: DNA intercalation
A specific hydrogen bond formation between an amide hydrogen atom of Benzamide and N-3 of adenine was reported  The authors have claimed that Benzamide also forms a hydrogen bond to another benzamide	

molecule. The aromatic ring of benzamide does not intercalate between adenine molecules, but lies nearly perpendicular to the planes of the latter [4]. • It has also been shown that the trifluoromethyl (-CF3) groups can act both as electrophile and nucleophile, to form non-covalent interactions. For instance, when C-F bonds interact with both the carbonyl- and NH- fragments of an amide unit, the multipolar C-F...C=O interaction is far more common [5]. • Bearing in mind that the triflоromethyl group is highly electron-withdrawing, it could be assumed that, apart from the additional polarization of the amide bond, this can also contribute to stronger hydrogen bonds with the adenine nucleobase of DNA, and to enhanced stability of the parent chemical-DNA complex. Apart from the –CF3 group, the introduction of other strong electron-withdrawing substituents such as –NO2 and –C#N would also promote this trend, increasing the positive mutagenicity outcome.	
Set of chemicals used for profile development	Trifluoromethyl Benzamide 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. EFSA (2011) Public consultation on the active substance cyflumetofen. https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-cyflumetofen. Last visited: March, 2024. 2. DAR public version - Risk assessment provided by the rapporteur Member State the Netherlands for the new active substance CYFLUMETOFEN. Prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Volume 1, February 2011. 3. p-Nitrobenzamide, Ames Conclusions, NTP, USA Deparment of Health and Human Services; https://cebs.niehs.nih.gov/datasets/search/ames?casrn=619-80-7. Last visited: March, 2024. 4. McLick, J., Hakam, A., Bauer, P.I., Kun, E., Zacharias, D.E., Glusker, J.P., Biochim. Biophys. Acta 909(1) (1987), 71 – 83; doi: 10.1016/0167-4781(87)90047-9. 5. Esterhuysen, K., A. Hesselmann, T. Klark, Trifluoromethyl: an amphiphilic noncovalent bonding partner, ChemPhysChem 10.1002/cphc.201700027; http://dx.doi.org/10.1002/cphc.201700027. Last visited: March, 2024.

Individual profile/alert	
Name	Trifluoromethylpyridinone Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Tautomers) (X is Cl, Br, F)

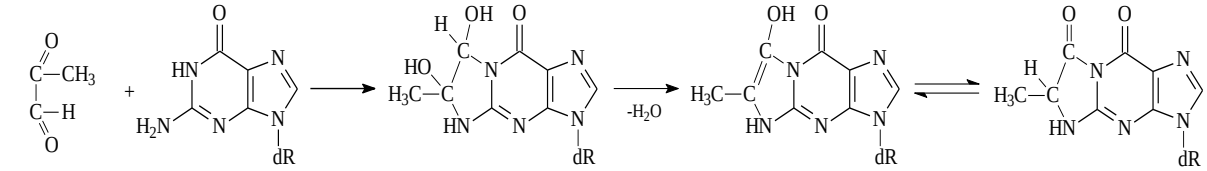
	 (Y is OH or H)
Mechanism	Structural sub-class A: Mechanistic domain: SN2 Mechanistic alert: Nucleophilic attack on labile C-halogen bond Structural sub-class B: Case 1: Mechanistic domain: AN2 Mechanistic alert: Nucleophilic addition on alpha,beta dicarbonyl compounds Case 2: Mechanistic domain: AN2 Mechanistic alert: 1,4-Michael-type addition on conjugated double bonds

The mutagenic activities in the Ames test against Salmonella typhimurium TA100 for a series of alpha-beta dicarbonyl compounds have been associated with the chemical reactivity of these compounds towards purine bases in DNA, more exactly, with the extent of stability of the adducts formed. The molecular basis of the mutagenic action of Glyoxal derivatives, for example, was suggested to be the AN2-type interaction between the dicarbonyl compound and the guanine fragments of nucleic acid with the formation of geminal carbinolamines and Shiff bases [9]. Concerning the adduct structure, a new ring was formed involving the 1 and N2 positions of the guanine ring and both carbonyl functionalities of Glyoxal, according to the following scheme, applicable to alpha,beta Ames-active dicarbonyl compounds



(Deoxyguanosine fragment) (Adduct)

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

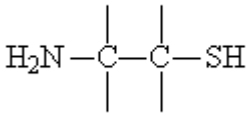
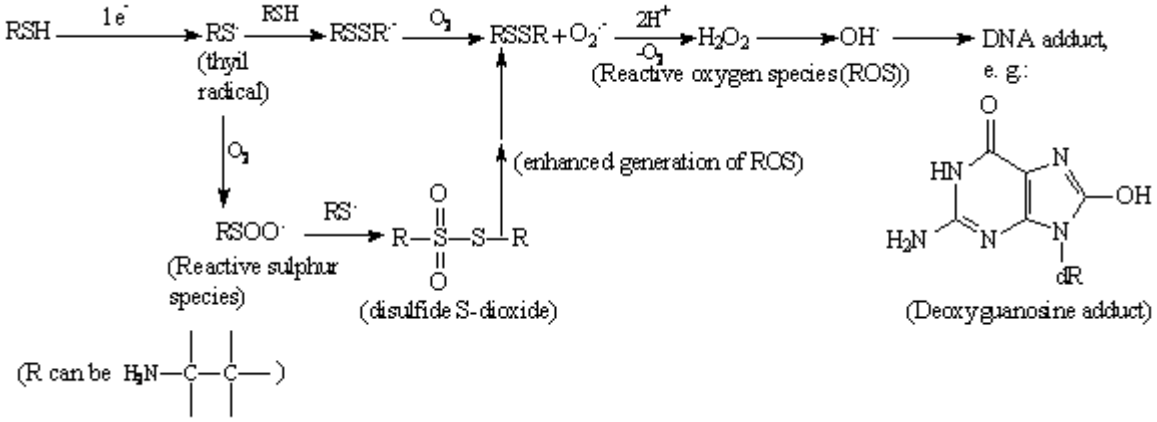


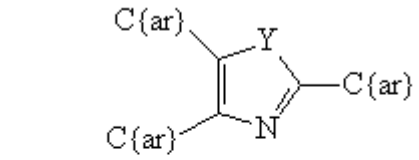
(Methylglyoxal) (Deoxyguanosine fragment) (Adducts)

Set of chemicals used for profile development	Trifluoromethylpyridinone 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.

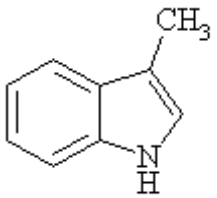
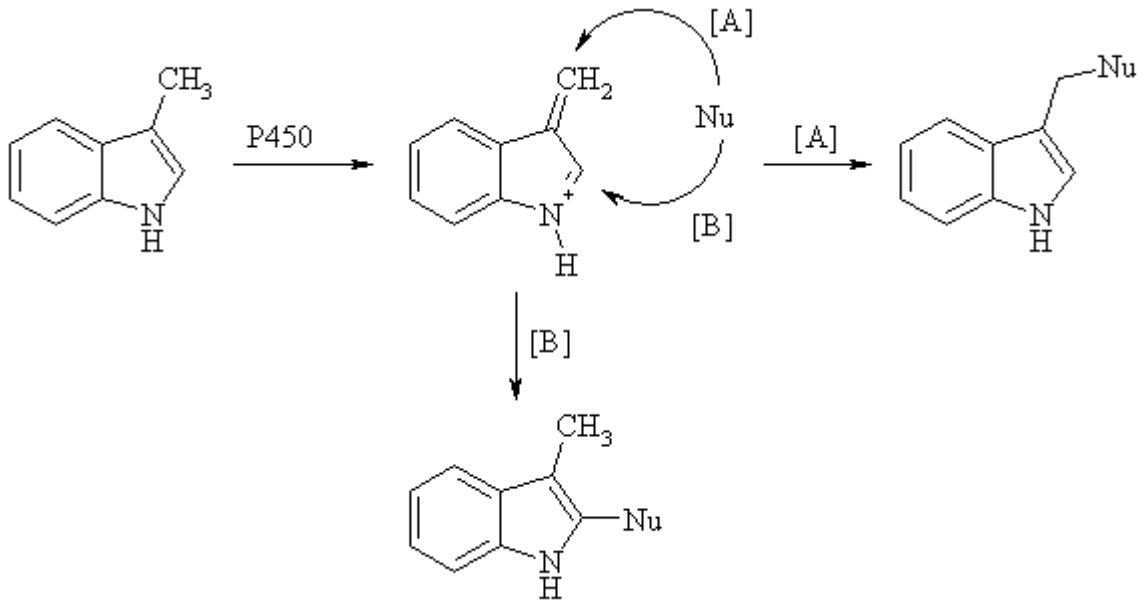
References	1. EFSA (2009) Public consultation on the active substance haloxyfop-P; https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-haloxyfop-p-0). Last visited: March, 2024. 2. Claxton L, K. Dearfield, R. Spanggord, E. Riccio, K. Mortelmans, Mutat Res. 176(2) (1987) 1987, 185 – 198; http://dx.doi.org/10.1016/0027-5107(87)90049-2. Last visited: March, 2024. 3. Conclusion on the peer review of the pesticide risk assessment of the active substance pyridalyl, European Food Safety Authority, EFSA Journal 11(8) (2013), 3240; https://www.efsa.europa.eu/en/efsajournal/pub/3240). Last visited: March, 2024. 4. US EPA (2009) Pyridalyl: Revised Human-Health Risk Assessment for Uses on Cotton, Fruiting Vegetables, Leafy Vegetables, Head & Stem Brassica Vegetables, Brassica Leafy Greens, and Turnip Greens, Shrubs, Ornamentals and Non-bearing Trees. https://www3.epa.gov/pesticides/chem_search/hhbp/R169200.pdf). Last visited: March, 2024. 5. Magahori, H., Y. Tomigahara, N. Isobe, H. Kaneko, J. Agric. Food Chem. 57 (2009), 10845 – 10851; DOI 10.1021/jf9026469. 6. European Food Safety Authority, Conclusion on the peer review of the pesticide risk assessment of the active substance pyridalyl. EFSA J., 2013, 11(8), 3240 [87 pp.]. doi: 10.2903/j.efsa.2013.3240. 7. EFSA (2012) Public consultation on the active substance fluazifop-P. https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-fluazifop-p. Last visited: March, 2024. 8. EFSA (2007) Public consultation on the active substance fluazifop-P-butyl. https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-fluazifop-p-butyl. Last visited: March, 2024. 9. Mellado, J. M. R., M. R. Montoya, Mutat. Res. 304(2) (1994), 261 – 264. 10. Shapiro, R., J. Hachmann, Biochem. 5(9) (1966), 2799 – 2807). 11. Frishmann, M., Cl. Bidmon, J. Angerer, M. Pischetsrieder, Chem. Res. Toxicol. 18 (2005), 1586 – 1592. 12. T.W. Schultz, J.W. Yarbrough, R.S. Hunter, A.O. Aptula, Chem. Res. Toxicol. 20(9), (2007), 1359–1363. 13. A.O. Aptula, G. Patlewicz, D.W. Roberts, T.W. Schultz, Toxicol In Vitro 20(2) (2006), 239–247. 14. R.M. LoPachin, D.S. Barber, T. Gavin, Toxicol. Sci 104(2), (2008), 235–249. 15. Eder, E., Chr. Hoffmann, H. Bastian, Chr. Deininger, S. Scheckenbach, Environ. Health Persp. 88 (1990), 99 – 106. 16. Hecht, S. S., E.J. McIntee, M. Wang, Toxicology 166 (1-2) (2001), 31 – 36.

Individual profile/alert	
Name	Thiols
Type of profile	Structural alert

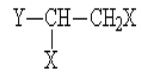
Description/applicability domain	
Mechanism	Radical ROS generation (indirect)
	
Set of chemicals used for profile development	Thiols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded 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	

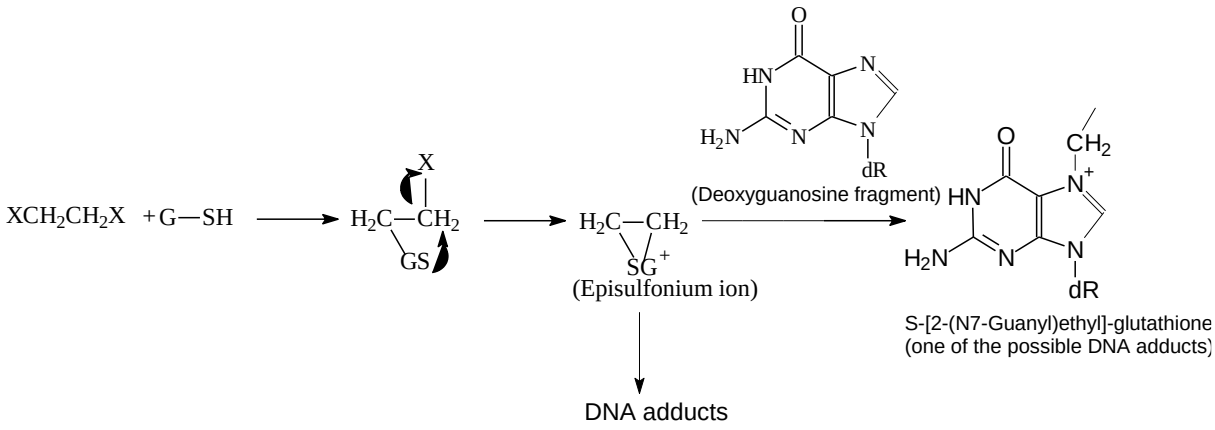
suggested to belong to the class of DNA intercalating agents [1], probably due to the multi-cyclic planar molecular system and conjugation effects.	
Set of chemicals used for profile development	Triarylimidazole and Structurally Related DNA Intercalators
Data/Knowledge used for profile development	An 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. 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	Tri-Methylindole 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	Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225

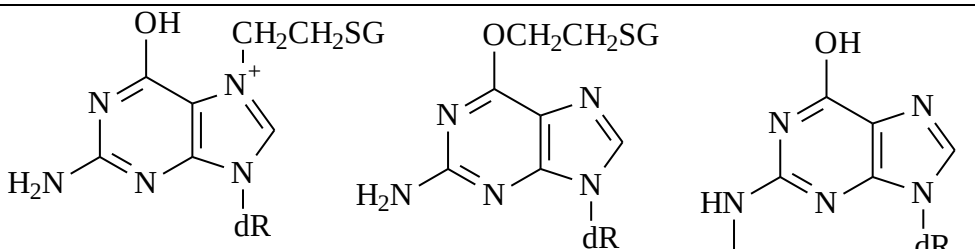
	Regal KA et al (2001) Chemical Research in Toxicology, 14, p1014-1024

Individual profile/alert	
Name	Vicinal Dihaloakanes
Type of profile	Structural alert
Description/applicability domain	 (Y is –H, -(CH$_2$)$_n$H (n = 1, 2), -O(CH$_2$)$_n$H (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]:

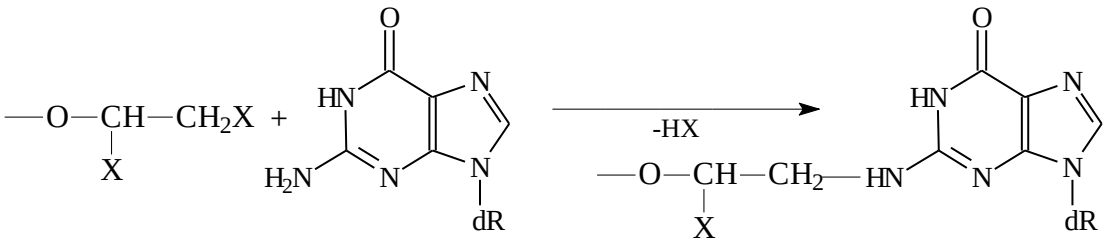


N7-alkylated adduct O6-alkylated adduct N2-alkylated adduc

(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].

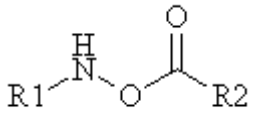
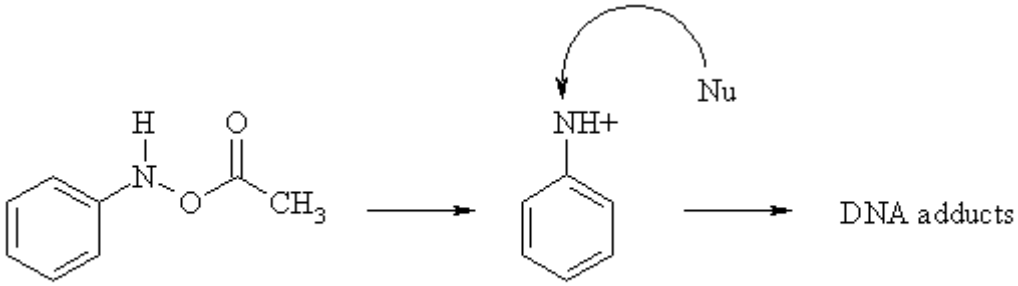
Beside 1,2-dichloroethane, 1,2-dibromoethane belonging to this class of compounds was also found to possess bacterial mutagenicity [7]. Short-chain vicinal dihalolakanes with halogen attached to terminal carbon atom are assumed to act by direct alkylation mechanism, too. Other short-chain vicinal haloalkane 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:

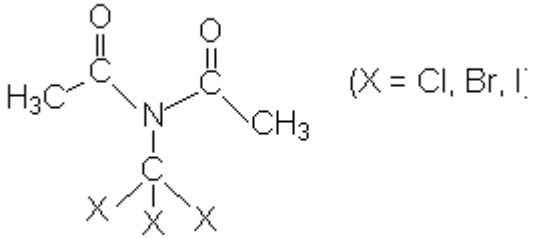


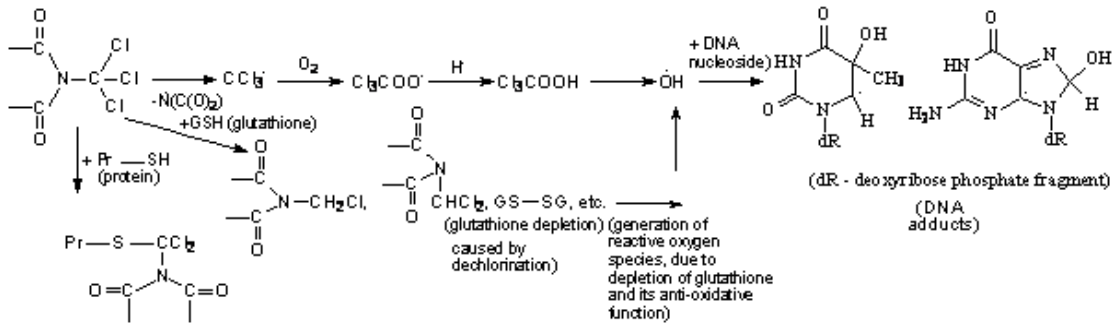
(Deoxyguanosine fragment) (One possible DNA alkylated adduct)

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

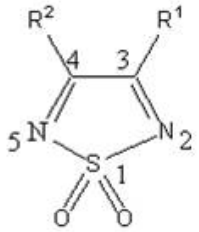
Name	Aromatic ester hydroxylamine
Type of profile	Structural alert
Description/applicability domain	 R1 = aromatic carbon atom R2 = any carbon atom
Mechanism	SN1 reaction Nitrenium ion formation
Desterification to produce a reactive nitrenium ion capable of reacting with DNA via an SN1 mechanism is the 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	
Mechanism	Acylation Direct acylation involving a leaving group

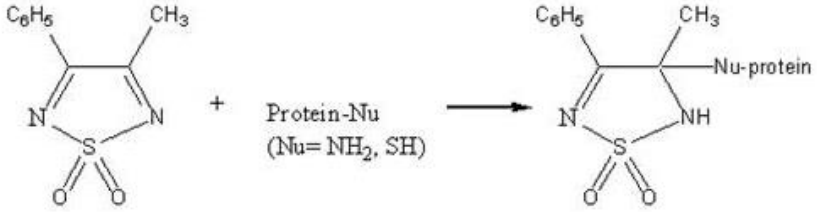


Set of chemicals used for profile development	Not Appliciable
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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.

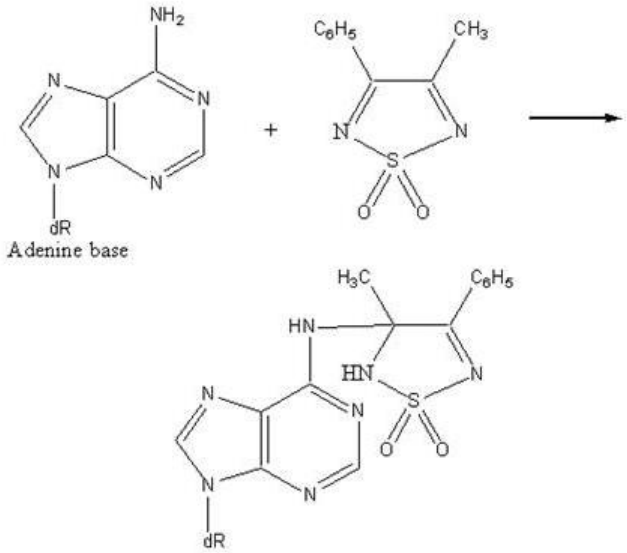
Individual profile/alert	
Name	Thiadiazoledioxide Derivatives
Type of profile	Structural alert

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:

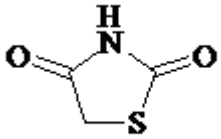


It may be assumed that thiadiazole dioxide derivatives can bind to the amino groups in DNA bases regardless of their lower nucleophility.

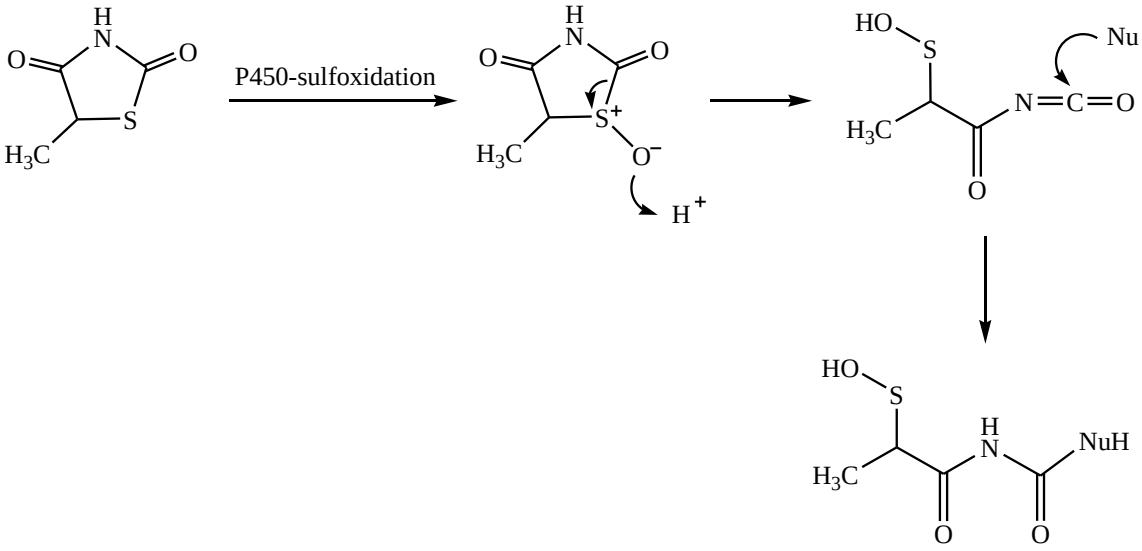


Set of chemicals used for	Not Appliciable

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. 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)



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	Bedir A et al (2008) Environmental and Molecular Mutagenesis, 49, p185-191.

	Kalgutkar AS et al (2005) Current Drug Metabolism, 6, p161-225.