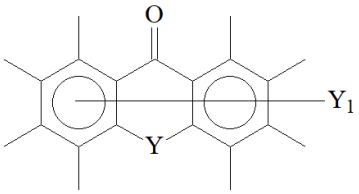
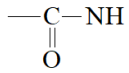
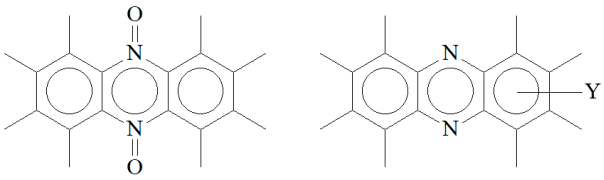
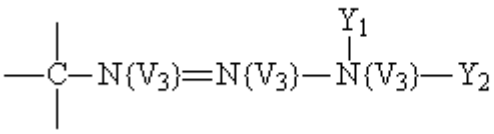
Individual profile/alert	
Name	Acridone, Thioxanthone, Xanthone and Phenazine Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is O, S{V_2}, N{V_3}) (Y_1 can be -OH, -O-CH_3, -NH{{sp3}{V3}, -CH_3, -CH_2OH,  No other substituents allowed, except for -H total number of substituents in both benzene rings: 2 - 5) (Note: Such substituents are believed to promote intercalation effects, due to electron-donating capability and/or enhanced conjugations)  (Y can be combinations between -H and -NH_2 or -H, NH_2 and -OH or OCH_3)
Mechanism	**Non-covalent interactions DNA intercalation and Radical ROS generation (indirect)**

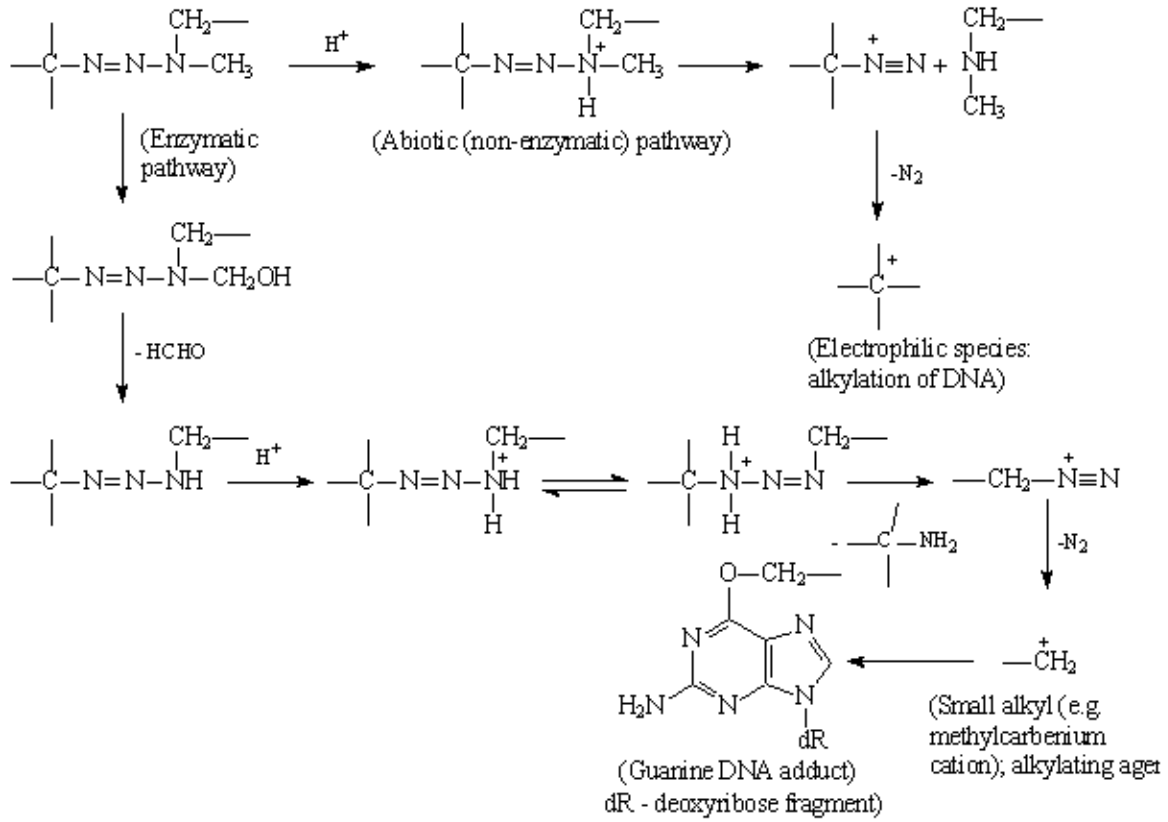
A number of tricyclic acridone, thioacridone and thioxanthene derivatives are known to act as DNA intercalating agents and possess *in vitro* bacterial mutagenicity in a broad range of intensity. Generally, acridones showed the highest bacterial mutagenicity [1].

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:
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].

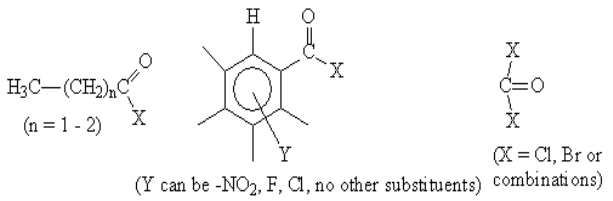
Set of chemicals used for profile development	Acridone, Thioxanthone, Xanthone and Phenazine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in

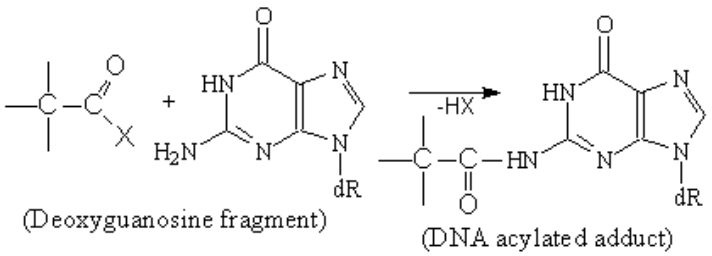
	this profiler according to the references listed below.
References	1. Denny, Mutat. Res. **232** (1990), 233 – 241. 2. Matsushima, Mutat. Res. **150** (1985), 141 – 146. 3. Harman, Mutat. Res./Environ. Mutag. Rel. Subjects **31**(2) (1975), 87 – 95. 4. Feng, J. Pharm. Biomed. Anal. **62** (2012), 228 – 234. 5. Double, J. Pharm. Pharmac. **28** (1976), 166 – 169. 6. Hoffman, Res. Toxicol. **10**(4) (1997), 347 – 359. 7. Sarrif, Mutat. Res. **321** (1994), 43 – 56. 8. Watanabe, Mutat. Res. **227** (1989), 135 – 145. 9. Chowdhury, Chem. Res. Toxicol. **25** (2012), 197 – 206.

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	**S_N1 Nucleophilic attack after carbenium ion formation**
On the basis of the literature data available, the following scheme of bioactivation of triazene derivatives can be expertly assumed:	



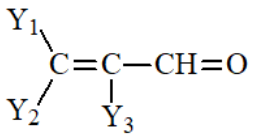
Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kazius, J. Med. Chem. **48** (2005), 312 – 320. 2. Thomas, Mutat. Res. **60** (1979), 25 – 32. 3. Malaveille, Canc. Res. **42** (1982), 1446 – 1453. 4. Marchesi, Pharmacol. Res. **56** (2007), 275 – 287. 5. Sieh, Mutat. Res. **73** (1980), 227 – 235.

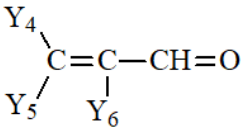
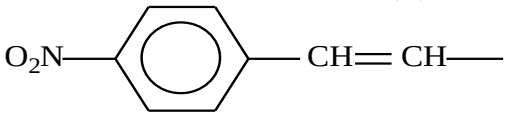
Individual profile/alert	
Name	Acyl Halides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N2 Direct acylation involving a leaving group**
A mixture of methylglyoxal and hydrogen peroxide has been found to react with 2'-deoxyguanosine to form N^2-acetyl-2'deoxyguanosine [3]. By analogy, direct DNA acylation mechanism by acyl halides such as acetyl chloride can be expertly suggested:	

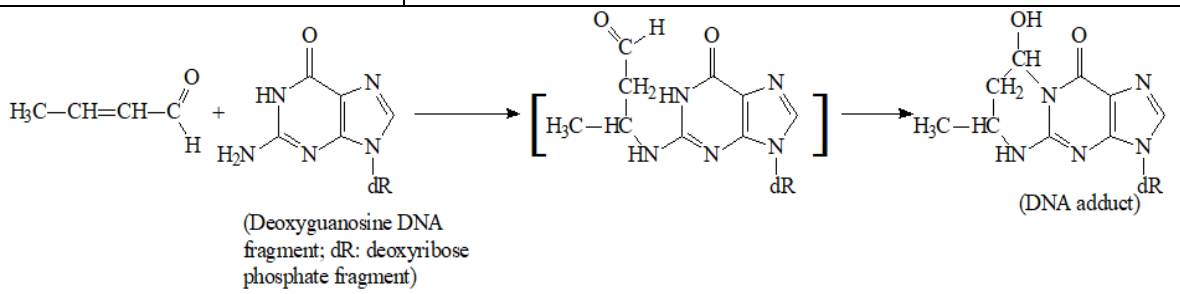


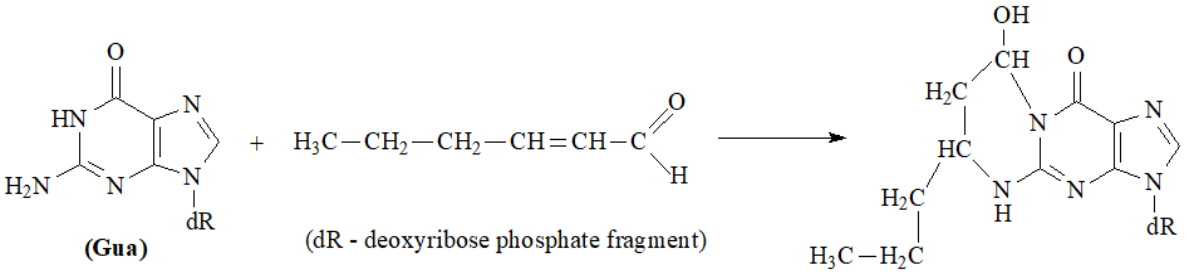
(Deoxyguanosine fragment) (DNA acylated adduct)

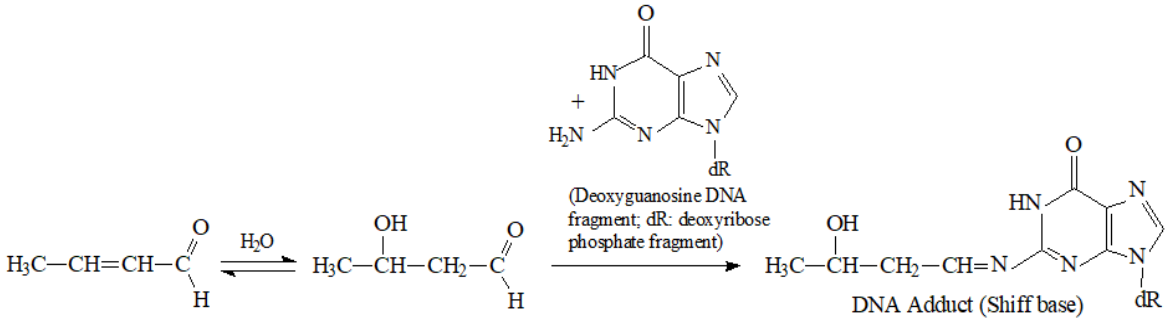
Set of chemicals used for profile development	Acyl Halides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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. ISBN-13 (Print Book) 978-92-832-1271-3; ISBN-13 (PDF) 978-92-832-1571-4; 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., 32P-Postlabeling analysis of a DNA adduct, an N2-acetyl derivative of guanine, formed in vitro by methylglyoxal and hydrogen peroxide in combination. Mutat. Res., 1996, 351(2), 173-180.

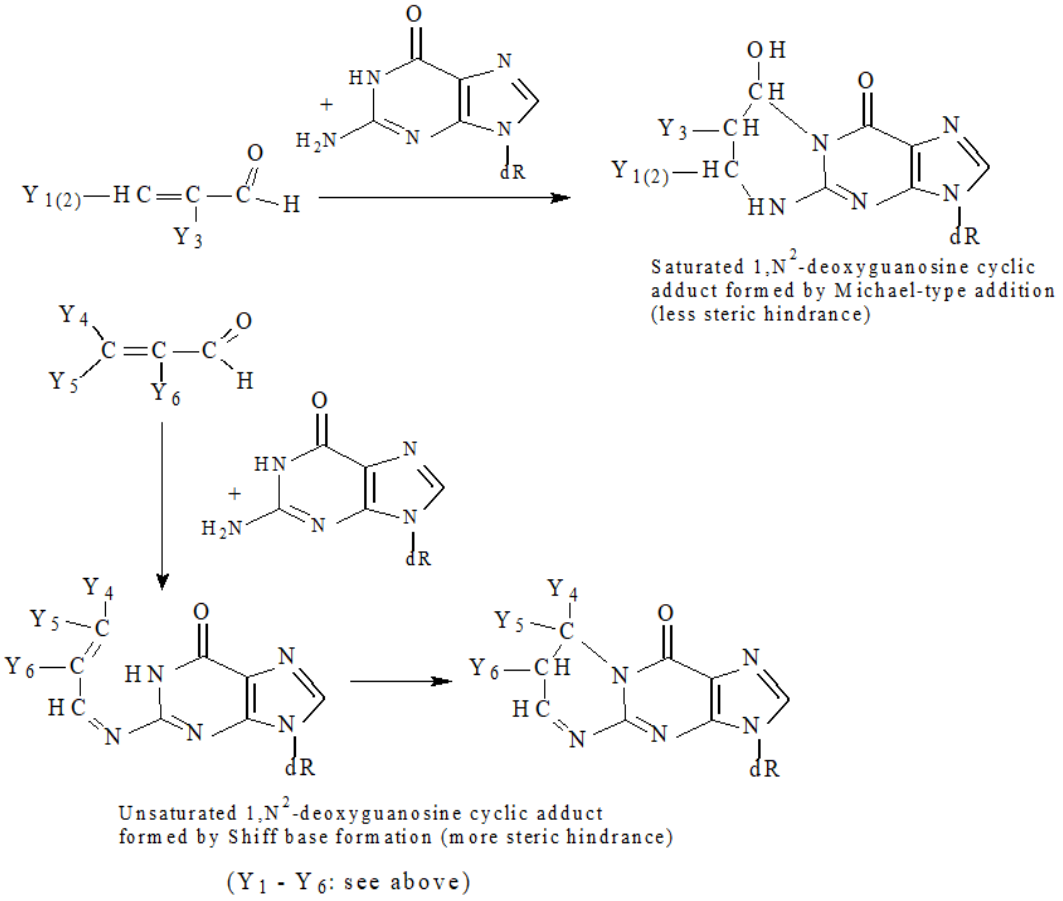
Individual profile/alert	
Name	Alpha,Beta-Unsaturated Aldehydes
Type of profile	Structural alert
Description/applicability domain	 Y_1, Y_2 are H (both); or CH_3 (both); or combination of H and n-C_nH_{2n+1} (n = 1 – 4); or combination of H and H_3C-CH=CH- ; Y_3 is H *(Notes: 1. If both Y_1 and Y_2 are 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)  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  ; Y_6 is –H, X or CH_3
Mechanism	**A_N2 Nucleophilic addition to α,β-unsaturated carbonyl compounds & A_N2 Shiff base formation**

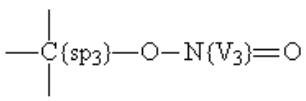


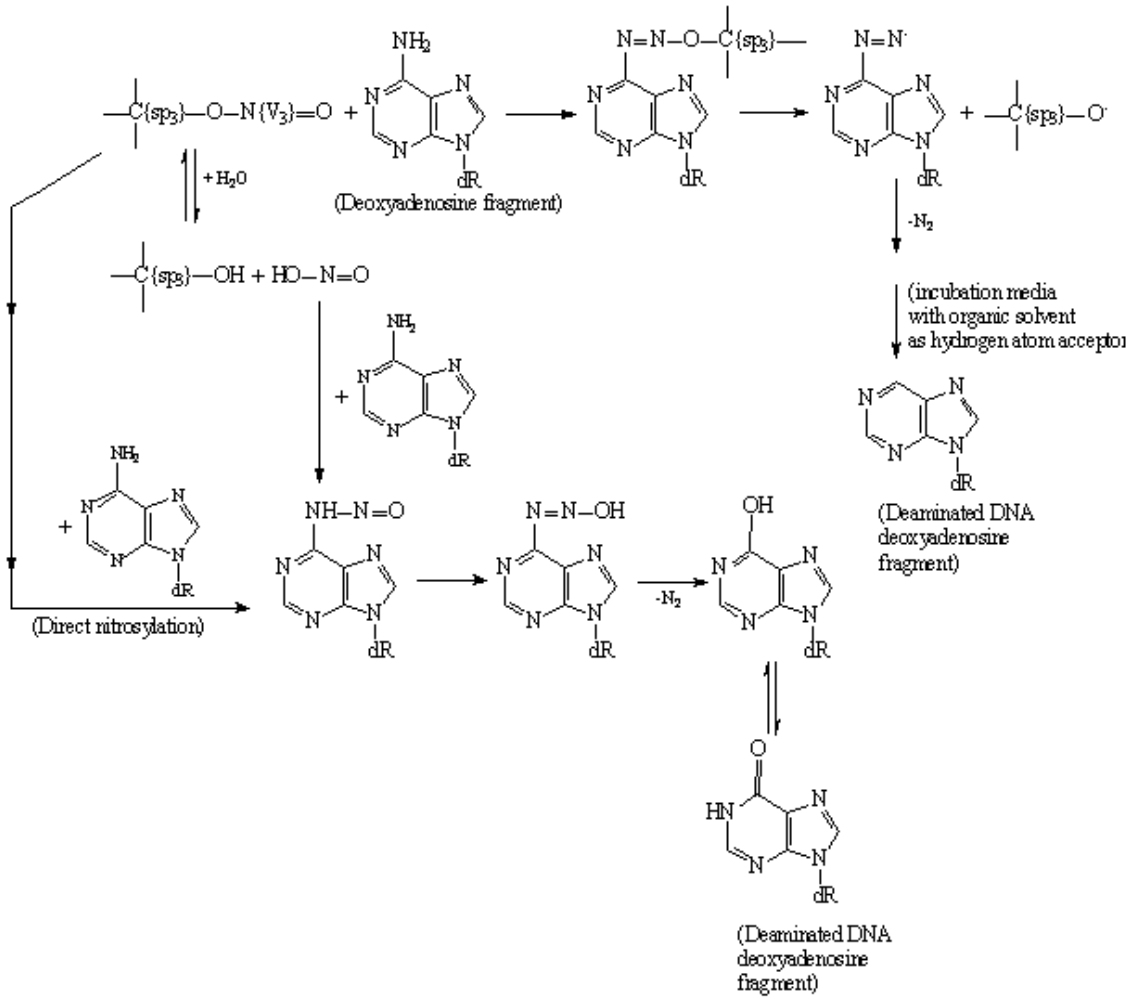






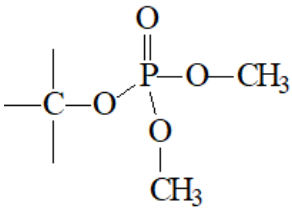
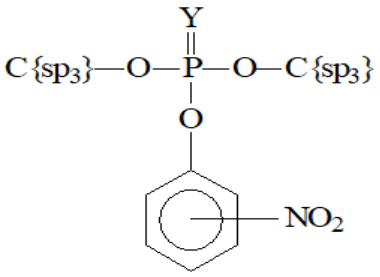
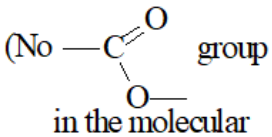
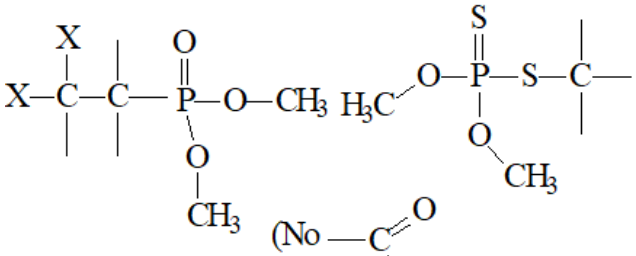
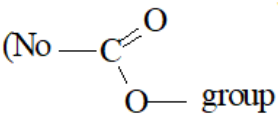
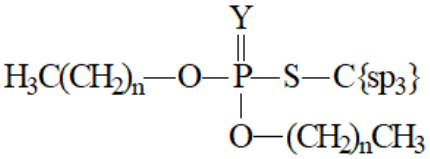
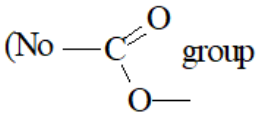
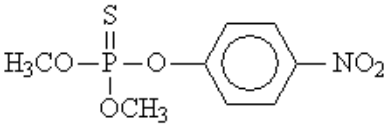
Set of chemicals used for profile development	Alpha,Beta-Unsaturated Aldehydes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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	Alkylnitrites
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N1 or S_N2 Nitrosation, A_N2 Formation of adducts similar to Shiff bases and Radical DNA base deamination after radical decomposition**
The following generalized scheme for the formation of mutagenic species by alkylnitrites can be suggested based on literature	

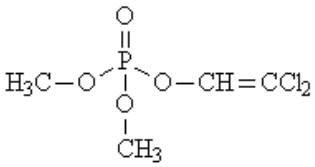


Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Tornqvist, Mutat. Res. **117** (1983), 47 – 54. 2. Dunkel, 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; ISBN-13: 978-0080423227, ISBN-10: 0080423221. 4. Wild, Fd. Chem. Toxicol. **21**(6) (1983), 707 – 719. 5. Ehrenberg, Hereditas 92(1) (1980), 127 – 130).

Individual profile/alert	
Name	Alkylphosphates, Alkylthiophosphates and Alkylphosphonates
Type of profile	Structural alert

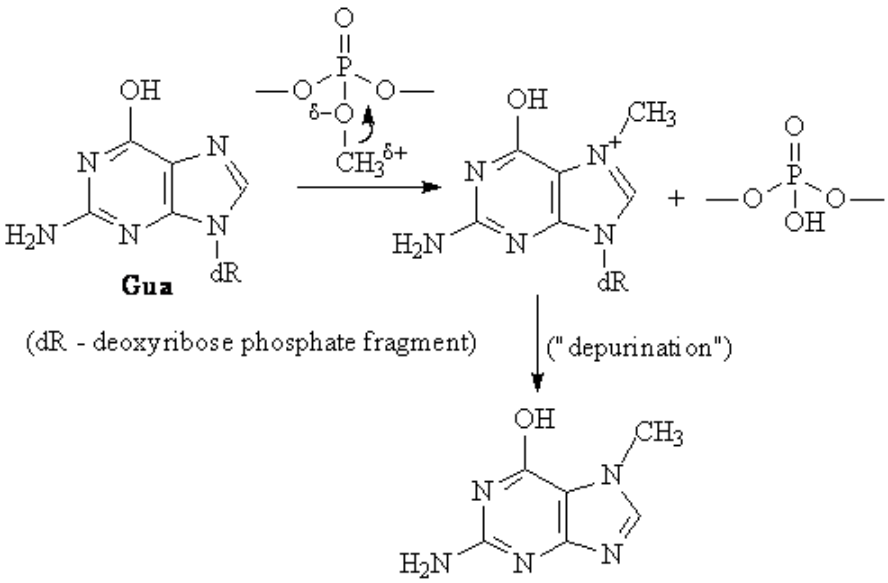
Description/applicability domain	  (Y is O, S {V2})  in the molecular structure)  (X is Cl, Br)  in the molecular structure)   in the molecular structure) (n = 0 - 1) (Y is O, S {V2})
Mechanism	**S_N2 Alkylation**
The compound methylparathion:	

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].

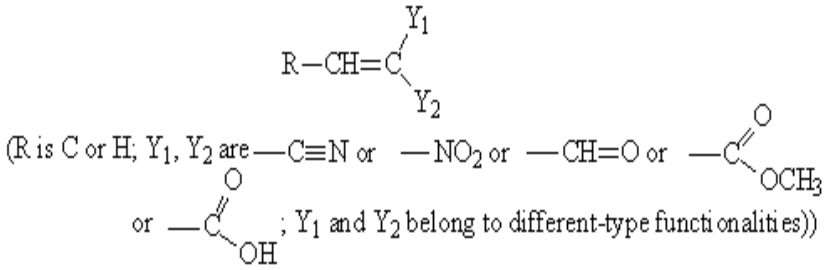
Therefore, the alkylation mechanism seems to be more plausible for this class of compounds, as expertly outlined below in Scheme 1



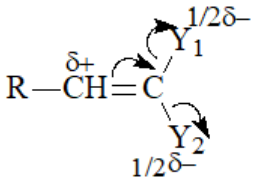
Scheme 1

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;

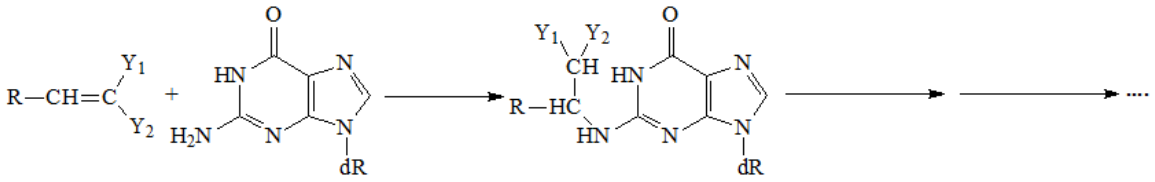
	http://www.inchem.org/documents/ehc/ehc/ehc145.htm). 2. Wang, T. C., Zool. Studies **42**(3) (2003), 462 – 469. 3. Braun, R., Chem. Biol. Interact., **39**(3) (1982), 339 – 350. 4. *Mutagenicity of Dichlorovos*, Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment, January 2002; Ashwood-Smith, J. Trevino, R. Ring, Mutagenicity of Dichlorvos, Nature, 240 (1972), 418-420 5. Lofroth, G., Naturwissenschaften **57**(8) (1970), 393 – 394. 6. Carere, A., Chem.-Biol. Interact. **22** (1978), 297 – 308. 7. Wiaderkiewicz, Acta Biochim. Pol. **33**(2) (1986), 73 – 85; https://www.ncbi.nlm.nih.gov/pubmed/3766014 last visited 10.2019. 8. Hour, Mutagen. **13**(2) (1998), 157 – 166.

Individual profile/alert	
Name	Alpha-Beta Conjugated Alkene Derivatives with Geminal Electron-Withdrawing Groups
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**A_N2 Michael-type conjugate addition to activated alkene derivatives**

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



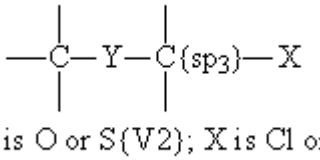
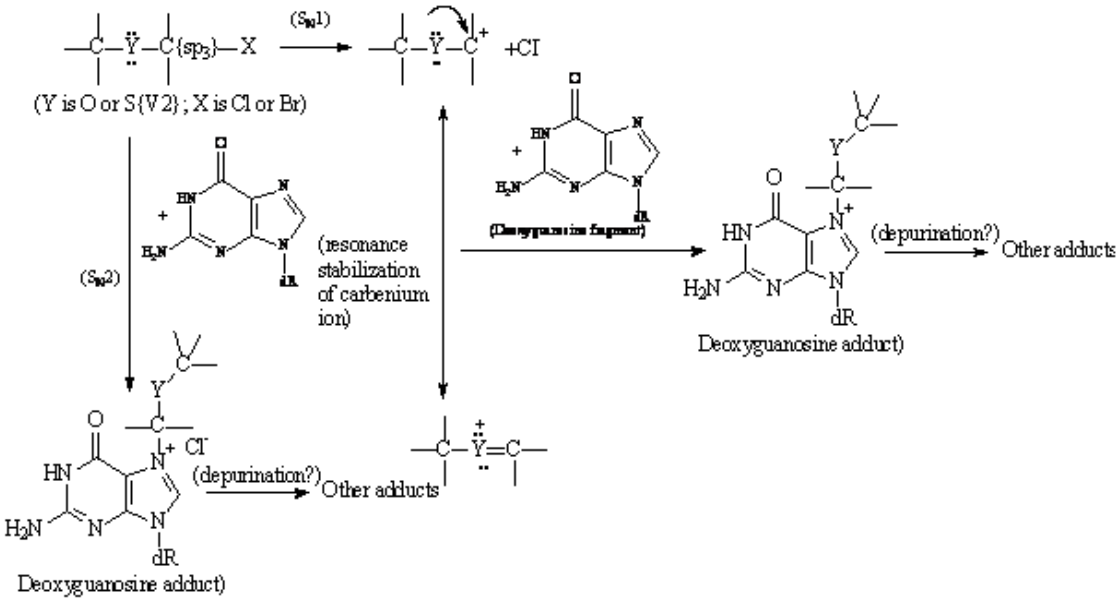
Thus some DNA alkylating capability becomes possible and it could materialize itself via mechanistic scheme, similar to Michael-type addition [4, 5], as follows:



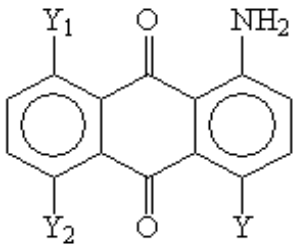
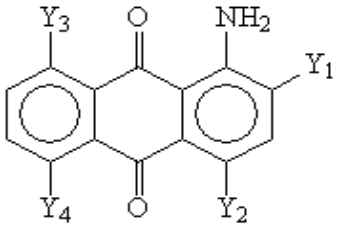
(Deoxyguanosine DNA fragment; dR: deoxyribose phosphate fragment)

(Possible initially formed DNA adduct)

Set of chemicals used for profile development	Alpha-Beta Conjugated Alkene Derivatives with Geminal 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. 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. 3. Andersen, Mutat. Res. **102** (1982), 373 – 381. 4. Hecht, Toxicology **166** (1-2) (2001), 31 – 36. 5. Solomon, Canc. Res. **45** (1985), 3465 – 3470.

Individual profile/alert	
Name	Alpha-Haloethers
Type of profile	Structural alert
Description/applicability domain	 (Y is O or S(V2); X is Cl or Br
Mechanism	**S_N1 after carbenium ion formation and S_N2 at an sp3 carbon atom**
The following mechanistic schemes can be expertly outlined: 	
Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was 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

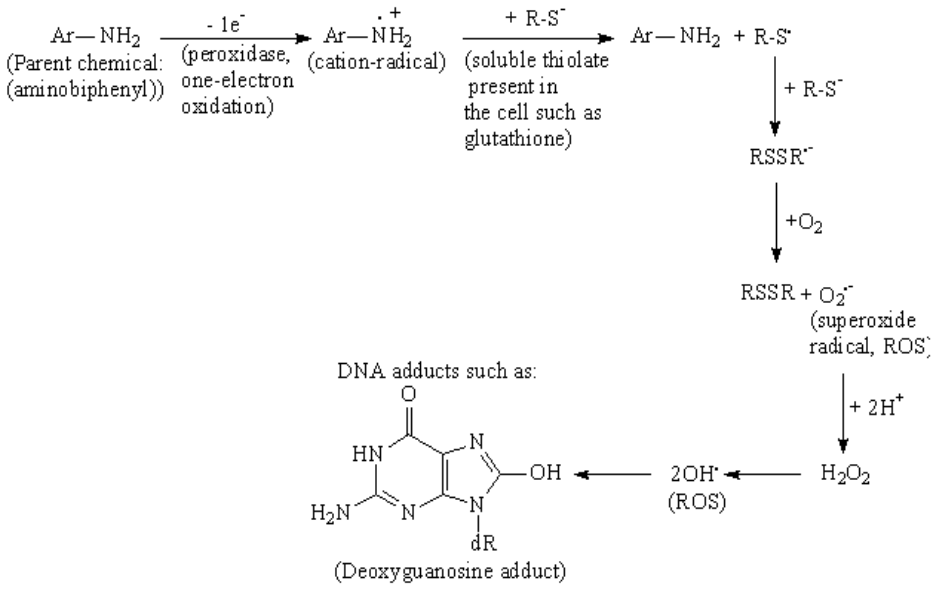
	visisted 09.2019 2. Van Duuren, Ann. New York Acad. Sci **163,** No. 2 (1969), 633 – 650; DOI: 10.1111/j.1749-6632.1969.tb24883.x. 3. Fishbein, Mutat. Res. **32** (1976), 267 – 308). 4. Zajdela, Canc. Res. **40** (1980), 352 – 356. 5. Enoch, ATLA **39** (2011), 131 – 145. 6. Enoch, Crit. Rev. Toxicol. **41**(9) (2011), 783 – 802. 7. Van Duuren, Ann. New York Acad. Sci **534** (1988), 620 – 634.

Individual profile/alert	
Name	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)  (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)
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

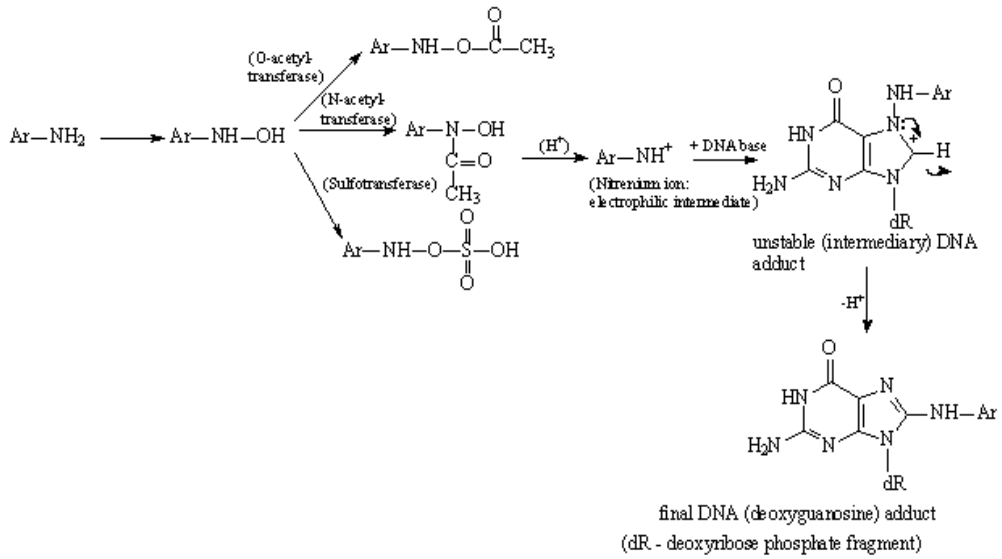
Endogenous generation of reactive oxygen species (ROS). Peroxidase enzymes might be present in *Salmonella typhimurium* bacterial strains, which are associated with endogenous generation of oxygen intermediates [7]. Generally, genotoxicity by oxygen intermediates may be caused by oxidative stress

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]:



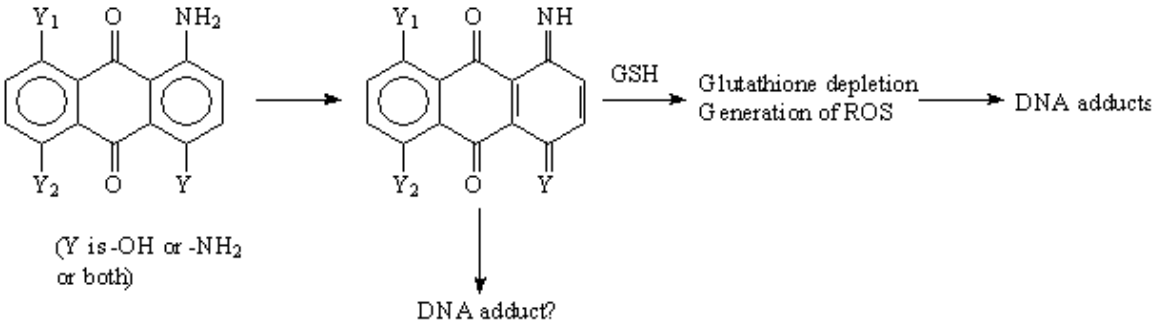
Scheme 1

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

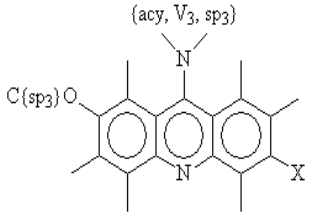
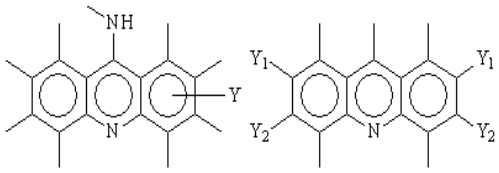
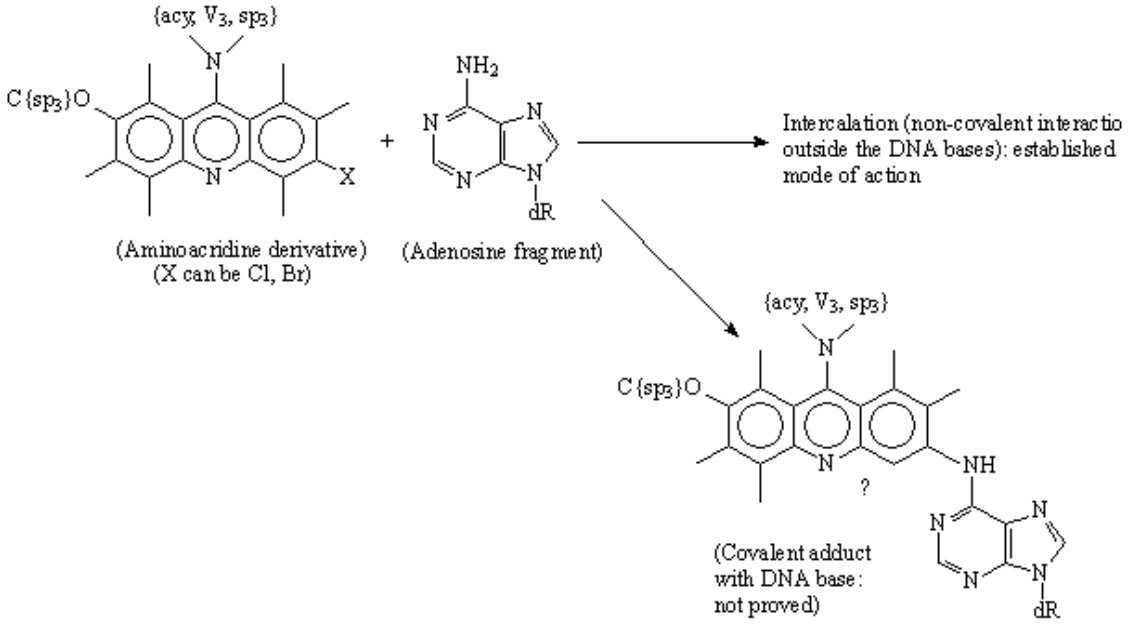
Not only is nitrenium ion chemistry implicated in the DNA damage. For some specific anthraquinone derivatives with electron-donating substituents mutually located at *p*- or *o*-positions, reactions associated with the formation of quinones, quinone imines or other quinoid structures could be involved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



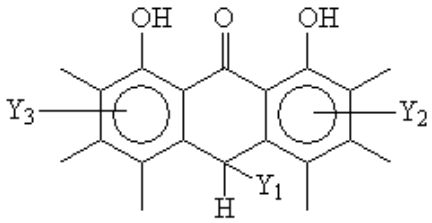
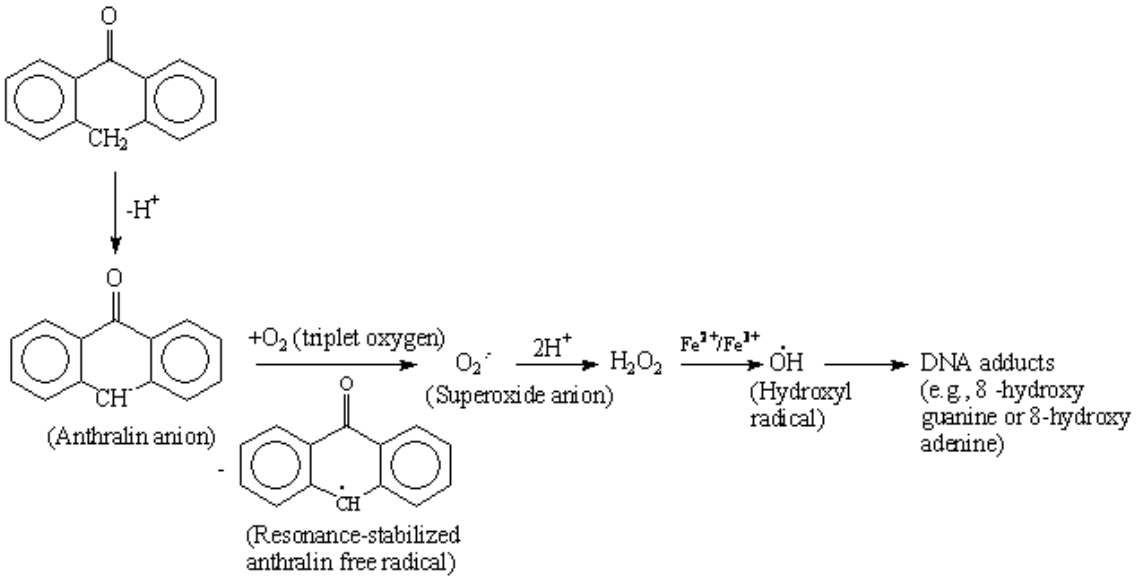
Scheme 3

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)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. 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.

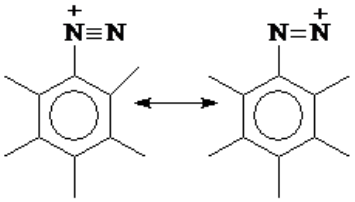
	11. Shamovsky, JACS **133** (2011), 16168 – 16185. 12. Skipper, Carcinog. **31**(10) (2010), 50 – 58.

Individual profile/alert	
Name	Aminoacridine DNA Intercalators
Type of profile	Structural alert
Description/applicability domain	 (X can be Cl, Br)  (Y can be H or -NH2) (Y1 can be H or -C(sp3); Y2 can be -NH2, -NHC(sp3) or -N(Csp3)2)
Mechanism	**Non-covalent interactions DNA intercalation**
	
Set of chemicals used for profile development	Aminoacridine DNA Intercalators
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kalinowska, Mutat. Res. **78** (1980), 7 – 15. 2. Yan, J. Med. Chem. **50** (2007), 4096 – 4104. 3. Wainwright, J. Antimicr. Chemother. **47** (2001), 1 – 13. 4. Hoffmann, Chem. Res. Toxicol. **10**(4) (1997), 347 – 359. 5. Fukui, Nucl. Acids Res. **24**(20) (1996), 3962 – 3967. 6. Asseline, Biocon. Chem. **7** (1996), 369 – 379.

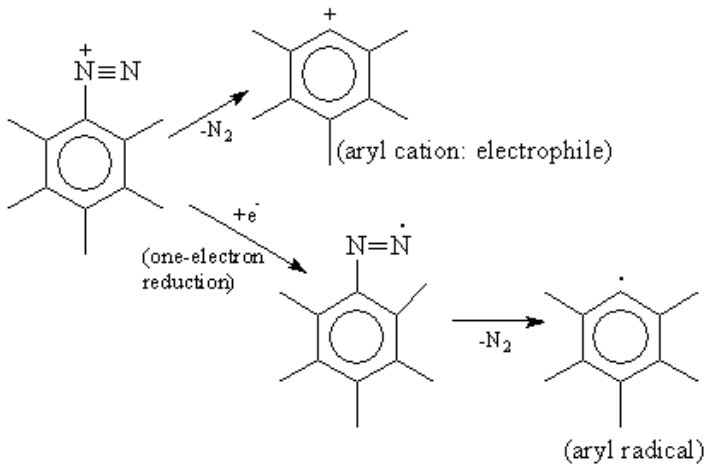
	7. Huang, Drug Metabol. Dispos. **34**(7) (2006), 1136 – 1144. 8. Denny, Mutat. Res. **232** (1990), 233 – 241. 9. Ferguson, Eur. J. Canc. **26**(6) (1990), 700 – 714.

Individual profile/alert	
Name	Anthrones
Type of profile	Structural alert
Description/applicability domain	 (Y_1 can be —H or —C(=O)—$(CH_2)_nH$ (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	N/A
Data/Knowledge used for profile development	An extensive review of the literature was 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).

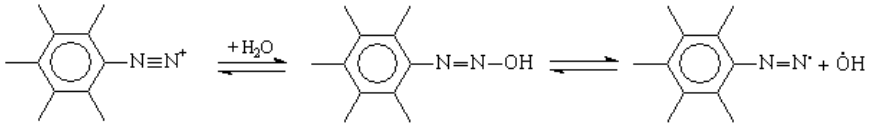
Individual profile/alert	
Name	Arenediazonium Salts
Type of profile	Structural alert

Description/applicability domain	
Mechanism	**S_N2 Direct nucleophilic attack on diazonium cation and Radical attack after one-electron reduction of diazonium cation**

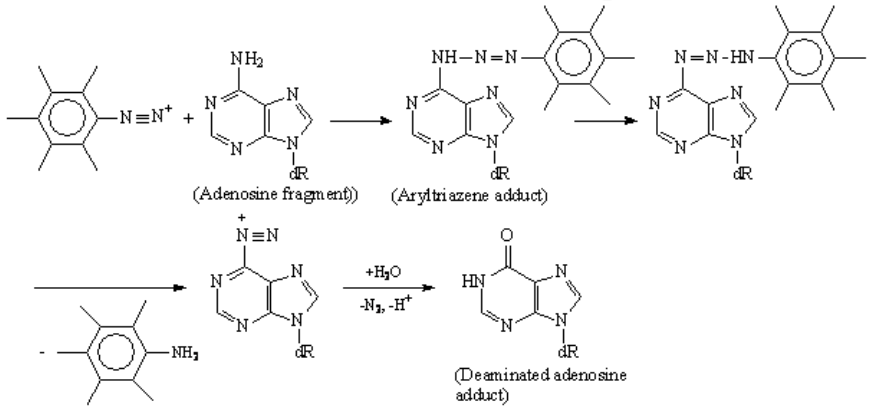
The decomposition pathways for arenediazonium ions can be expressed as follows:



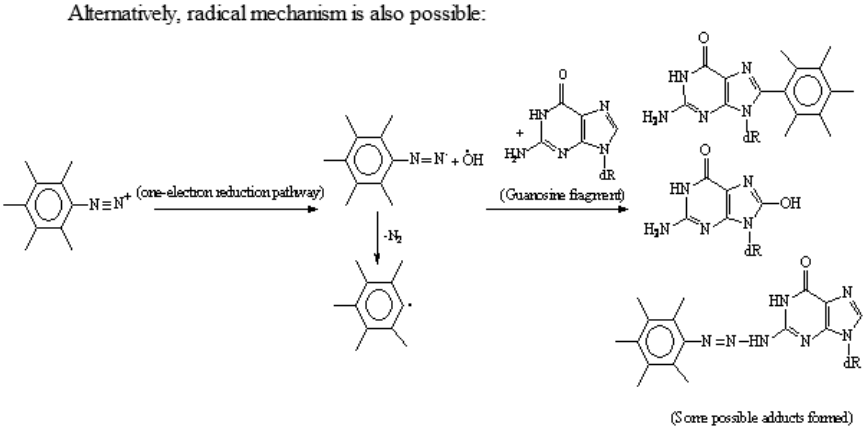
Also, hydroxyl radicals can be formed under these conditions:



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

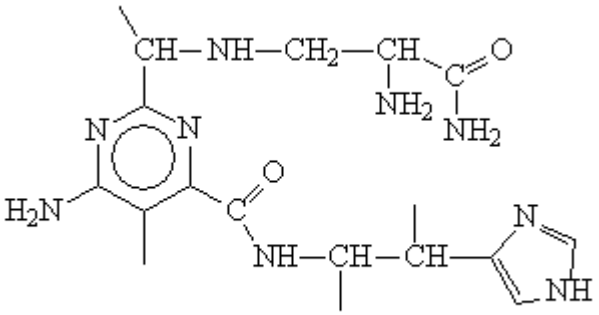
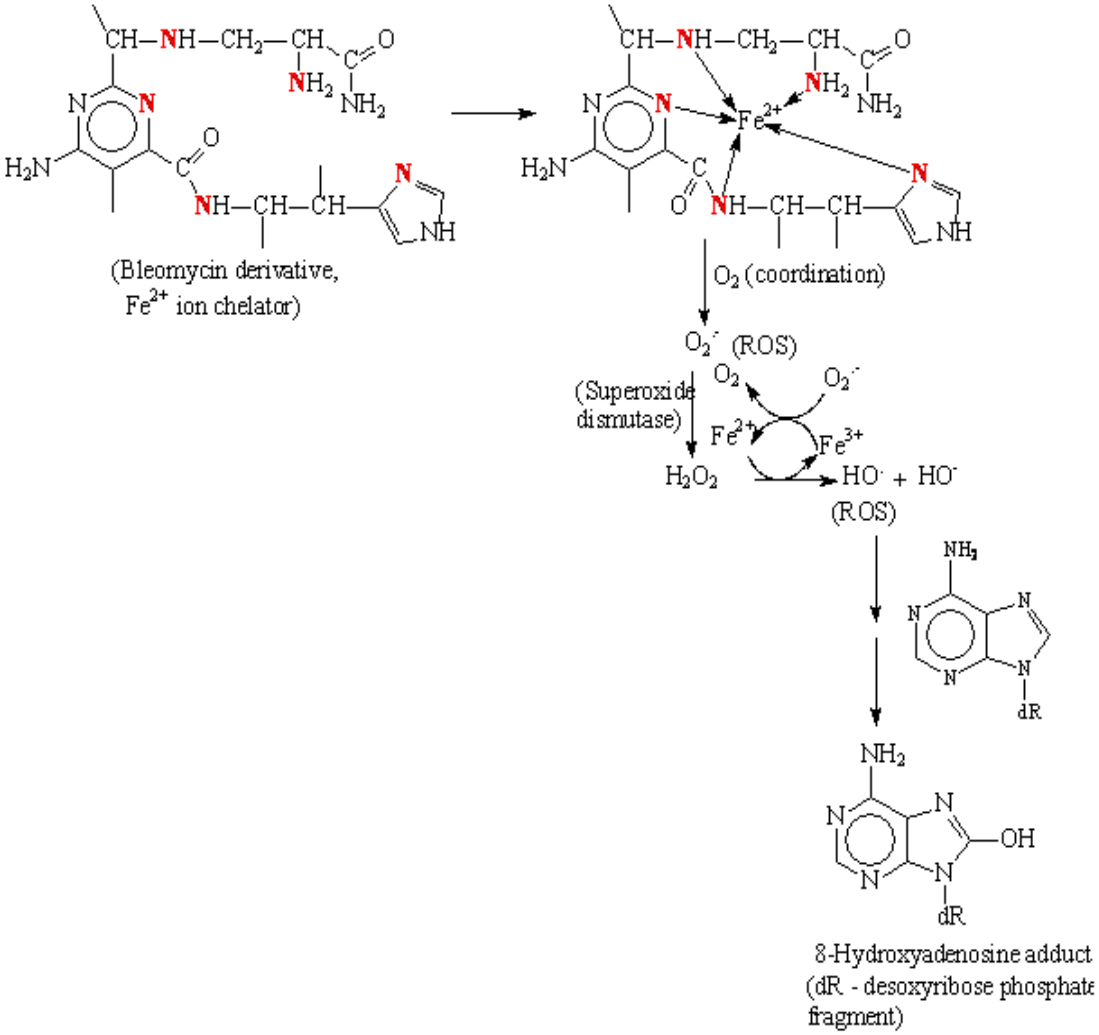


Alternatively, radical mechanism is also possible:

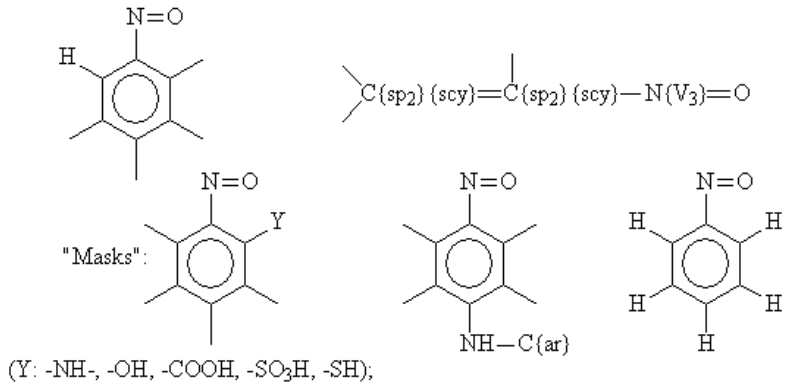


Set of chemicals used for profile development	Arenediazonium Salts
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Lawson, J. Agric. Food Chem. **43** (1995), 2627 – 2635. 2. Malaveille, Canc. Res. **42** (1982), 1446 – 1453.

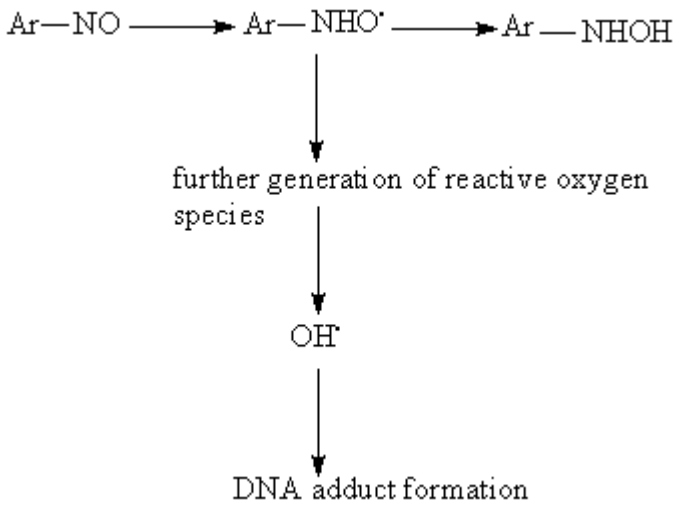
Individual profile/alert	
Name	Bleomycin and Structurally Related Compounds
Type of profile	Structural alert

Description/applicability domain	
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). 8. Podger, D. M., Mutat. Res. **117** (1983), 9 – 19. 9. Dixon, Sc. J., Nature Chemical Biology **10** (2014), 9 – 17.

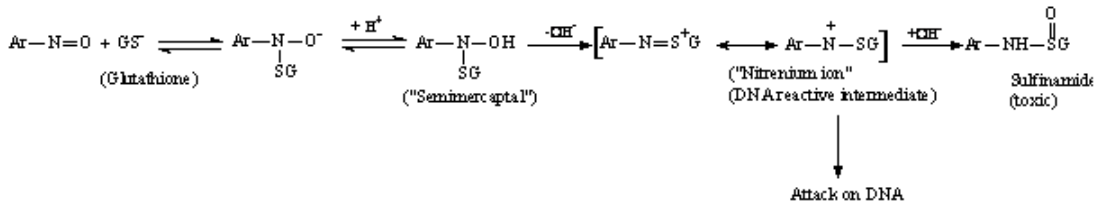
Individual profile/alert	
Name	C-Nitroso Compounds
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N1 Nucleophilic substitution after glutathione-induced nitrenium ion formation and Radical ROS generation (indirect)**

Radical mechanism - the formation of reactive entities such as ArNHO· 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]:



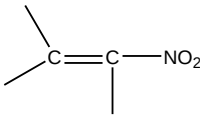
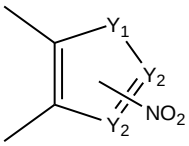
Scheme 1

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



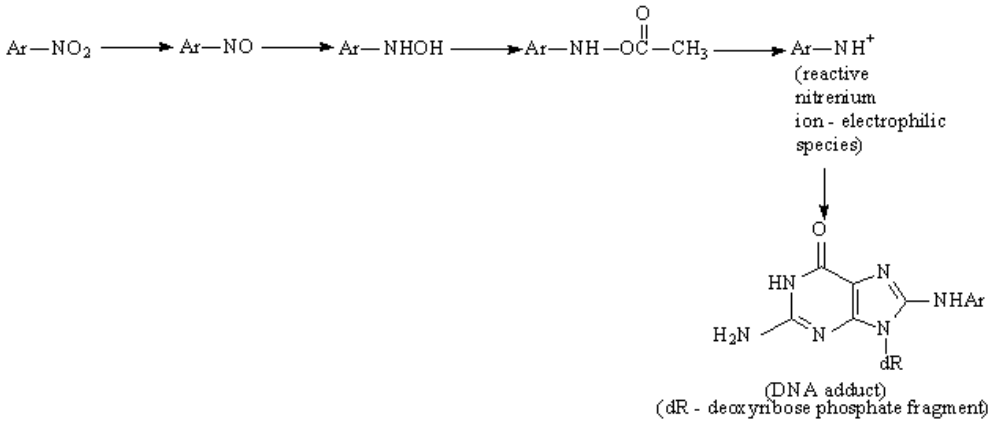
Scheme 2

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)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. 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.

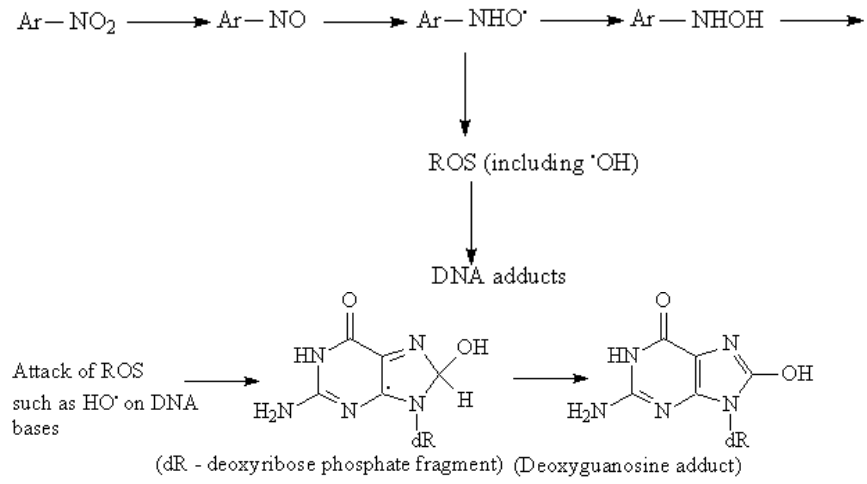
Individual profile/alert	
Name	Conjugated Nitroalkenes and Five Membered Aromatic Nitroheterocyclics
Type of profile	Structural alert
Description/applicability domain	**Characteristic active structural fragment**  **More specifically defined active structural fragment**  **R_1= N(V3)(sp^3) or S(V2) or O** **R_2= N(V3)(sp^2) or C(sp^2)**
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

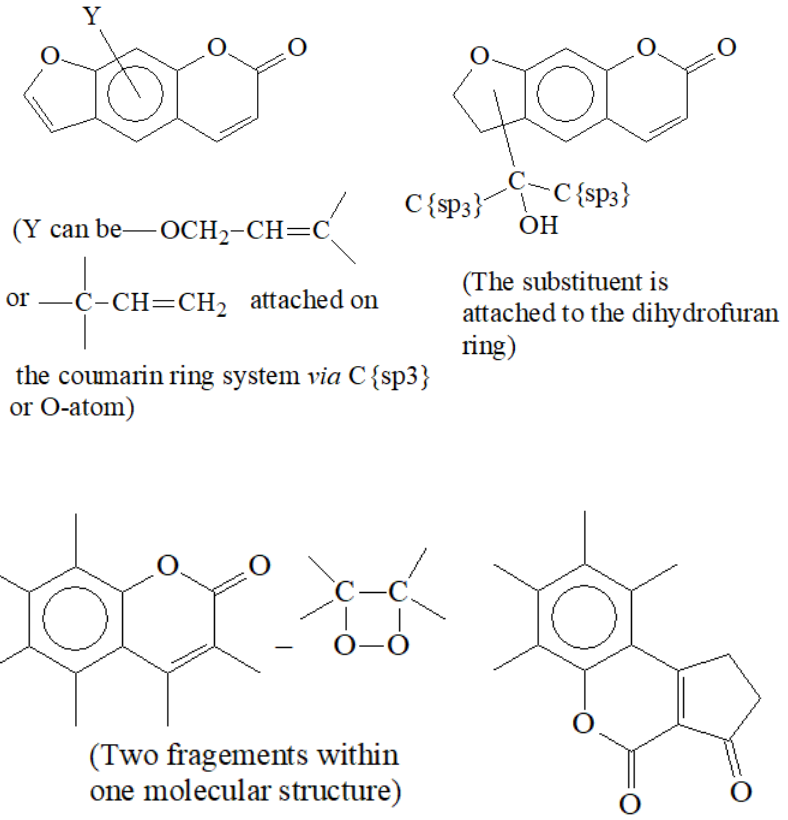
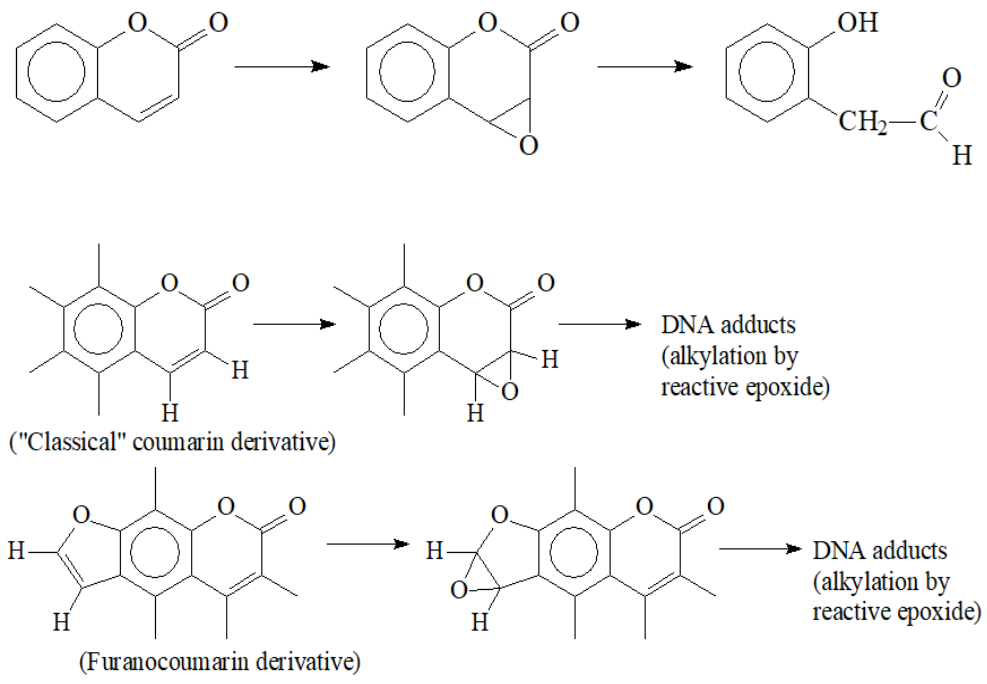


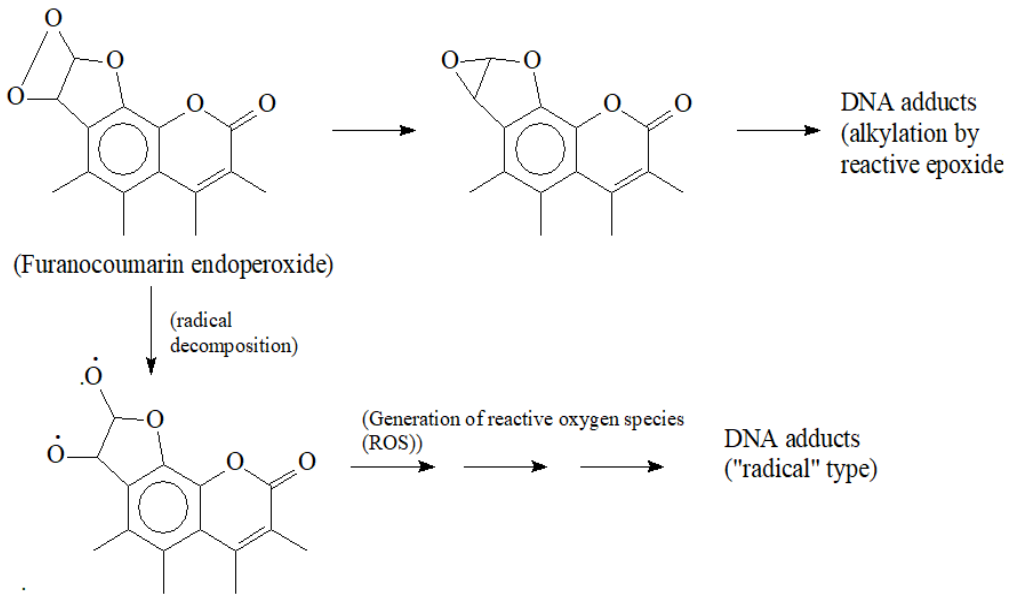
Homolytic



Set of chemicals used for profile development	Conjugated Nitroalkenes and Five-Membered Aromatic Nitroheterocyclics
Data/Knowledge used for profile development	An extensive review of the literature was 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*, Chemical Carcinogenesis Research Information System; http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+ccris:@term+@rn+443-48-1.". 11. Wang, Canc. Res. **35** (1975), 3611 – 3617. 12. Ramos, Mutat. Res. **390** (1997), 233 – 238. 13. CCRIS: Benznidazole CASRN: 22994-85-0, Toxicology Data Network, U.S. National Library of Medicine**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+22994-85-0. 14. Gene-Tox: Misonidazole CASRN: 13551-87-6, Toxicology Data Network, U.S. National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+genetox:@term+@rn+@rel+13551-87-6 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.

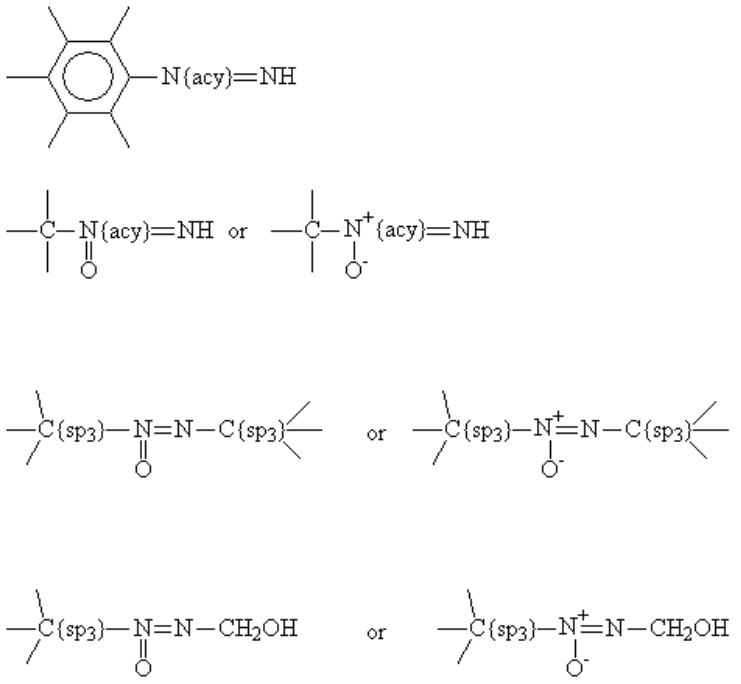
Individual profile/alert	
Name	Coumarins
Type of profile	Structural alert

Description/applicability domain	
Mechanism	**S_N2 Direct acting epoxides formed after metabolic activation, Radical ROS generation, Non-covalent interactions DNA intercalation & S_N1 DNA alkylation**
	

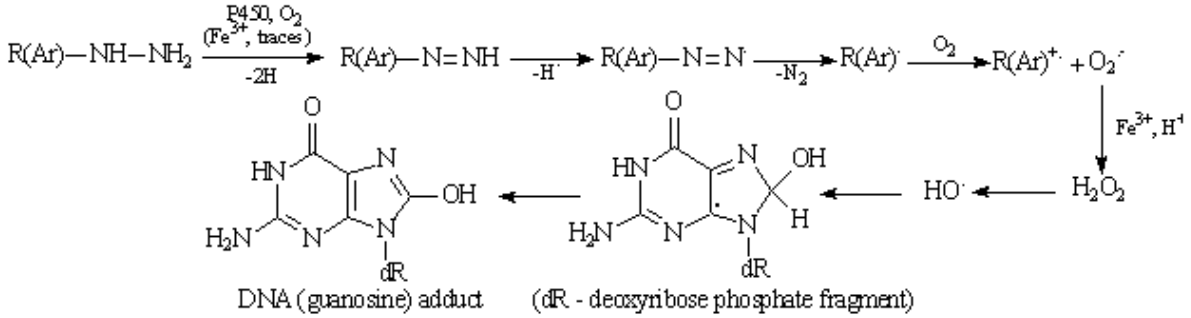


Set of chemicals used for profile development	Coumarins
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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 10.2019 . 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 10.2019. 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)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. 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 and Azoxyalkanes
Type of profile	Structural alert

Description/applicability domain	
Mechanism	**Radical ROS generation (indirect) and S_N1 Direct nucleophilic attack on diazonium cation (DNA alkylation)**

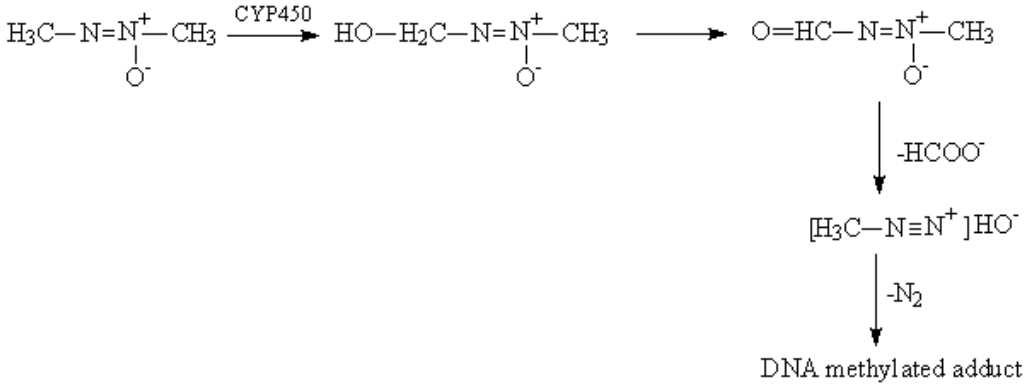
On the basis of the available literature data, the following generalized scheme, similar to those suggested for *Hydrazine Derivatives* and *Arenediazonium Salts* can be assumed to operate *via* radical mechanism by reactive oxygen species (ROS) formation [2 – 6] as shown in Scheme 1



Scheme 1

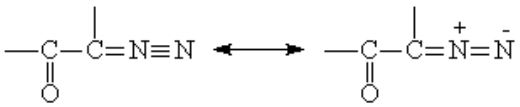
ROS can be also generated as a result from oxidation/reduction processes in bacteria without addition of exogenous S9 system. In such a case, the radical mechanism discussed above is likely to operate.

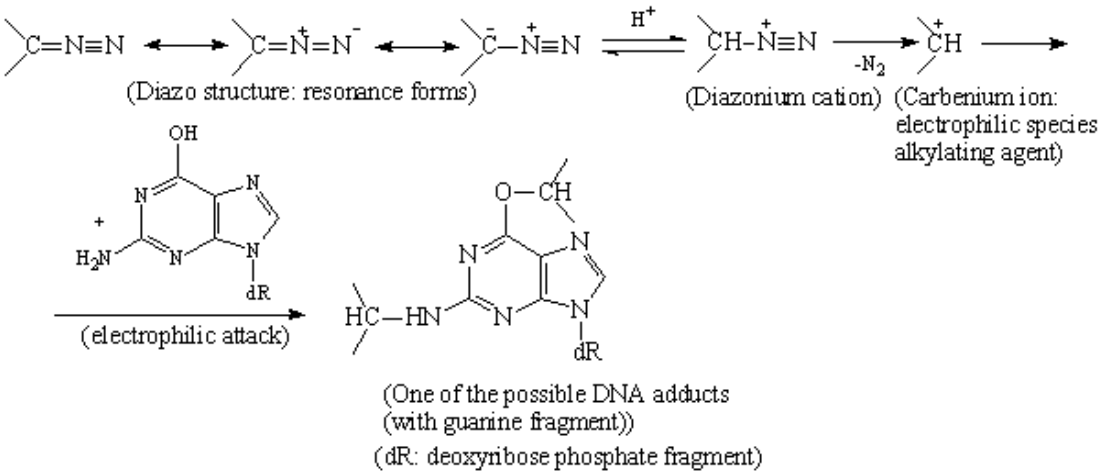
The metabolism of both the azoxymethane and methylazoxymethanol acetate is associated with an ester hydrolysis (for methylazoxymethanol acetate only), and microsomal oxidative N-dealkylation. The mutagenicity and DNA reactivity effects could be mainly due to generation of diazene and alkyl radicals or carbenium and alkanediazonium ions. The following mechanistic scheme of generation of reactive species has been suggested [9, 10] as shown in Scheme 2



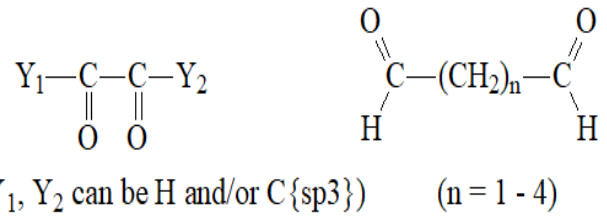
Scheme 2

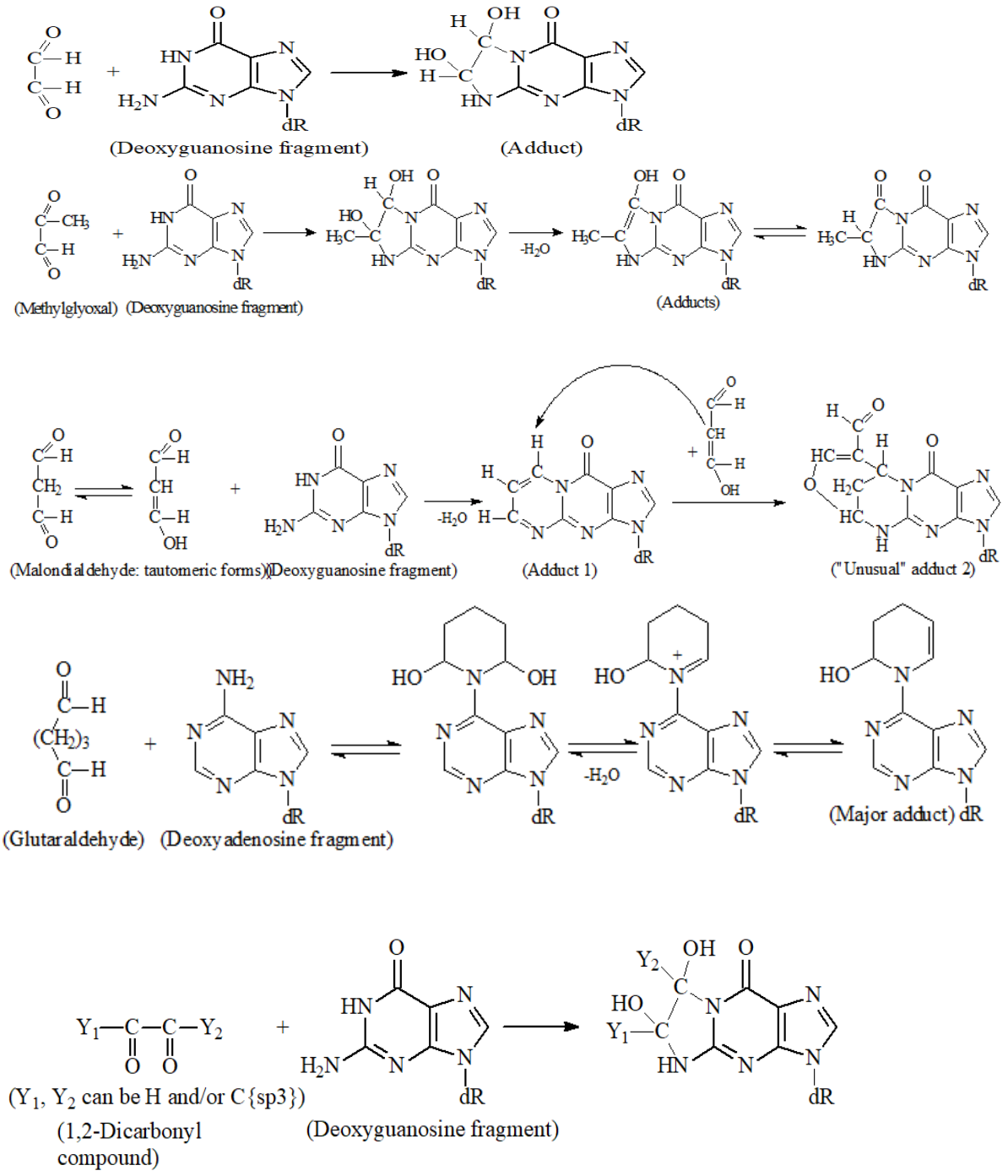
Set of chemicals used for profile development	Diazenes and Azoxyalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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. 7. CCRIS: *Methylazoxymethanol Acetate*, Toxicology Data Network, U.S. National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+592-62-1. 8. CCRIS: *Azoxymethane*, Toxicology Data Network, U.S. National Library of Medicine**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+25843-45-2). 9. Sohn, O. S., Carcinog. **12**(1) (1991), 127 – 131. 10. Campbell, Canc. Res. **38** (1978), 4585 – 4590. 11. Xiao, Mutagen. 11(3) (1996), 241 – 245.

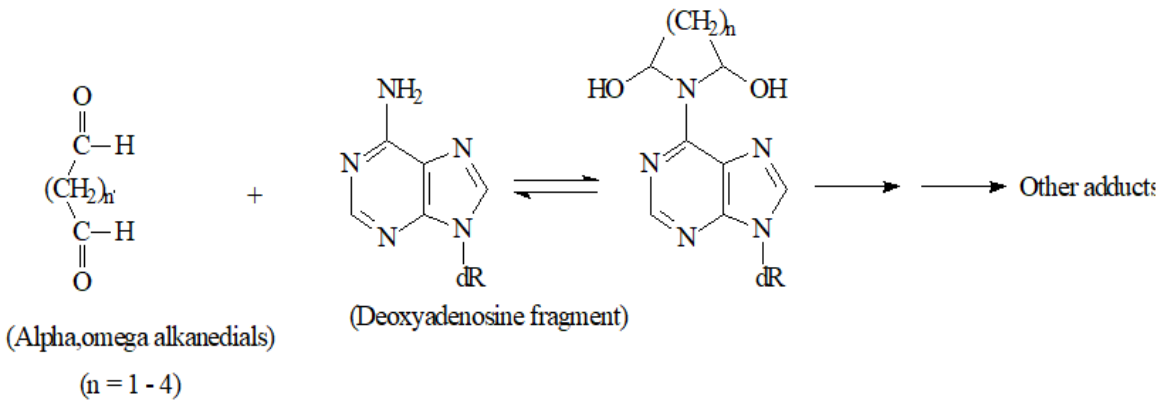
Individual profile/alert	
Name	Diazoalkanes
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N1 Alkylation by carbenium ion formed**
The following mechanistic scheme for DNA alkylation by this class of compounds can be assumed based on literature:	



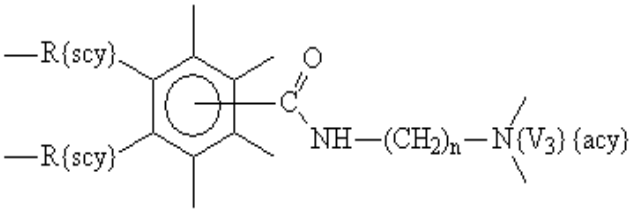
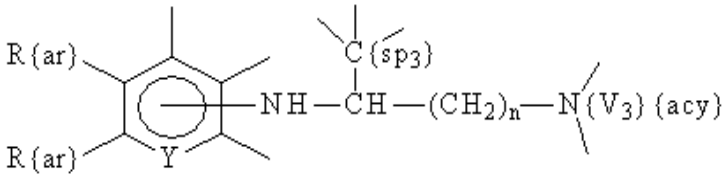
Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.L. Fishbein, 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 10.2019. 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. http://www.collectionscanada.gc.ca/obj/s4/f2/dsk1/tape7/PQDD_0028/NQ51008.pdf. Last visited 10.2019.. 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	 (Y_1, Y_2 can be H and/or C{sp3}) (n = 1 - 4)
Mechanism	**A_N2 Shiff base formation**

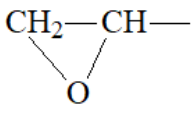

(Deoxyguanosine fragment)
(Adduct)
(Methylglyoxal)
(Deoxyguanosine fragment)
(Adducts)
(Malondialdehyde: tautomeric forms)
(Deoxyguanosine fragment)
(Adduct 1)
("Unusual" adduct 2)
(Glutaraldehyde)
(Deoxyadenosine fragment)
(Major adduct)
(Y1, Y2 can be H and/or C{sp3})
(1,2-Dicarbonyl compound)
(Deoxyguanosine fragment)

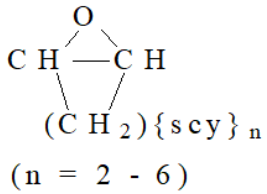


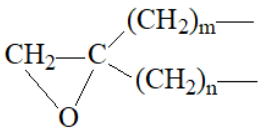
Set of chemicals used for profile development	Dicarbonyl Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
qReferences	1. Bjeldanes, L. F., Mutat. Res. **67** (1979), 367 – 371. 2. Dorado, L., Mutat. Res. **269** (1992), 301 – 306. 3. Mellado, J. M. R., Mutat. Res. **304** (1994), 261 – 264. 4. Shapiro, R., Biochem. **5**(9) (1966), 2799 – 2807). 5. Frishmann, M., Chem. Res. Toxicol. **18** (2005), 1586 – 1592). 6. More, S. S., J. Agric. Food Chem. **60** (2012), 3311 – 3317. 7. Marnett, L. J., J. Am. Chem. Soc. **108** (1986), 1348 – 1350). 8. Olsen, R., Chem. Res. Toxicol. **20** (2007), 965 – 974.

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.

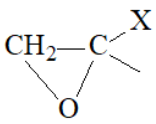
Individual profile/alert	
Name	Epoxides and Aziridines
Type of profile	Structural alert
Description/applicability domain	



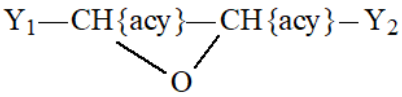


(m = 1 - 3; n = 1 - 3)

(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)



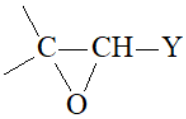
Y_1 and Y_2 can be the following structural moeties:

(a) (-CH_2)$_n$H (n = 1 - 2)

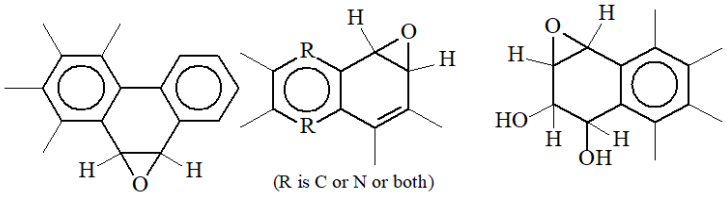
(b) CH_2{scy} and -CH{scy}=CH{scy}-

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

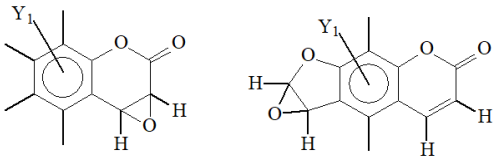
(d) Y_1 is Cl or Br; Y_2 is C



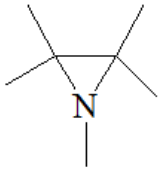
(Y can be Cl, Br or -CHO)

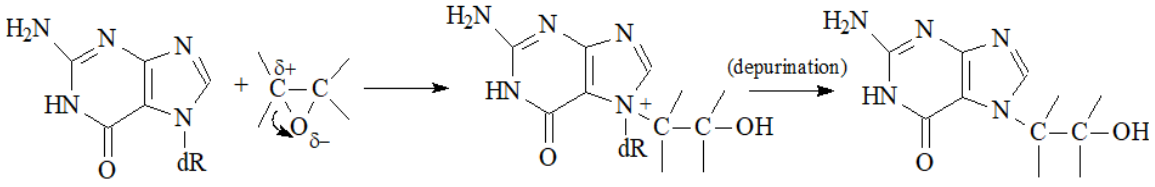


(R is C or N or both)

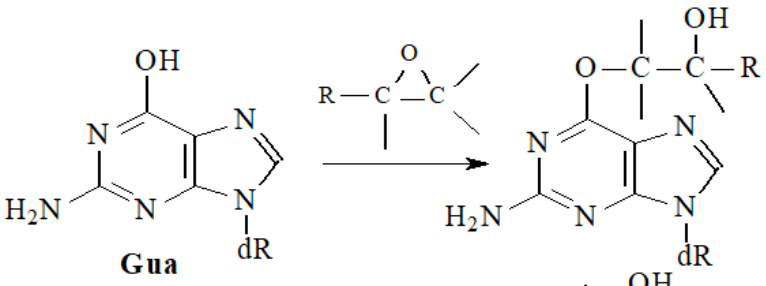


(Y_1 is –H (all) or combinations of H and –OCH_3, -NH_2, -NO_2, -NHOH, -CH_3, -CH_2X (X is Cl, Br); no more than one substituent

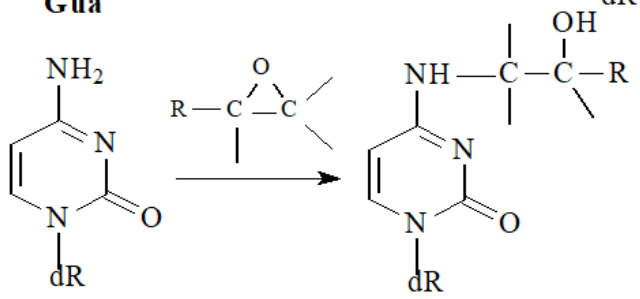
	
Mechanism	**S_N2 Alkylation, direct acting epoxides and related**



(DNA fragment)
(dR - deoxyribose phosphate fragment)

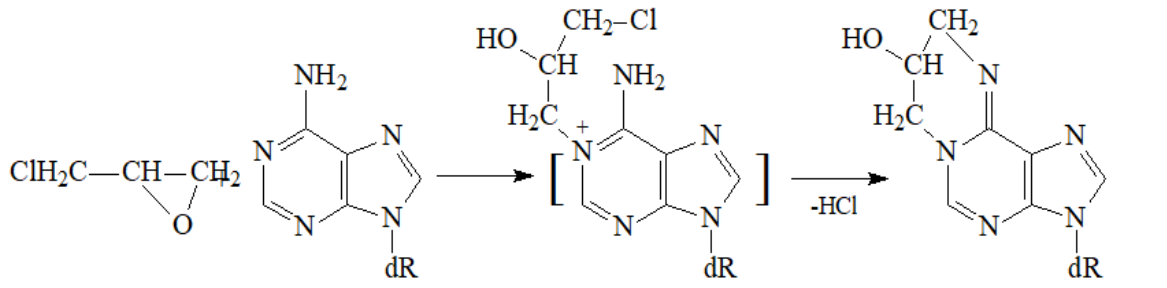


Gua

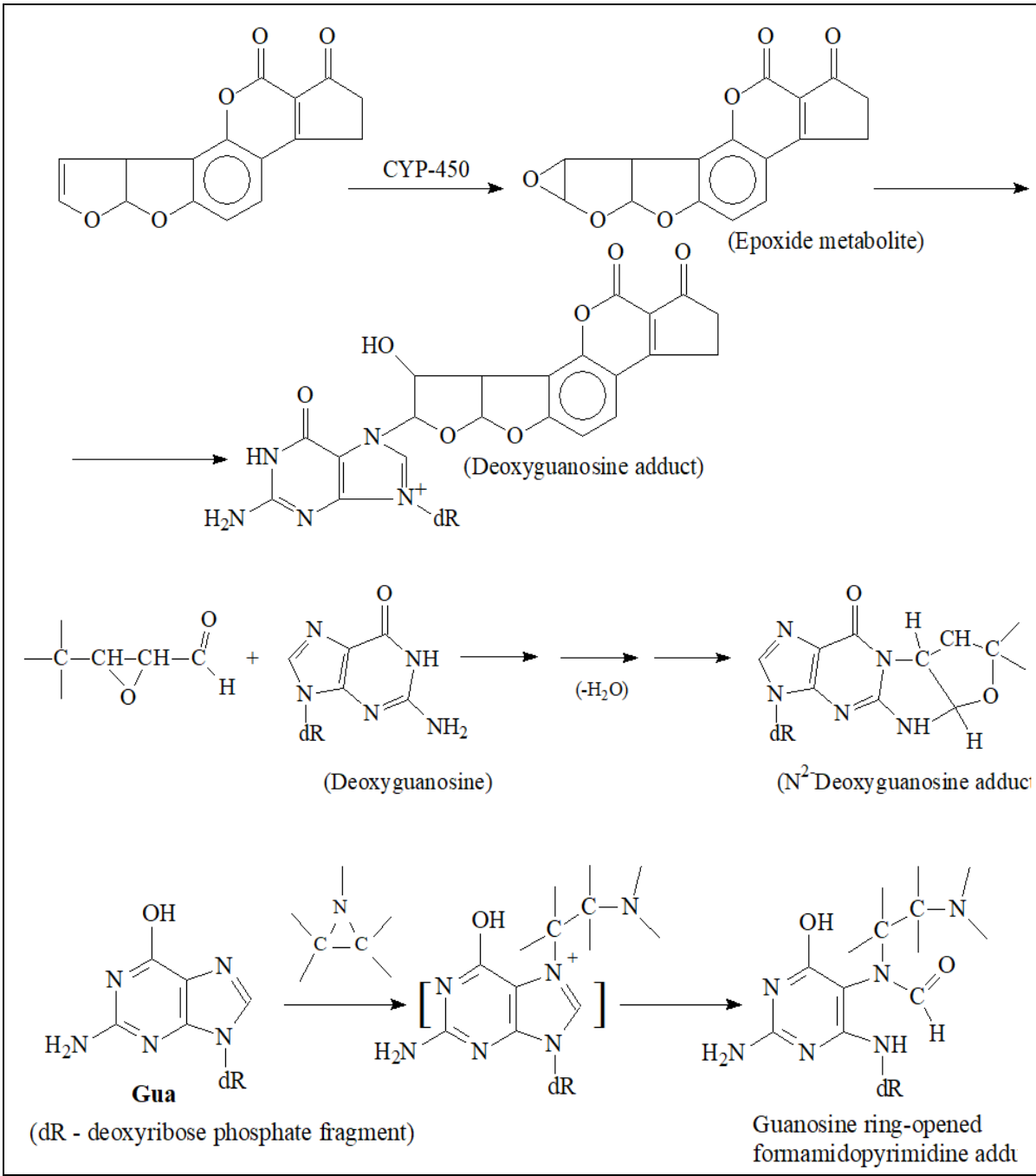


Cyt

(dR - deoxyribose phosphate fragment)

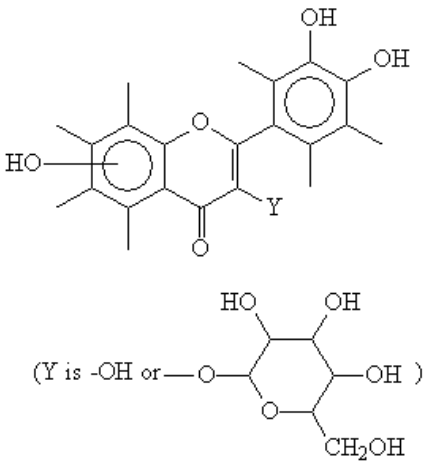


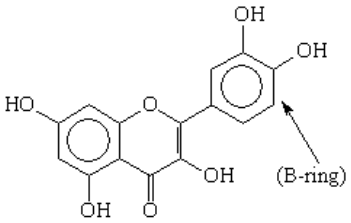
1,N6-2-hydroxypropanoadenine adduct



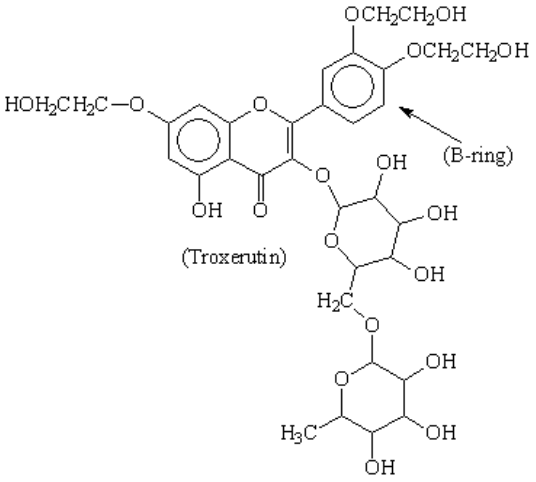
Set of chemicals used for profile development	Epoxides and Aziridines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
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*, Chemical Carcinogenesis Research Informartion System (CCRIS), US National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. 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. 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; http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+ccris:@term+@rn+1321-74-0

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]:	

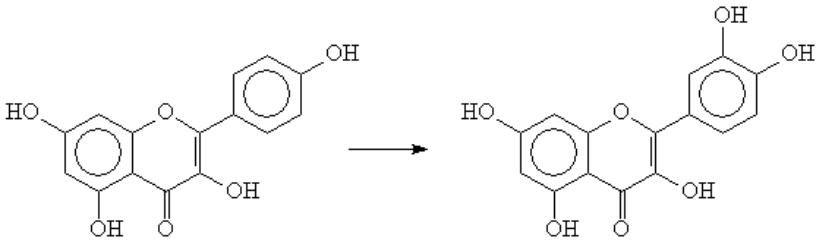


(Quercetin)



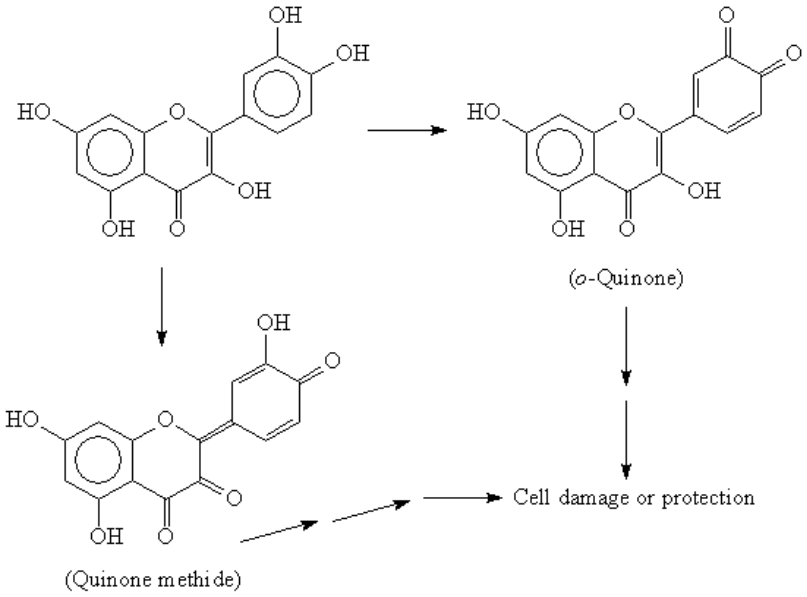
(Troxerutin)

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:

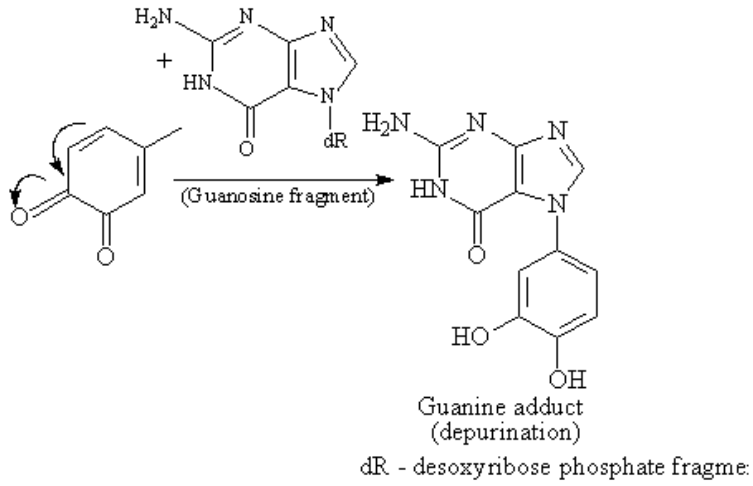


(Kaempferol)

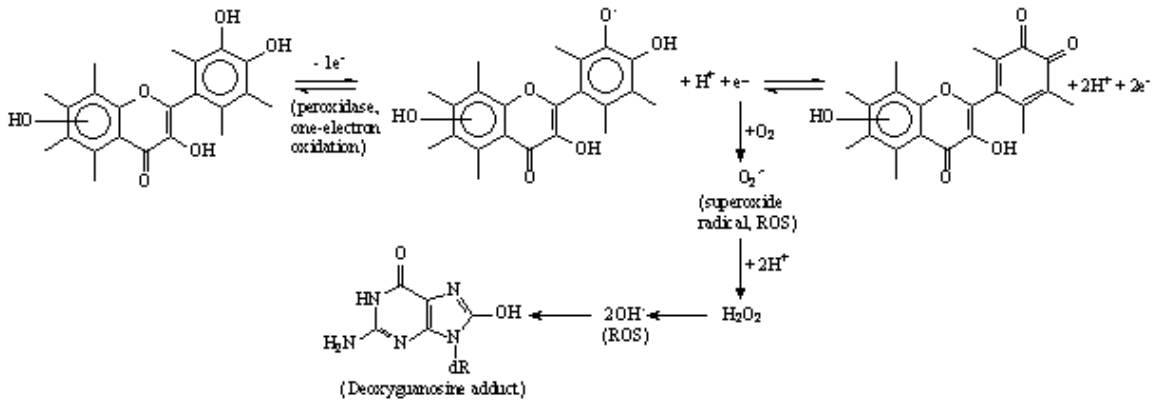
For example, quercetin can generate active *o*-quinone/quinone methide metabolites by the following pathways [7]:



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]:

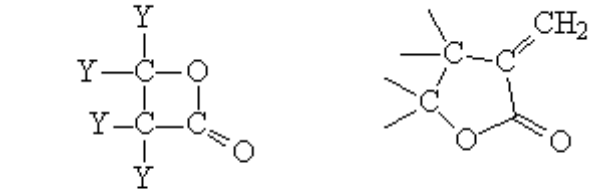


If the presence of endogenous peroxidase enzymes in the "classical" *Salmonella typhimurium* strains is assumed, the following mechanistic scheme involving the formation of ROS could explain the observed positive *in vitro* bacterial mutagenicity results for a few flavonoids such as quercetine as parent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

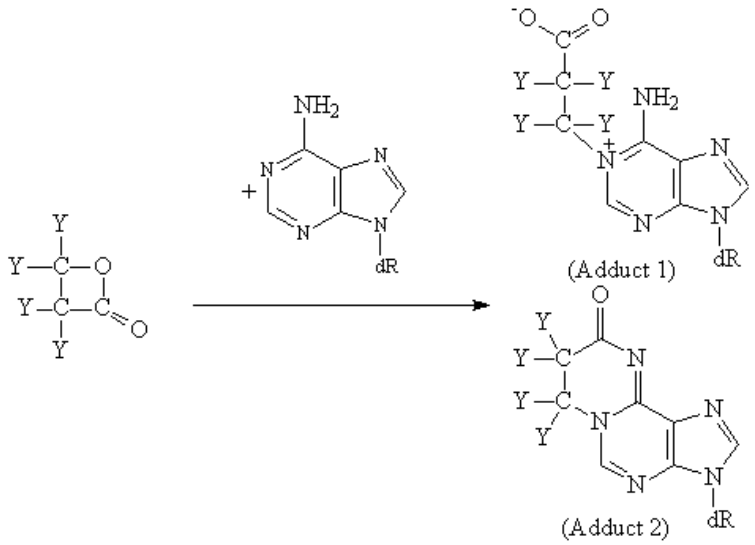


Set of chemicals used for profile development	Flavonoids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.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

	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?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.

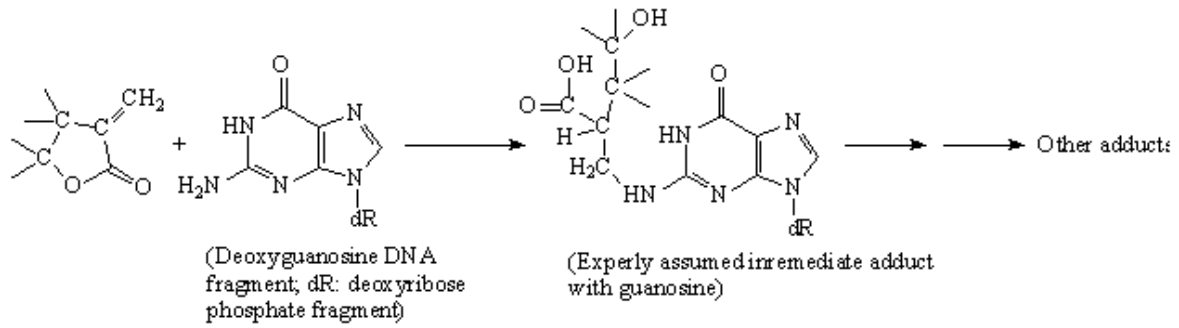
Individual profile/alert	
Name	Four- and Five- membered Lactones
Type of profile	Structural alert
Description/applicability domain	 (Y can be -H and/or $-CH_3$, C_2H_5)
Mechanism	**Ring opening S_N2 reaction (alkylation) and A_N2 Michael-type addition on α,β-unsaturated carbonyl compounds**

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:

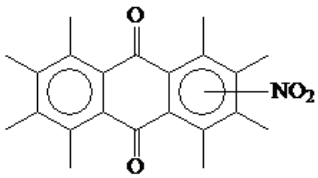
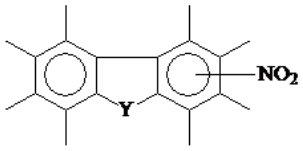


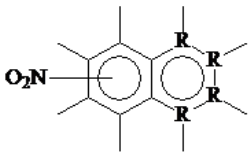
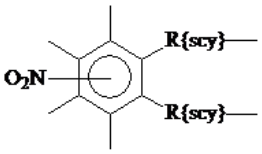
Scheme 1

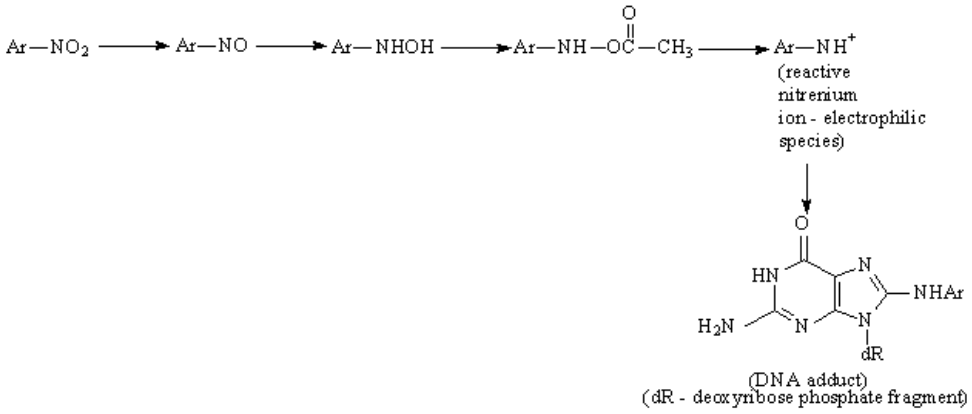
The conjugated system in the molecular structure of some alpha-methylidene-γ-butyrolactone derivatives might actually cause bacterial mutagenicity by expertly assumed (hypothetic) mechanistic Scheme 2, similar to that for some α,β-unsaturated systems:



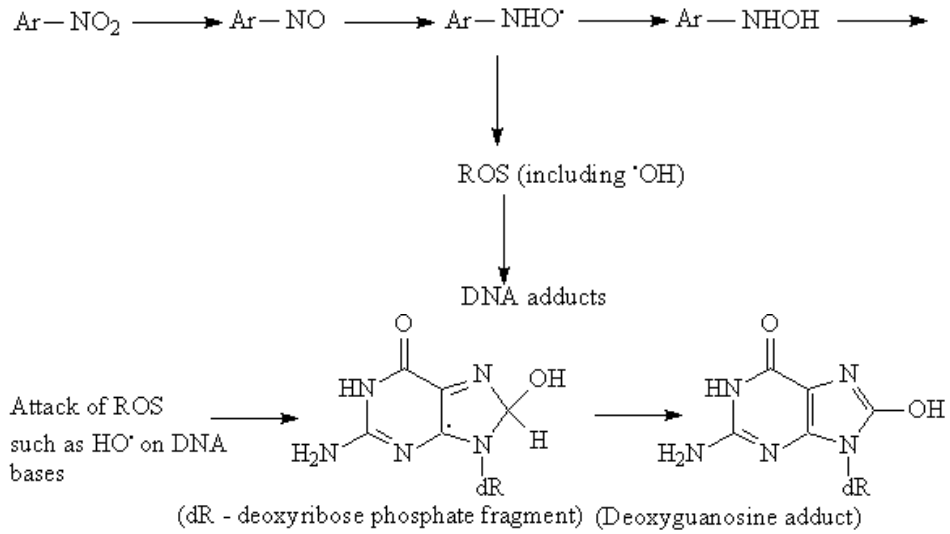
Set of chemicals used for profile development	Four- and Five-Membered Lactones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed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

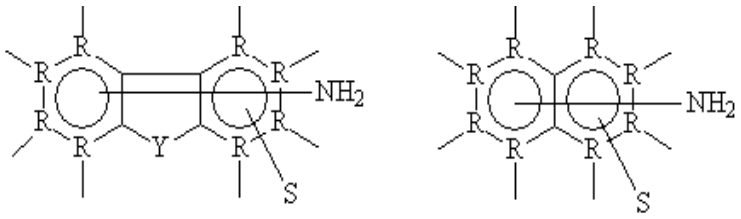


Homolytic

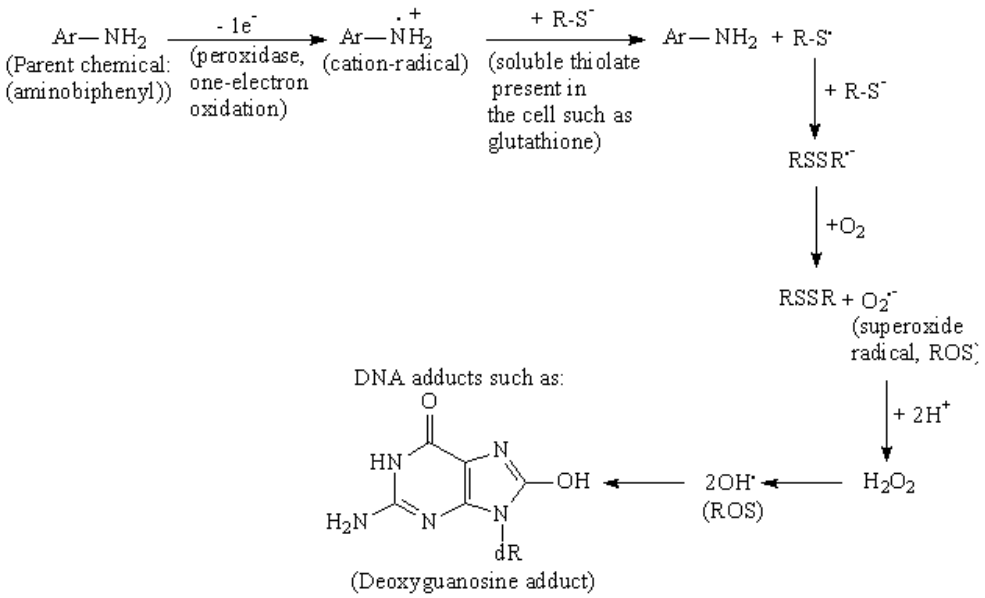


Set of chemicals used for profile development	Fused-Ring Nitroaromatics
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
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 $-NO_2$; 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**

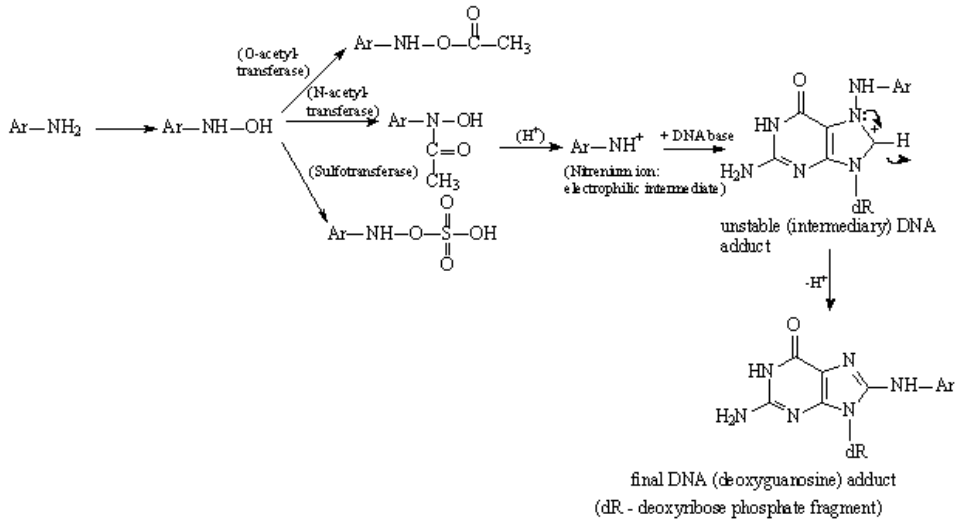
It is expertly assumed that the presence of electron-donating substituents with either +I or +M-effects, together with the planar structure and conjugation effects may determine the positive mutagenicity of some polycyclic aromatic amines as parent chemicals. In addition, endogenous generation of reactive 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

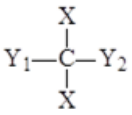
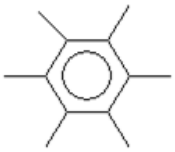
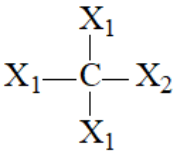
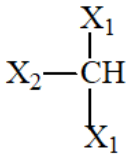
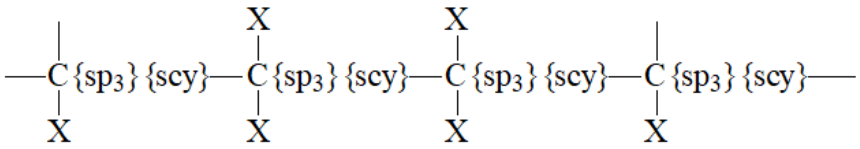
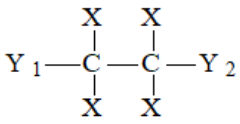
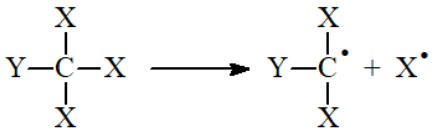
formation of *N-hydroxylamine* intermediate. These reactive N-hydroxylamine derivatives (metabolites) can undergo phase II conjugation, to generate the more reactive N-O sulfate and/or N-O acetyl conjugates. The excellent leaving group capability of sulfonyloxy- and acetoxy-functionalities in these conjugates 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]:

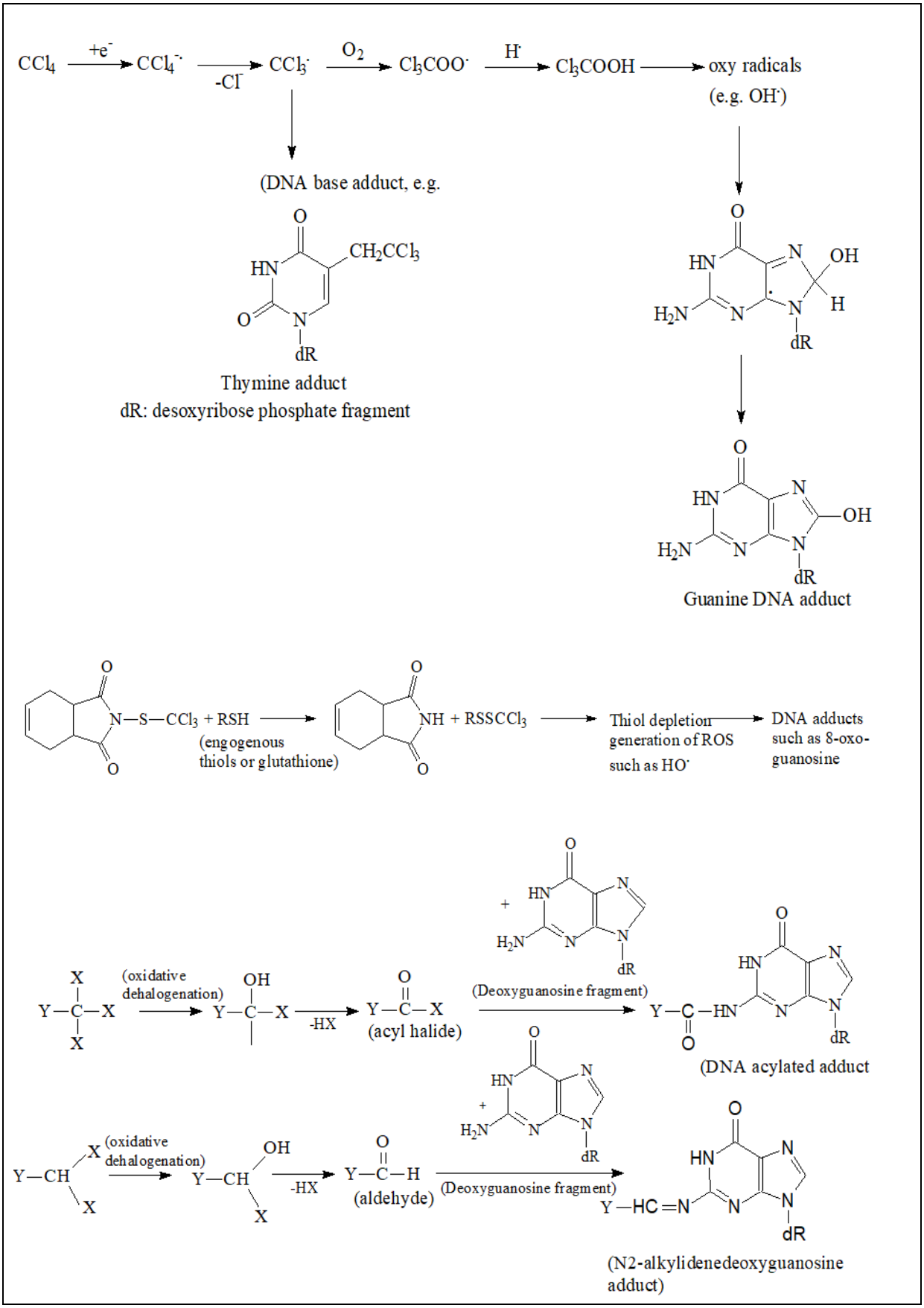


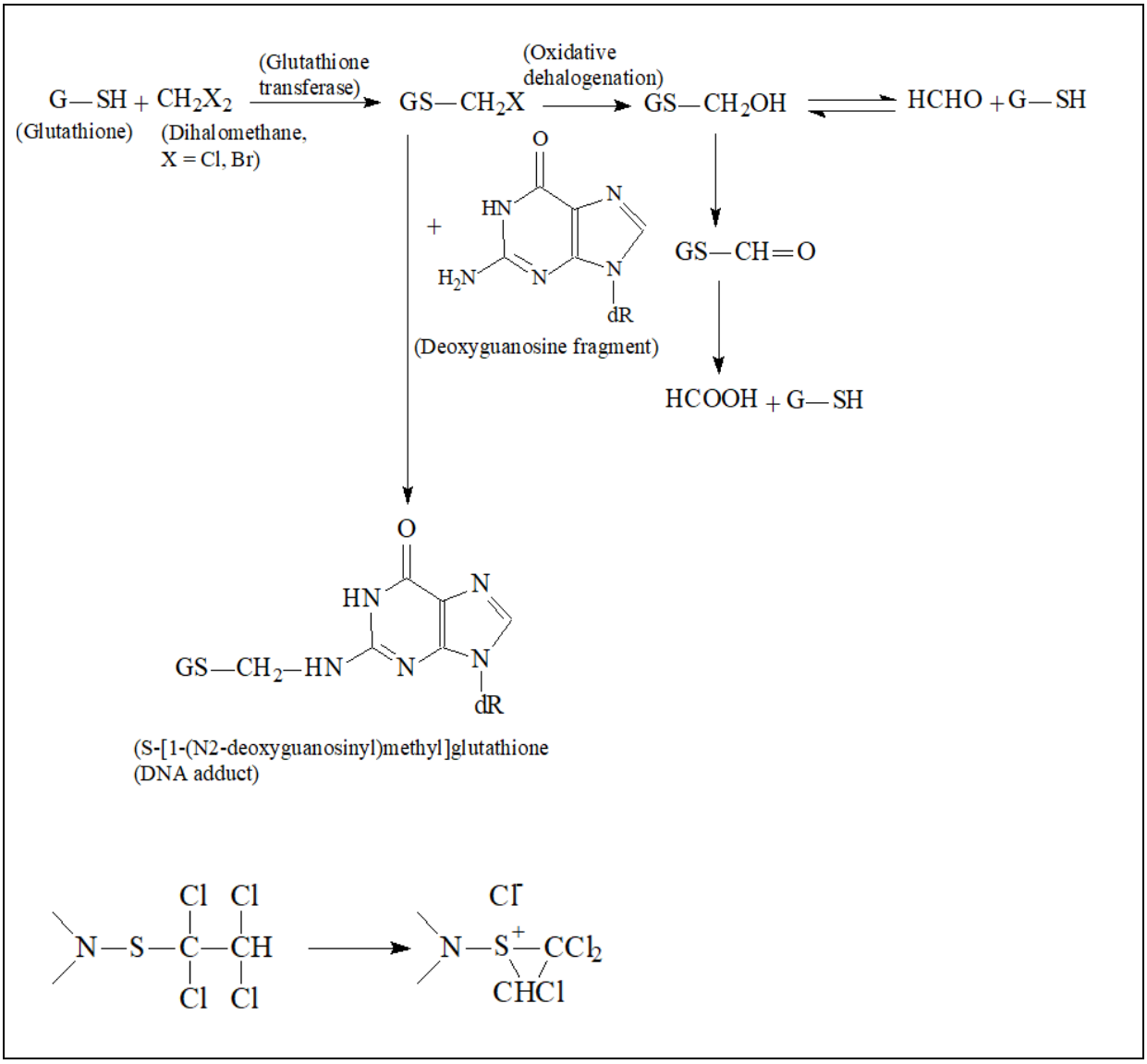
Scheme 2

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)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS). 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.

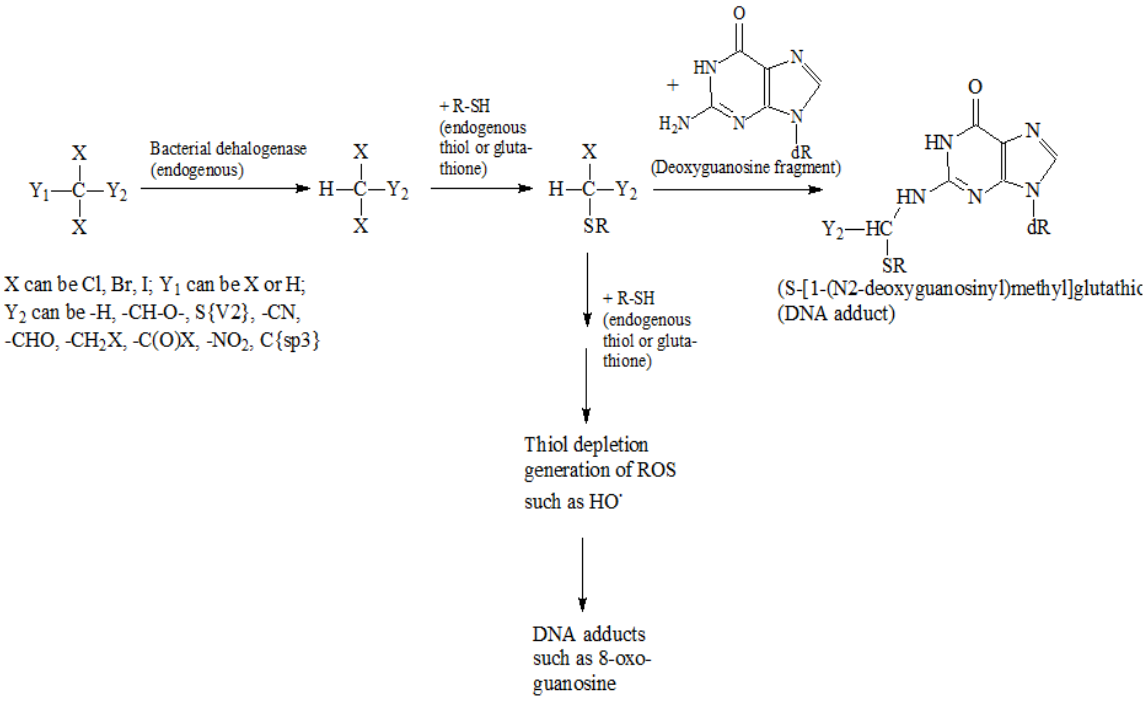
Individual profile/alert	
Name	Geminal Polyhaloalkane Derivatives
Type of profile	Structural alert

Description/applicability domain	 X can be Cl, Br, I; Y_1 can be X or H; Y_2 can be -H, -CH-O-, S{V2}, -CN, -CHO, $-CH_2X$, -C(O)X, $-CH_3$, -C(O)-O- (carboxyl group attached *via* C-atom); Y_2 can be also:  (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  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
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**
	


CCl4
+e-
CCl4-·
-Cl-
CCl3·
O2
Cl3COO·
H·
Cl3COOH
oxy radicals
(e.g. OH·)
(DNA base adduct, e.g.
CH2CCl3
HN
O
N
dR
Thymine adduct
dR: desoxyribose phosphate fragment
OH
H2N
Guanine DNA adduct
N—S—CCl3 + RSH
(engogenous
thiols or glutathione)
NH + RSSCCl3
Thiol depletion
generation of ROS
such as HO·
DNA adducts
such as 8-oxo-
guanosine
(oxidative
dehalogenation)
-HX
(acyl halide)
(Deoxyguanosine fragment)
(DNA acylated adduct
(aldehyde)
(N2-alkylidenedeoxyguanosine
adduct)

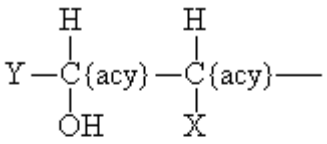
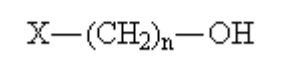

G—SH + CH2X2
(Glutathione)
(Dihalomethane, X = Cl, Br)
(Glutathione transferase)
GS—CH2X
(Oxidative dehalogenation)
GS—CH2OH
HCHO + G—SH
(Deoxyguanosine fragment)
GS—CH=O
HCOOH + G—SH
GS—CH2—HN
(S-[1-(N2-deoxyguanosinyl)methyl]glutathione
(DNA adduct)

Cl Cl
N—S—C—CH
Cl Cl
Cl⁻
N—S—CCl2
CHCl



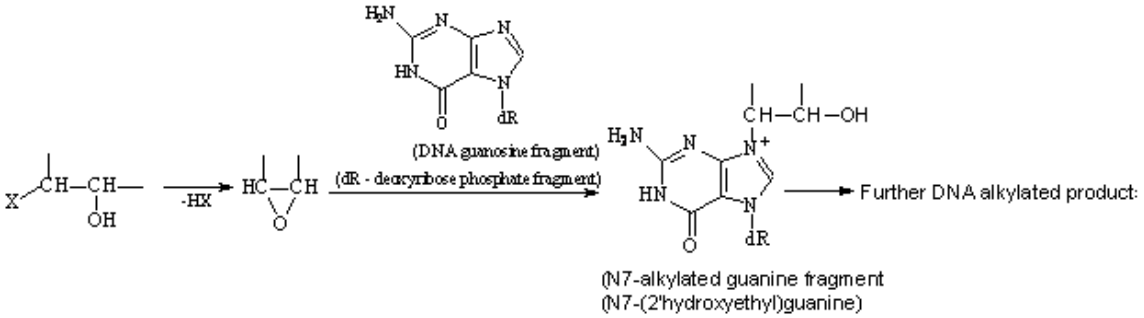
Set of chemicals used for profile development	Geminal Polyhaloalkane Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Strubel, K., Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128. 2. *Chemical Carcinogenesis Research Information System (CCRIS)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS 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**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+2314-97-8. 8. CCRIS: 1,1,1-Trichloroethane CASRN: 71-55-6, Toxicology Data Network, U.S. National Library of Medicine**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+71-55-6. 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; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+2425-06-1 . 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). 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; http://oehha.ca.gov/water/phg/pdf/carbtet.pdf. 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. 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 10.2019. 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; http://www.biomedsearch.com/nih/Mutagenicity-some-commercially-available-nitro/362191.html. 28. Wang, M., Chem. Res. Toxicol. **13**(11) (2000), 1149 – 1157; http://pubs.acs.org/doi/abs/10.1021/tx000118t. 29. Anders, M. W., Drug Metabol. Rev. **36** (3 – 4) (2004), 583 – 594. 30. Bernard, Br. K., Inter. J. Toxicol. **19** (2000), 43 – 61. 31. Mejer, J., Chem.-Biol. Interact. **31** (1980), 247 – 254. 32. Morita, T., Mutat. Res. **802** (2016), 1 – 29. 33. Sato, T., The Science of Total Environment **46** (1985), 229 – 241.

Individual profile/alert	
Name	Haloalcohols
Type of profile	Structural alert
Description/applicability domain	 (Y can be C(sp3) or -H) (X = Cl, Br, J)  (X is Cl, Br, n = 3 - 10
Mechanism	**S_N2 Alkylation, direct-acting epoxide formed after E2 reaction and**

	Radical ROS formation after GSH depletion (indirect)

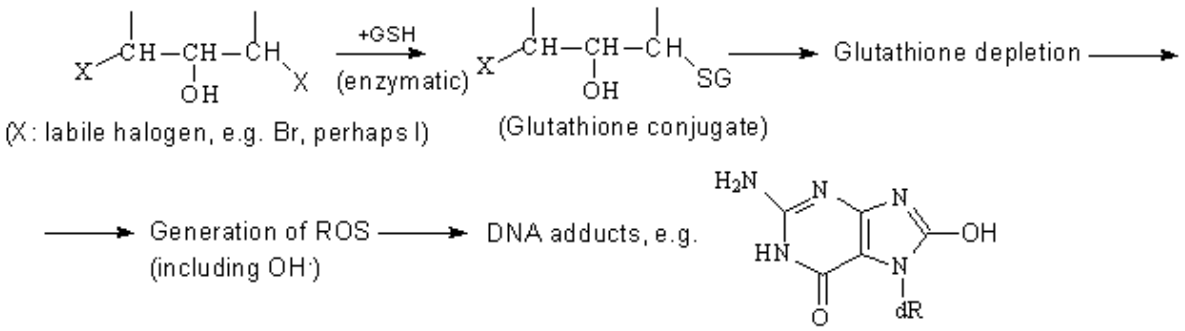
The metabolism of 1,3-dichloropropan-2-ol is likely to produce a reactive epoxide intermediate that could damage DNA, and this compound was found to be mutagenic to *Salmonella typhimurium* strains TA1535 and/or TA 100. 2,3 Dichloropropan-1-ol, on the other hand, was also mutagenic *in vitro* in *Salmonella 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]:



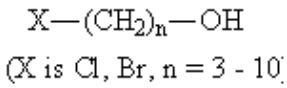
Scheme 1

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:

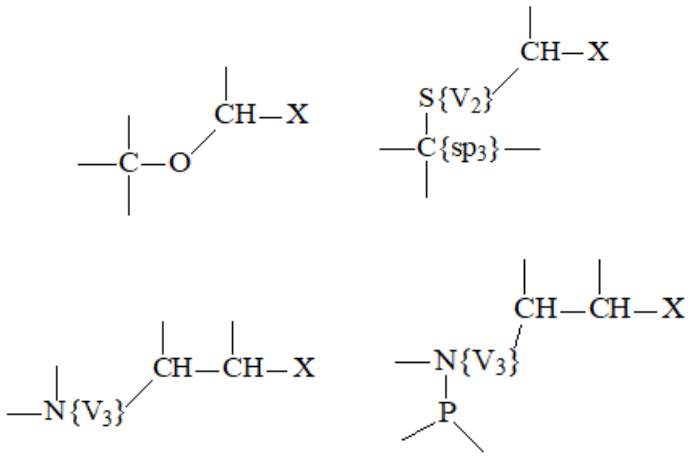


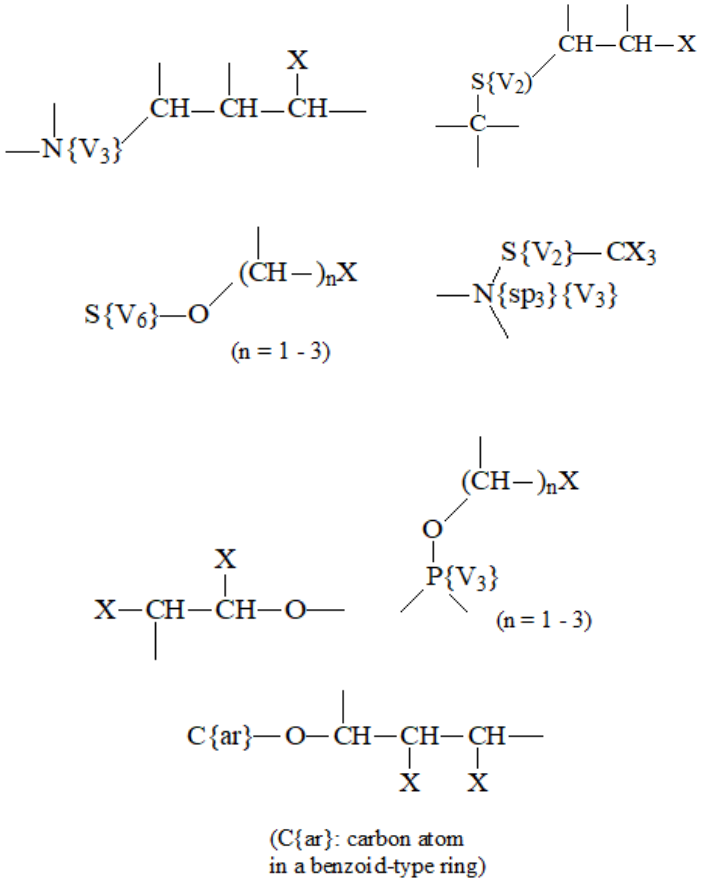
Such mechanistic scheme could also apply to haloalcohols of the type:



Scheme 2

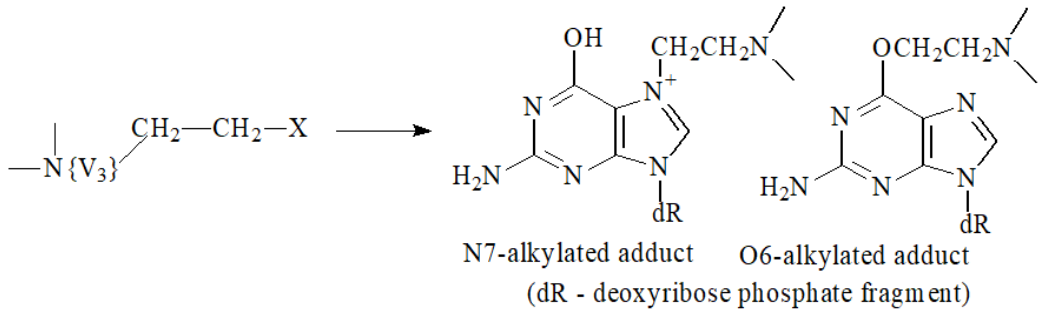
Set of chemicals used for profile development	Haloalcohols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

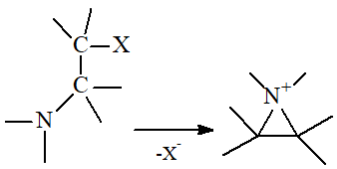
Individual profile/alert	
Name	Haloalkane Derivatives Containing Chain Heteroatom
Type of profile	Structural alert
Description/applicability domain	



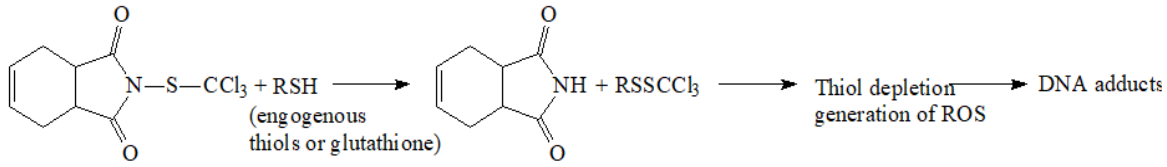
Mechanism	**S_N2 Alkylation, nucleophilic substitution at sp3 carbon atom & Radical Generation of ROS by glutathione depletion (indirect)**

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

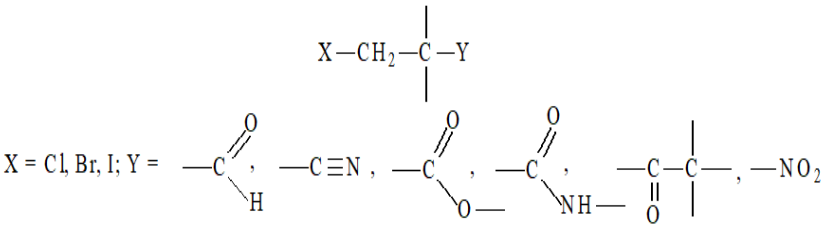




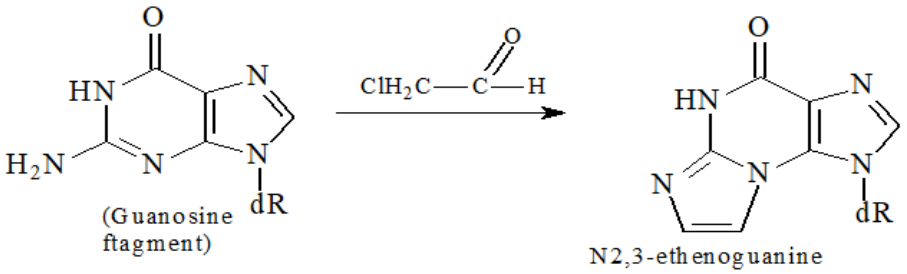
2. Compounds with halogen in α-position with respect to a heteroatom



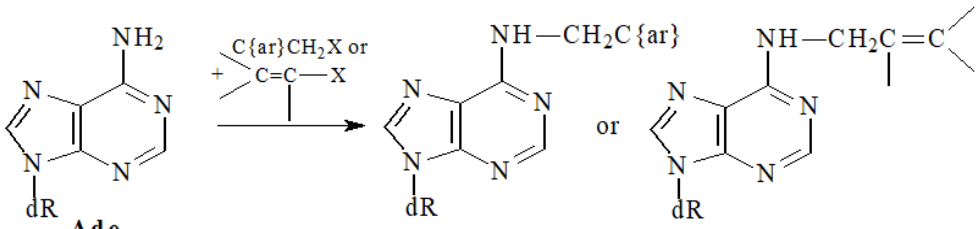
Set of chemicals used for profile development	Haloalkane Derivatives Containing Chain Heteroatom
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kovacic, P., Medical Hypoth.**64** (2005), 104 - 111. 2. *Evidence on the Carcinogenicity of Technical Grade Bis(2-Chloro-1-Methylethyl) Ether*, Final November 1999 (Reproductive and Cancer Hazard Assessment Section Office of Environmental Health Hazard Assessment, California EPA; https://oehha.ca.gov/media/downloads/crnr/bcmeef_1.pdf,, last visited 09.2019. 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); http://www.stormingmedia.us/72/7294/A729492.html 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 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 11. CCRIS: Mephalan, Toxicology Data Network, U.S. National Library of Medicine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+148-82-3 12. CCRIS: Chlomaphazine, Toxicology Data Network, U.S. National Library of Medicine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+494-03-1 13. CCRIS: Uracil Mustard, Toxicology Data Network, U.S. National Library of Medicine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+66-75-1 14. CCRIS: Acrylic Acid, 2-Bromoethyl Ester, Toxicology Data Network, U.S. National Library of Medicine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+4823-47-6

Individual profile/alert	
Name	Haloalkane Derivatives with Labile Halogen
Type of profile	Structural alert
Description/applicability domain	$Y—CH_2X$ (Y can be C{ar}(no X attached to C{ar}, no more than two substituents attached on C{ar} (condensed rings not to be counted)); C=C; NO_2, C(O)O, C(O)H; X is Cl, Br, I) 
Mechanism	**S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom, A_N2 Shiff base formation for aldehydes & S_N2 Acylation involving a leaving group**

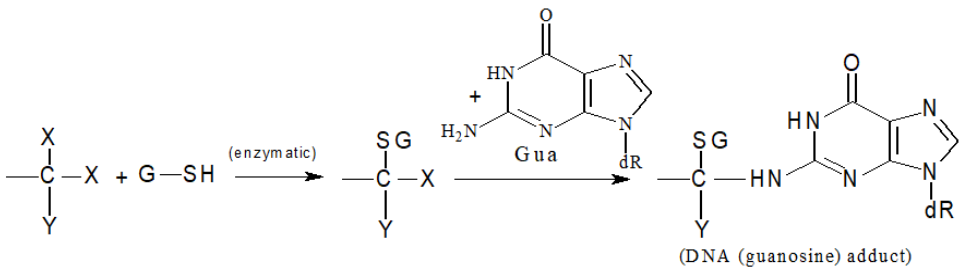
1. Haloalkane derivatives with labile halogen at alpha-position towards other groups



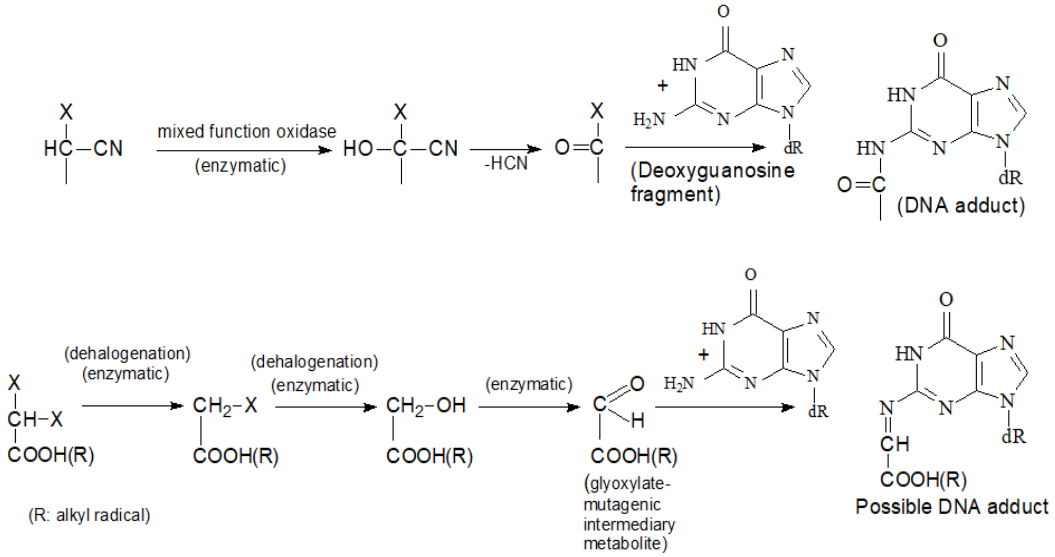
(dR - deoxyribose phosphate fragment)



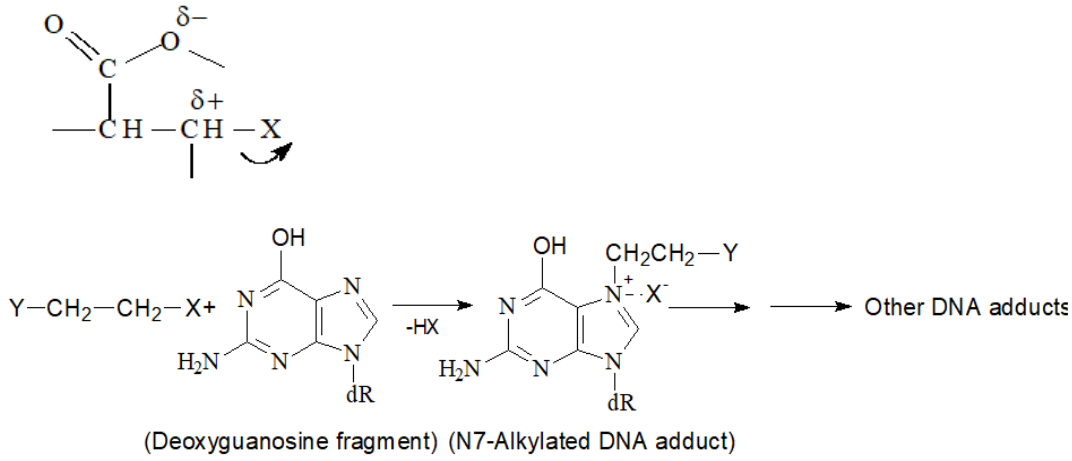
(dR- deoxyribose phosphate fragment)



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

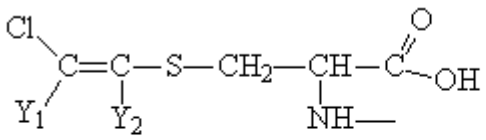


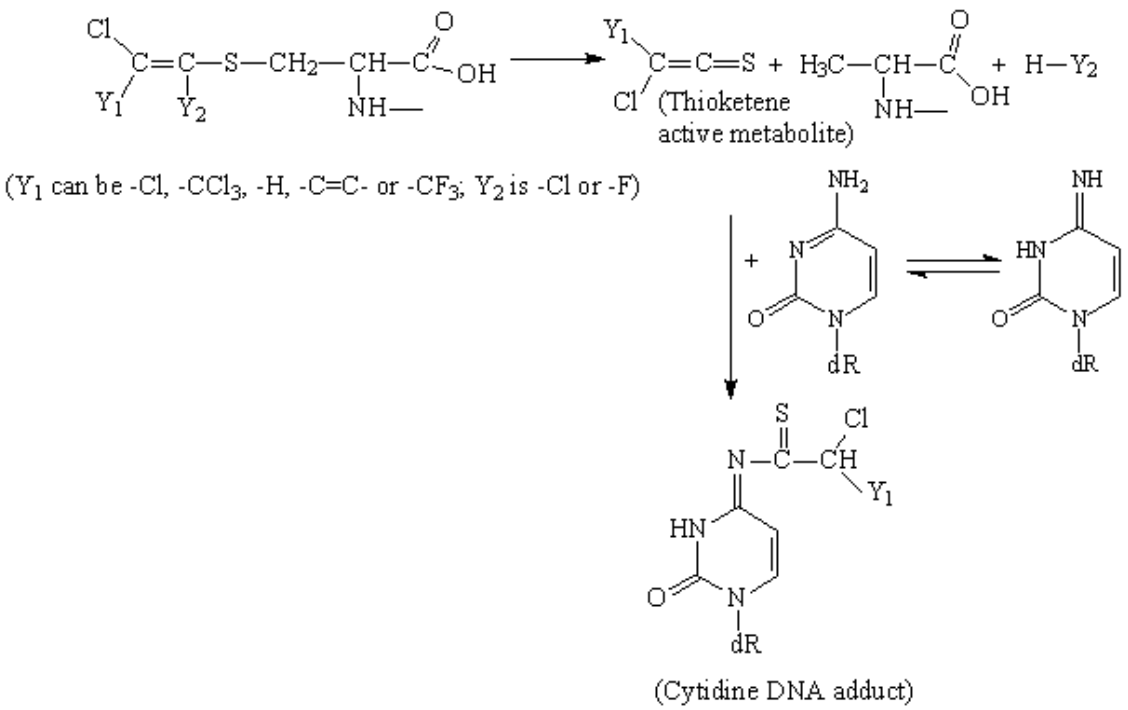
2. Haloalkane derivatives with labile halogen at beta-position towards other groups



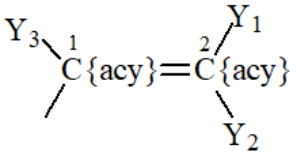
Set of chemicals used for profile development	Haloalkane Derivatives with Labile Halogen
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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; http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=86035, last visited 09.2019. 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. 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. 8. Eder, E., Xenobiotica **12**(12), 1982, 831-848;

	DOI: 10.3109/00498258209038955. 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); http://www.who.int/water_sanitation_health/dwq/chemicals/brominatedaceticacids.pdf 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; http://www.epa.gov/iris/toxreviews/0654tr.pdf. 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; http://www.who.int/water_sanitation_health/dwq/chemicals/monochloroaceticacid.pdf. 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 19. Eder, E., Xenobiotica 12(12), 1982, 831-848; DOI: 10.3109/00498258209038955. 20. McCoy, E. C., Mutat. Res./Fund. Molec. Mechan. Mutag. 57(1) (1978), 11 – 15; http://www.sciencedirect.com/science/article/pii/0027510778902294.

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**

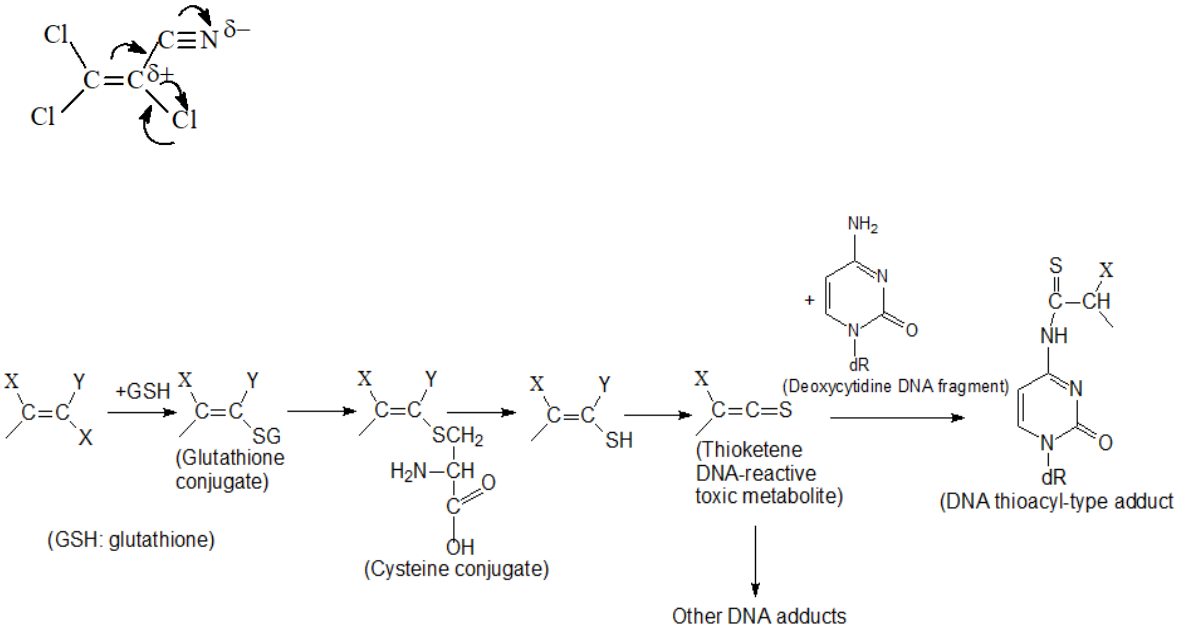


Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Evidence 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 09.2019 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.

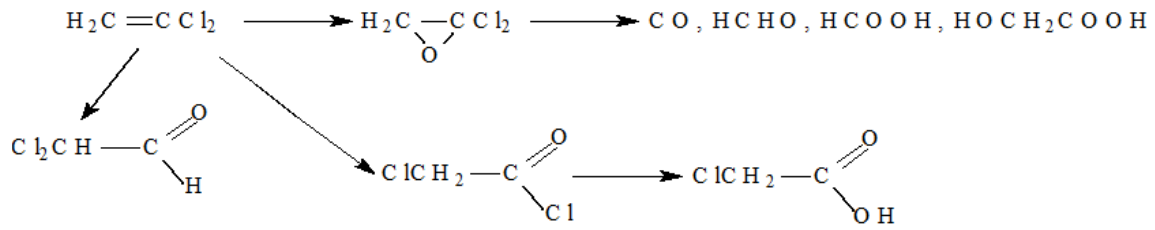
Individual profile/alert	
Name	Haloalkene Derivatives with Electron-Withdrawing Groups
Type of profile	Structural alert
Description/applicability domain	 Y_2 can be $–NO_2$ or –CN or **-C=C-** or Cl or Br or -C(O)O- (attached *via* the carbon of carbonyl group C(O)), or -C(O)C (attached *via* the carbon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

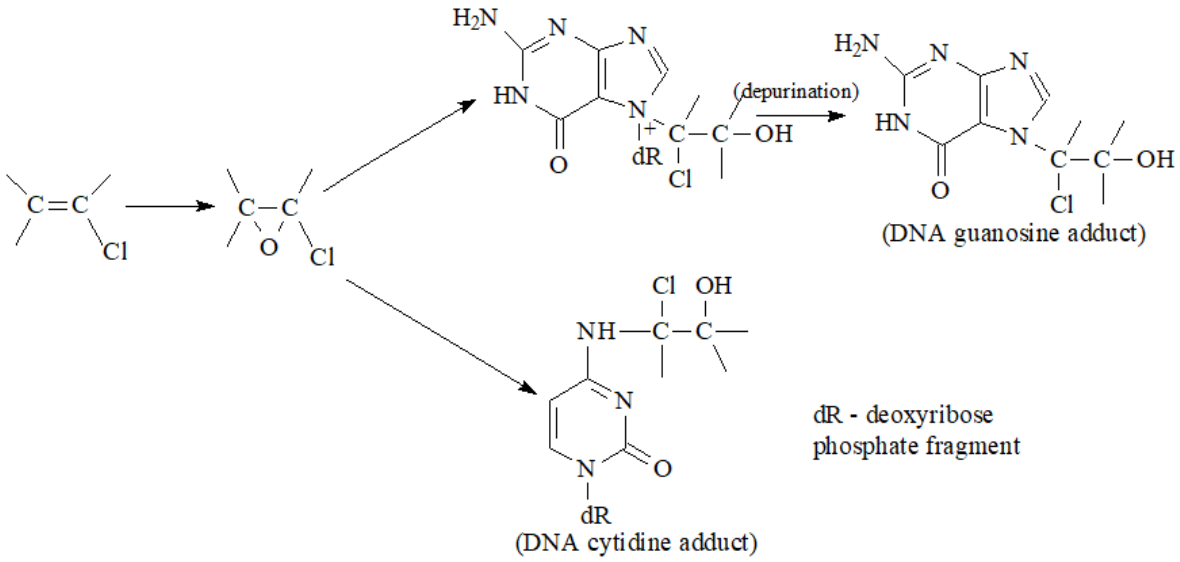
1. Haloalkenes containing halogen(s) and other electron-withdrawing group(s) (EWG).



2. Vinyl-type haloalkenes, not containing other EWGs

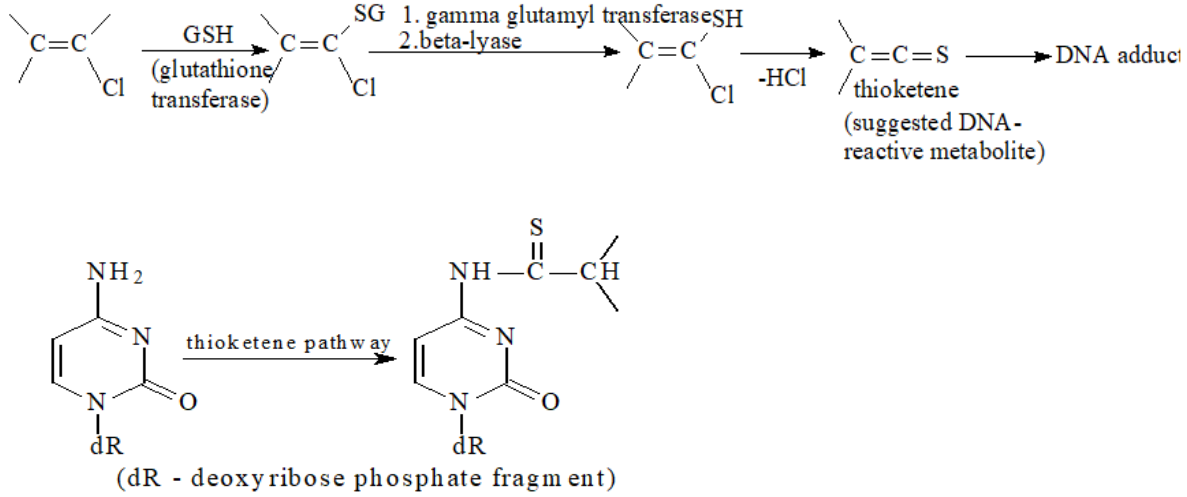


3. Formation of epoxide intermediate that binds covalently to DNA *via* electrophilic mechanism of alkylation towards the biological macromolecule:



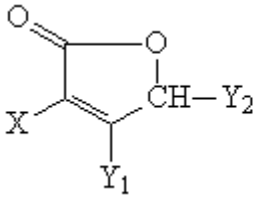
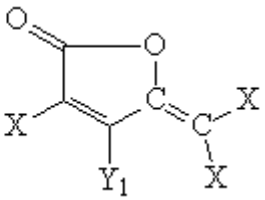
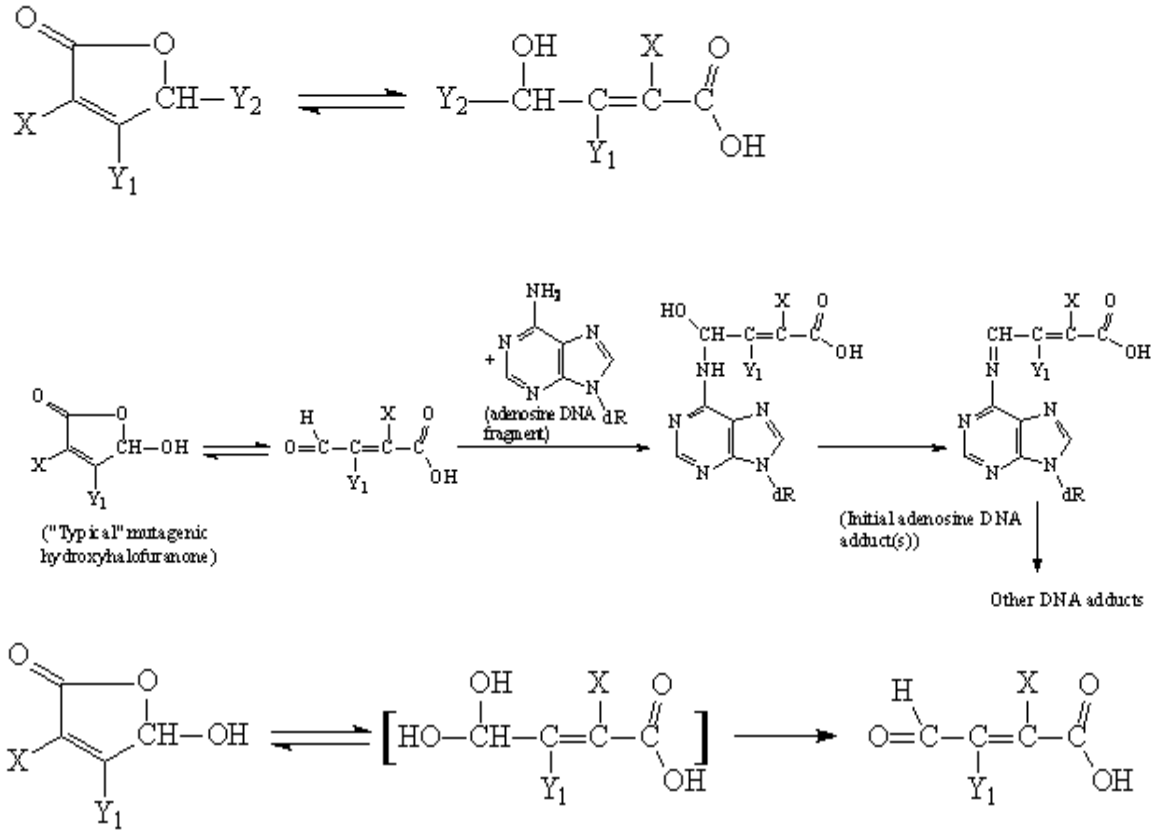
4. Glutathione or thiol activation pathway. In this case, the formation of reactive product that binds to

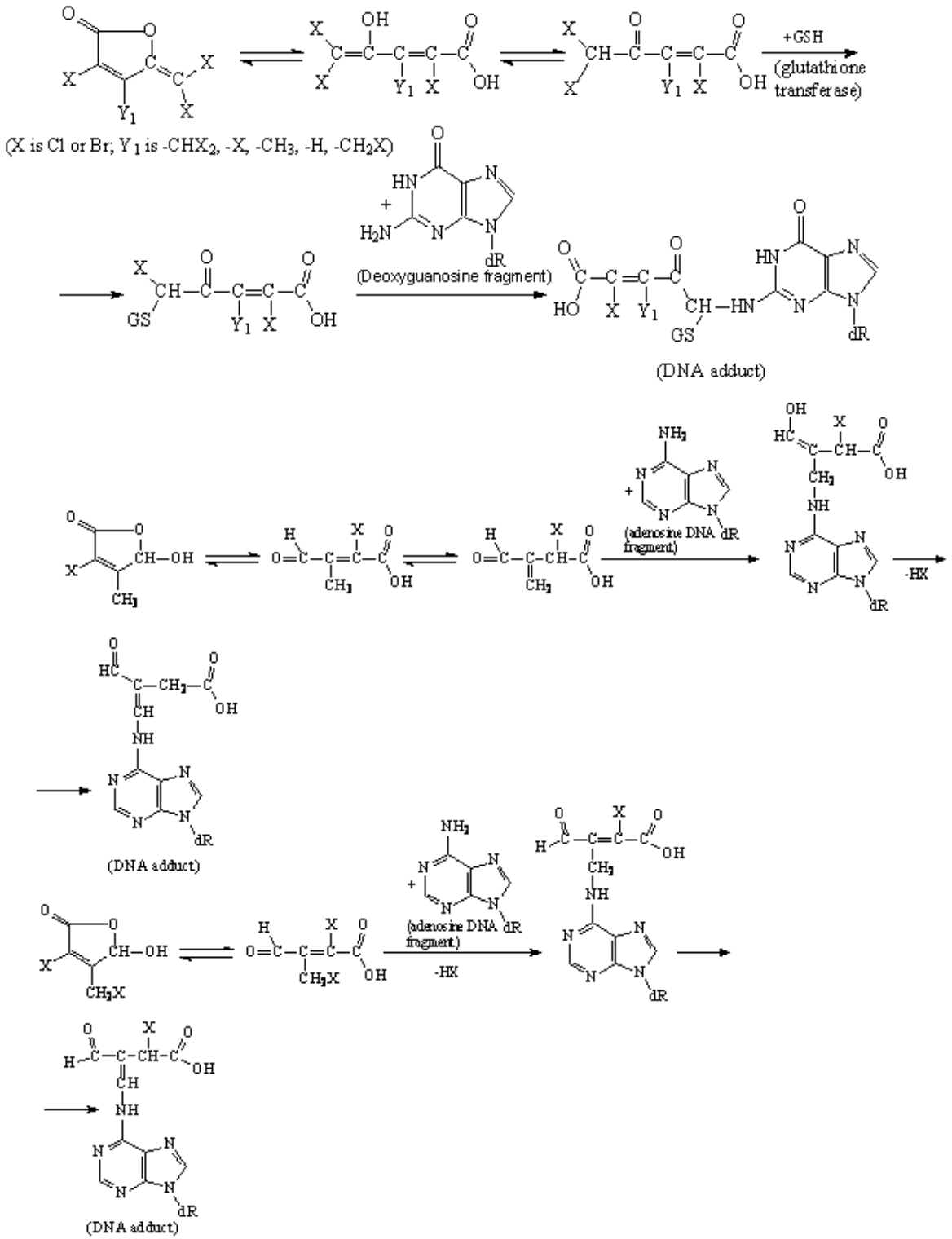
DNA *via* electrophilic mechanism [11] takes place:



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; http://www.ene.gov.on.ca/stdprodconsume/groups/lr/@ene/@resources/documents/resource/std01_079011.pdf 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 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 11. Muller, M., Chem. Res. Toxicol. **11**(5) (1998), 464 – 470; DOI: 10.1021/tx9701440. 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 15. Dichlorvos, ChemPlus, A Tooxnet Database, U.S. National Library of

	Medicine; https://chem.nlm.nih.gov/chemidplus/rn/62-73-7 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

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)
Mechanism	**S_N2 Nucleophilic substitution at sp3 carbon atom & A_N2 Shiff base formation**
	



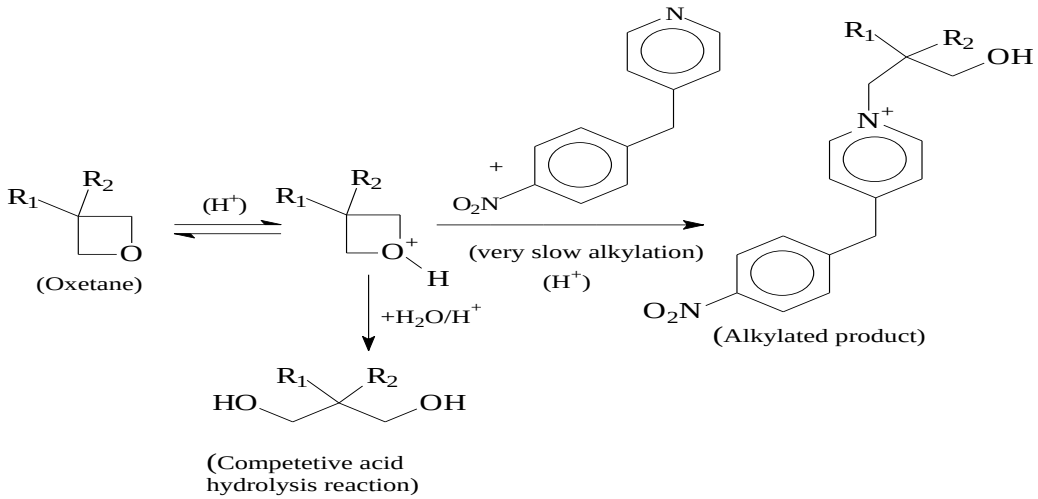
Set of chemicals used for profile development	Halofuranones
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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; http://web.usal.es/~jucali/papers/PHD20111.pdf. Last visited 10.2019

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

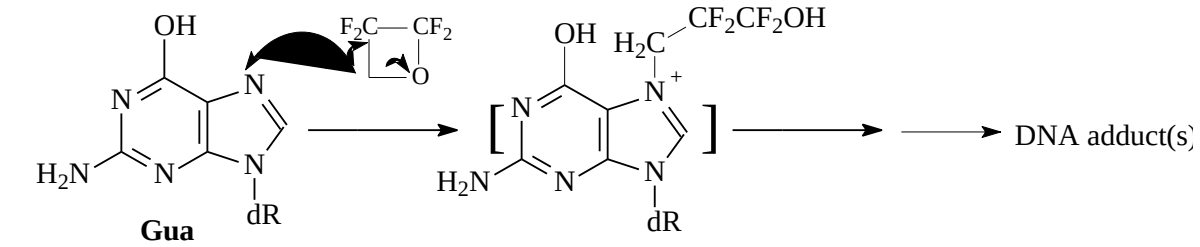
I. Halogenated Oxetanes

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



(Scheme 1)

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

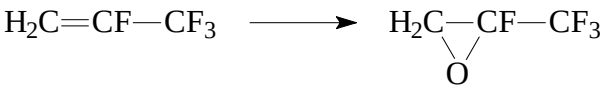


(dR - deoxyribose phosphate fragment;
Gua: Guanine nucleosides)

(Scheme 2)

II. Haloepoxides

The chemical 2,3,3,3-tetrafluoropropene (HFO-1234yf) was reported positive in the bacterial mutagenicity test with Salmonella typhimurium strain TA100 and E. coli (WP2 uvrA) with metabolic activation only [4]. On the other hand, the biotransformation studies showed the epoxide (Scheme 3) as the primary active metabolite of HFO-1234yf [5] (Scheme 3):

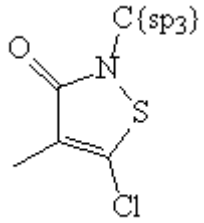
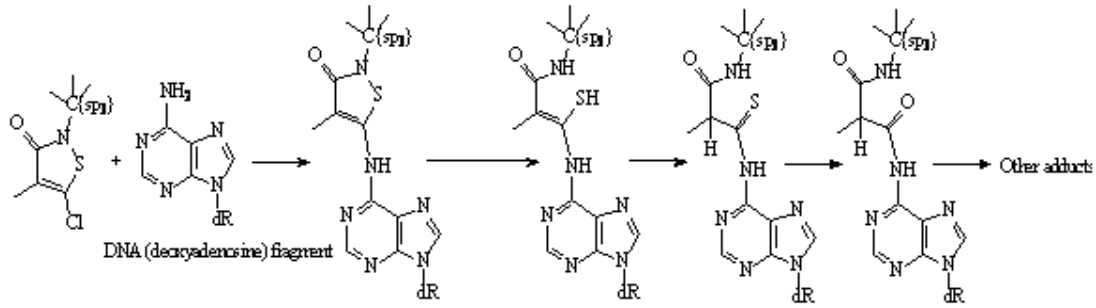


2,3,3,3-Tetrafluoropropene (HFO-1234yf)

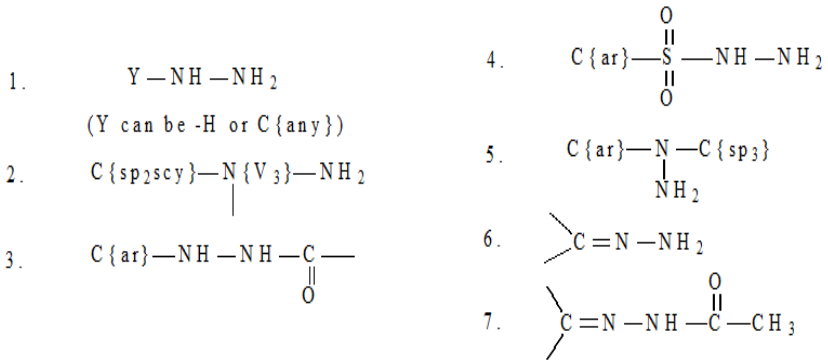
2,3,3,3-Tetrafluoropropene epoxide (active metabolite)

(Scheme 3)

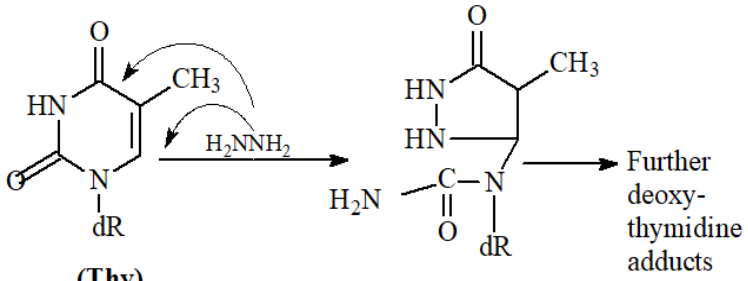
Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Bombarelli,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

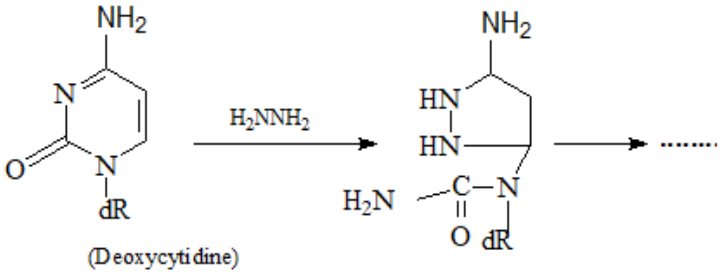
The mechanism of the direct formation of the initial DNA adduct with hydrazine is complex, accompanied by an array of DNA adducts [3]:



(Thy)

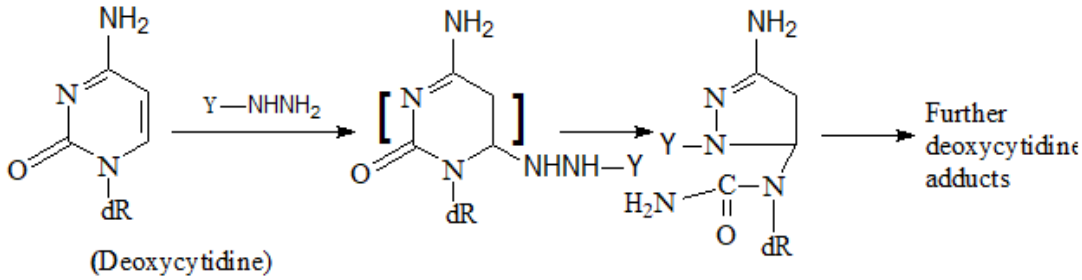
(dR - deoxyribose phosphate fragment)

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:



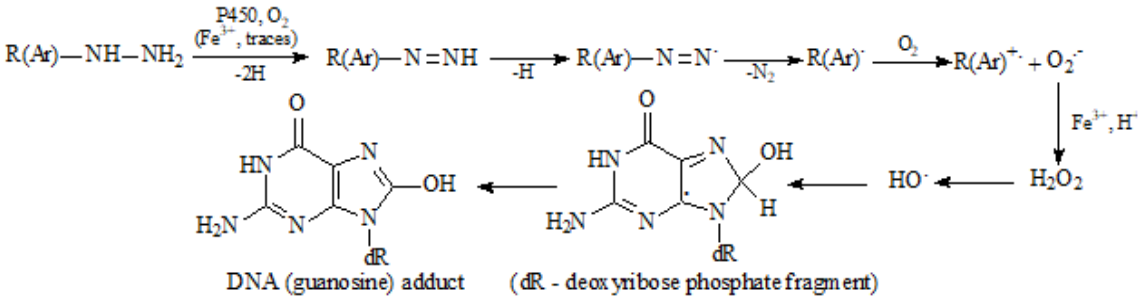
(Deoxycytidine)
(dR - deoxyribose phosphate fragment)

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:

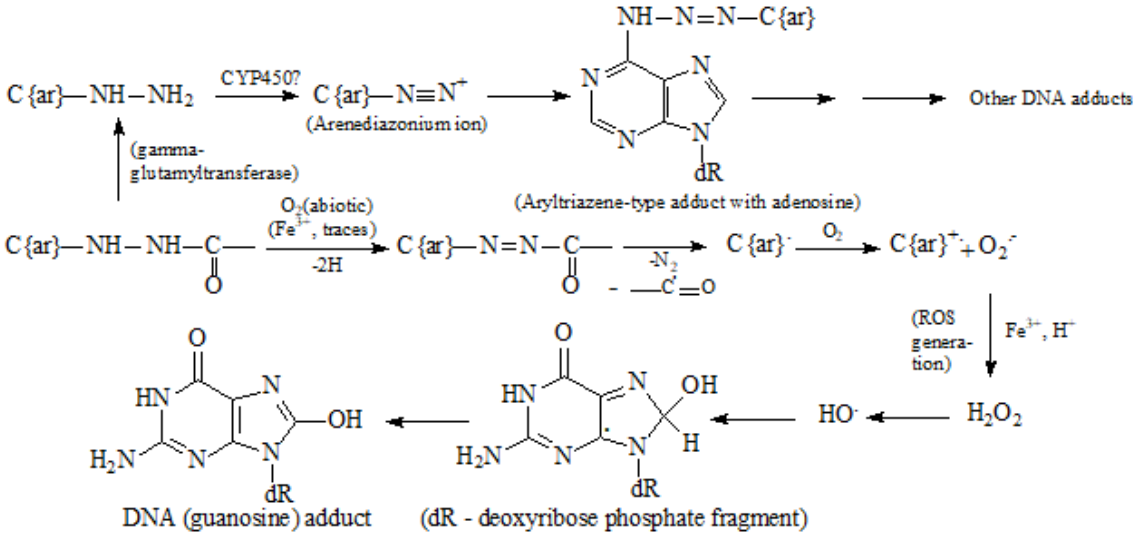


(Deoxycytidine)
(dR - deoxyribose phosphate fragment)
(Y can be alkyl, aryl or -H)

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]:

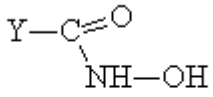


Based on the established abiotic oxidative consumption of agaritine and structurally similar chemicals, the following mechanistic scheme for the explanation of its mutagenicity can be expertly suggested:

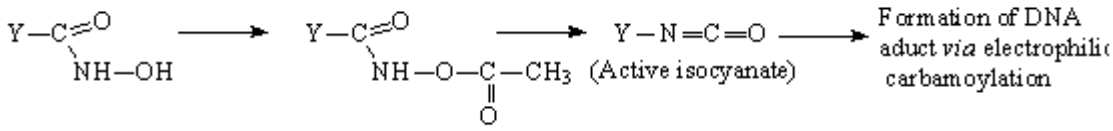


Set of chemicals used for profile development	Hydrazine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Phenylhydrazine, ICPS Inchem, Concise International Chemical Assessment Document 19; http://www.inchem.org/documents/cicads/cicads/cicad_19.htm#PartNumber:7 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://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+86-54-4 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.

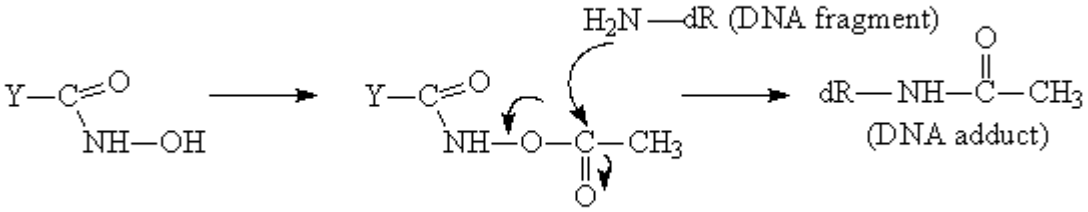
Individual profile/alert	
Name	Hydroxamic Acids
Type of profile	Structural alert

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]:

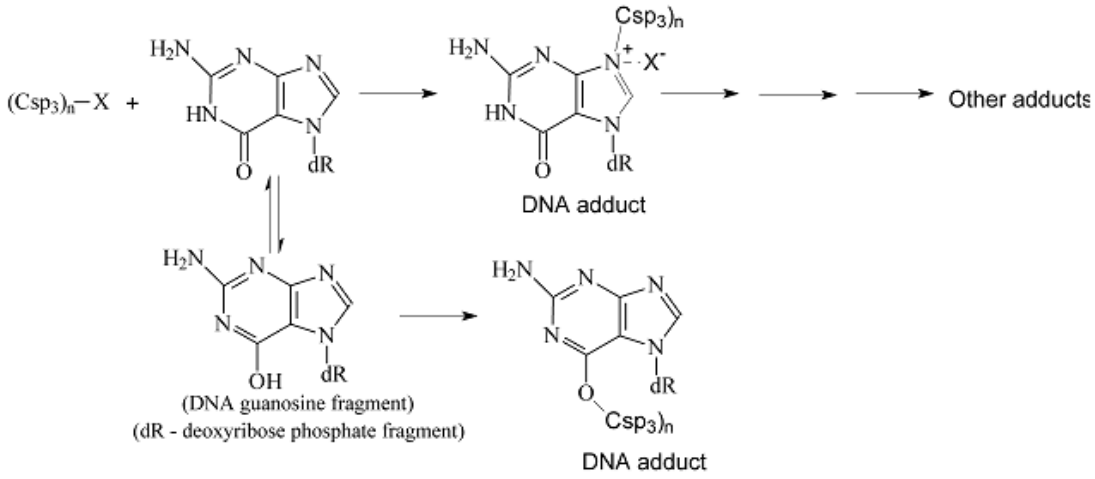


Set of chemicals used for profile development	Hydroxamic Acids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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	Monohaloalkanes
Type of profile	Structural alert
Description/applicability	**$(C(sp^3)(acy))_n$-X(n=1-4; X=-Cl, -Br, -I)**

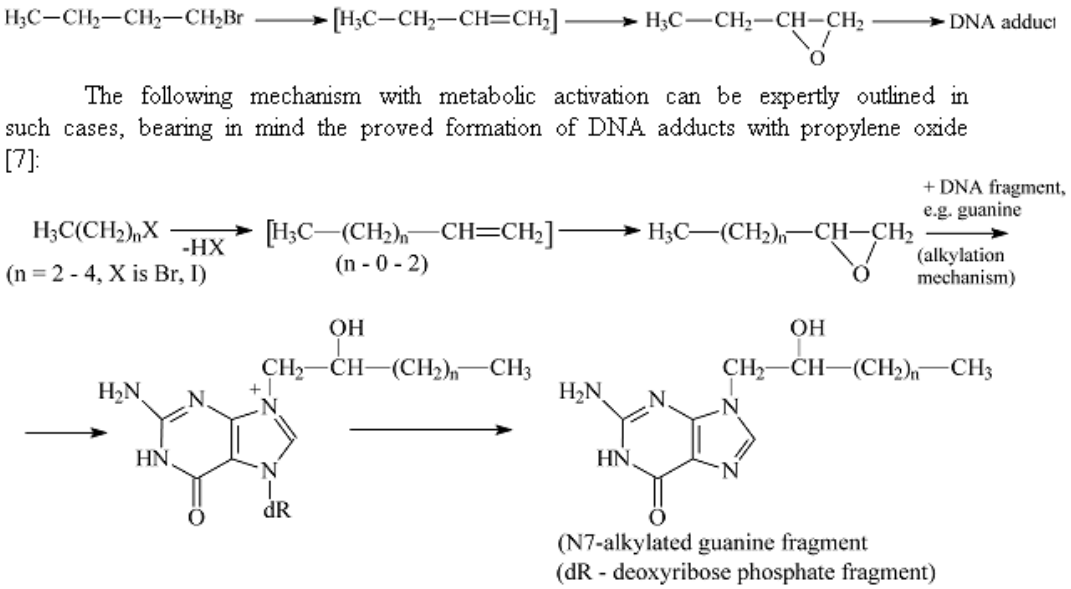
domain	
Mechanism	**S_N2 Alkylation, nucleophilic substitution at sp3-carbon atom, S_N1 Nucleophilic substitution after carbenium ion formation and S_N2 Alkylation by epoxide metabolically formed after E2 reaction**

Direct-Acting Mutagens – DNA alkylation in Scheme 1:



Scheme 1

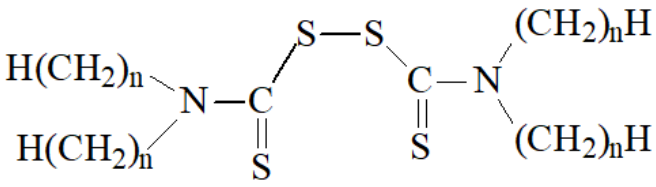
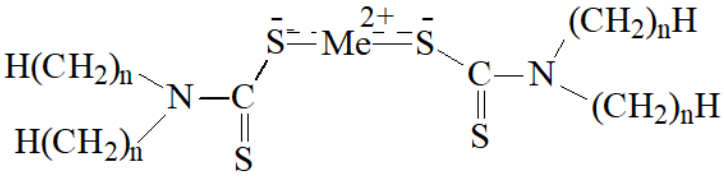
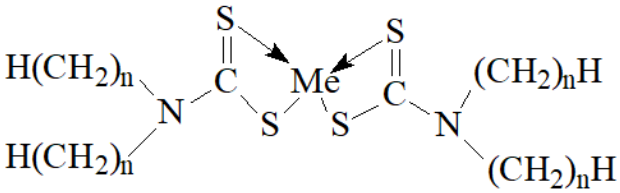
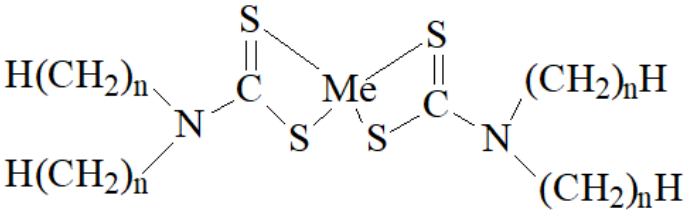
Metabolic Activation (Bioactivation) (Exogenous S9 System Added) in Scheme 2:

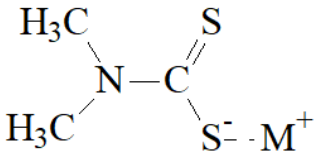
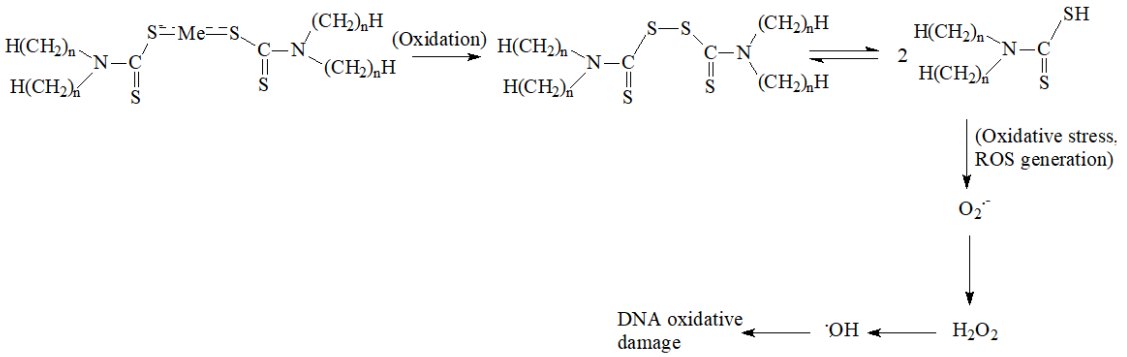


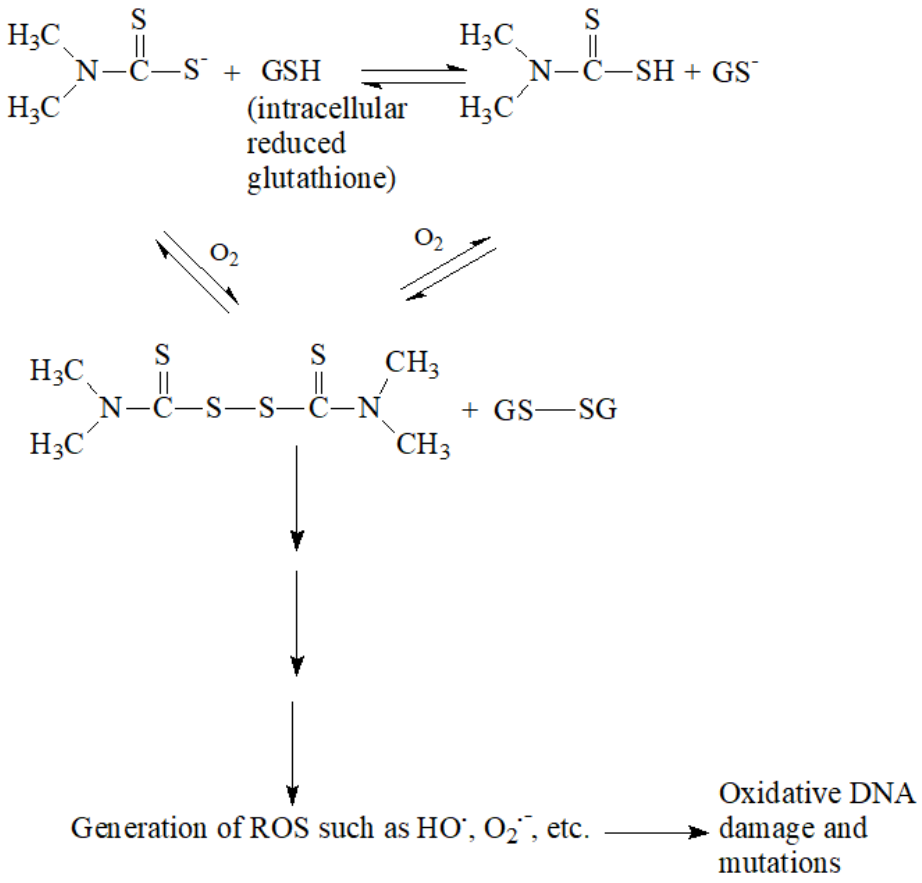
Scheme 2

Set of chemicals used for profile development	Monohaloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Woo, Environ. Health Persp. **110** (2002), 75 – 87. 2. Ballering, Mutagenesis **9**(4) (1994), 387 – 389; DOI:

	10.1093/mutage/9.4.387. 3. *Toxicology and Carcinogenesis Studies of Bromoethane (Ethyl Bromide) (CAS No. 74-96-4) in F344/N Rats and B6C3F₁ Mice (Inhalation Studies)*, NTP Technical Report Series No. 363, US Department of Health and Human Services, Public Health Service, National Institute of Health, October 1989; http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr363.pdf. 4. Guengerich, Jap. J. Toxicol. Environ. Health **43**(2) (1997), 69-82; http://sc.chat-shuffle.net/paper/uid:110003642293. 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.

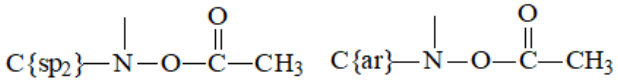
Individual profile/alert	
Name	N,N-Dialkyldithiocarbamate Derivatives
Type of profile	Structural alert
Description/applicability domain	  or  or 

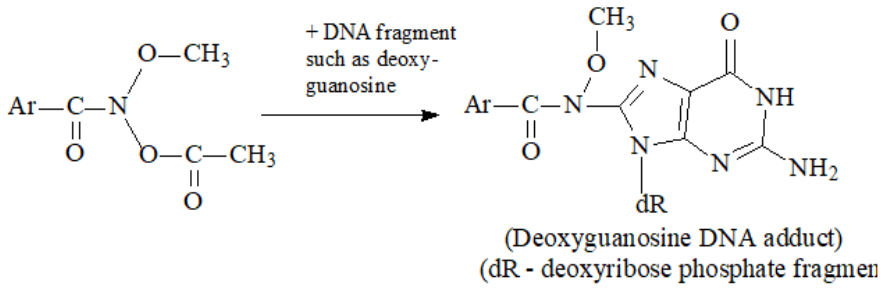
	(n = 1, 2; Me^{2+} can be Zn^{2+}, Cd^{2+}, Cu^{2+} or Pb^{2+} or Me can be Zn, Cd(II), Cu(II) or Pb(II) (depending on the structural representation of metal complexes))  (M^+ can be Na^+, K^+, Li^+)
Mechanism	**Radical ROS generation**
 Mutagenicity of tetramethylthiuram disulfide (thiram), which can 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:	

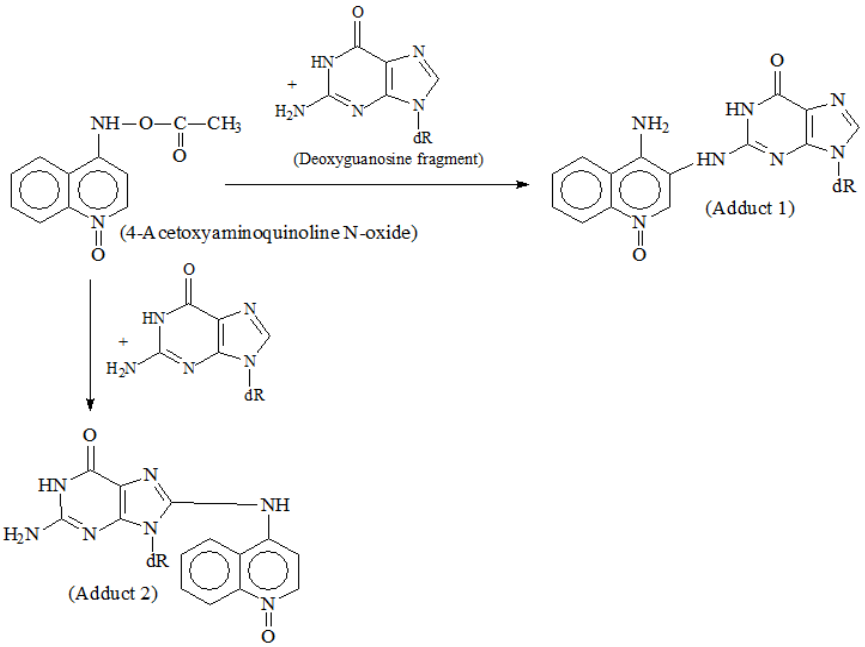


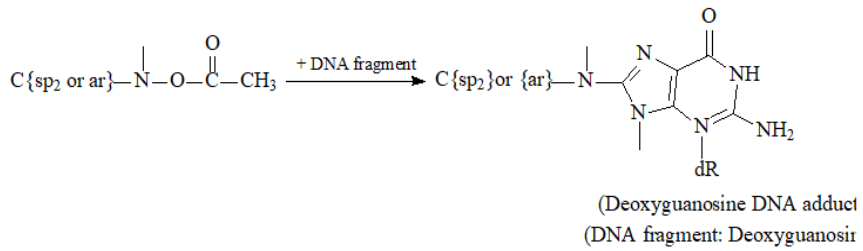
Set of chemicals used for profile development	N,N-Dialkyldithiocarbamate Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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**;** https://toxnet.nlm.nih.gov/cgi-bin/sis/search2, last visited 10.2019 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

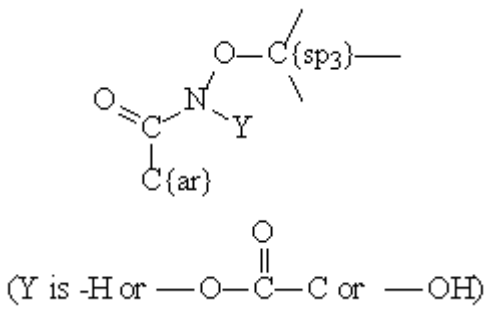
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N2 reaction on a nitrogen-atom bound to a good leaving group**

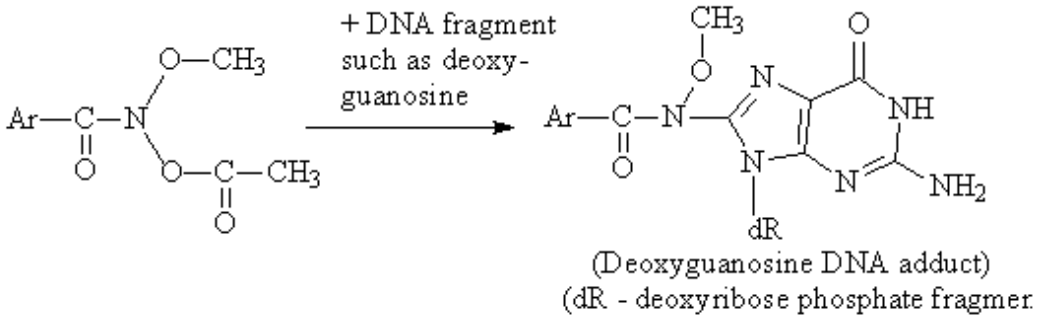




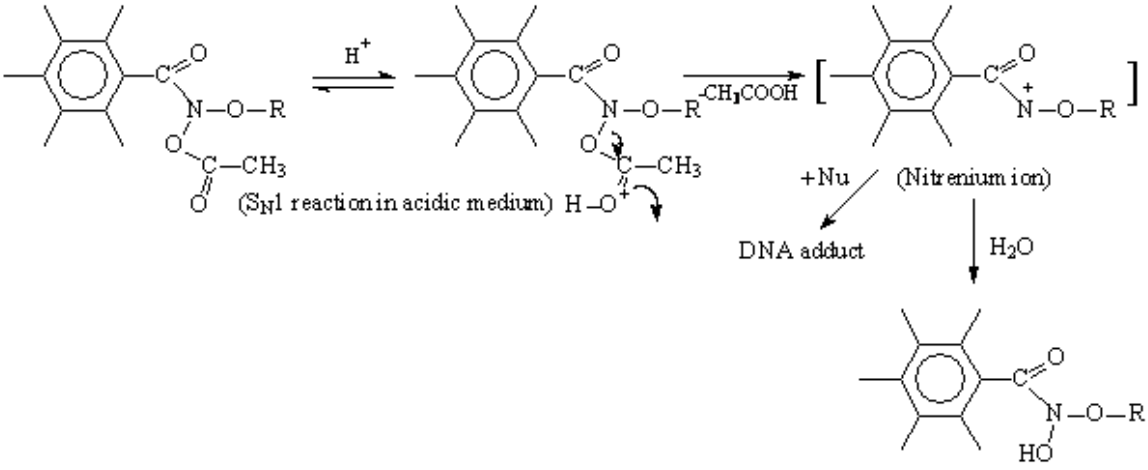


Set of chemicals used for profile development	N-Acetoxyamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

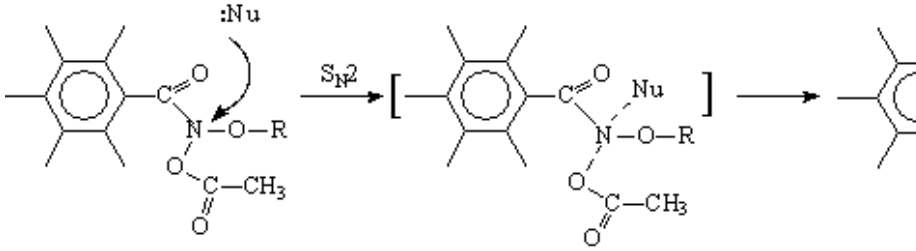
Individual profile/alert	
Name	N-Acyloxy(Alkoxy) Arenamides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N2 or S_N1 reaction at nitrogen-atom bound to a good leaving group or on nitrenium ion**



(Deoxyguanosine DNA adduct)
(dR - deoxyribose phosphate fragmer

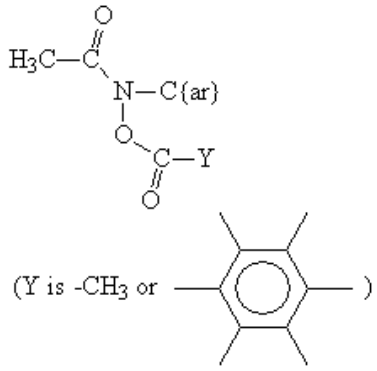


(SN1 reaction in acidic medium)

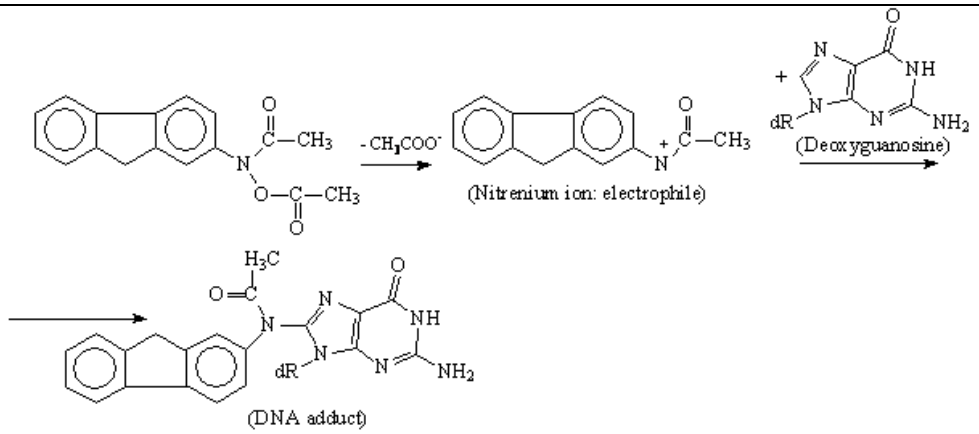


(S_N2 or S_N1 reactions with nucleophile Nu such as primary amine and amino group in DNA purine bases such as adenine)

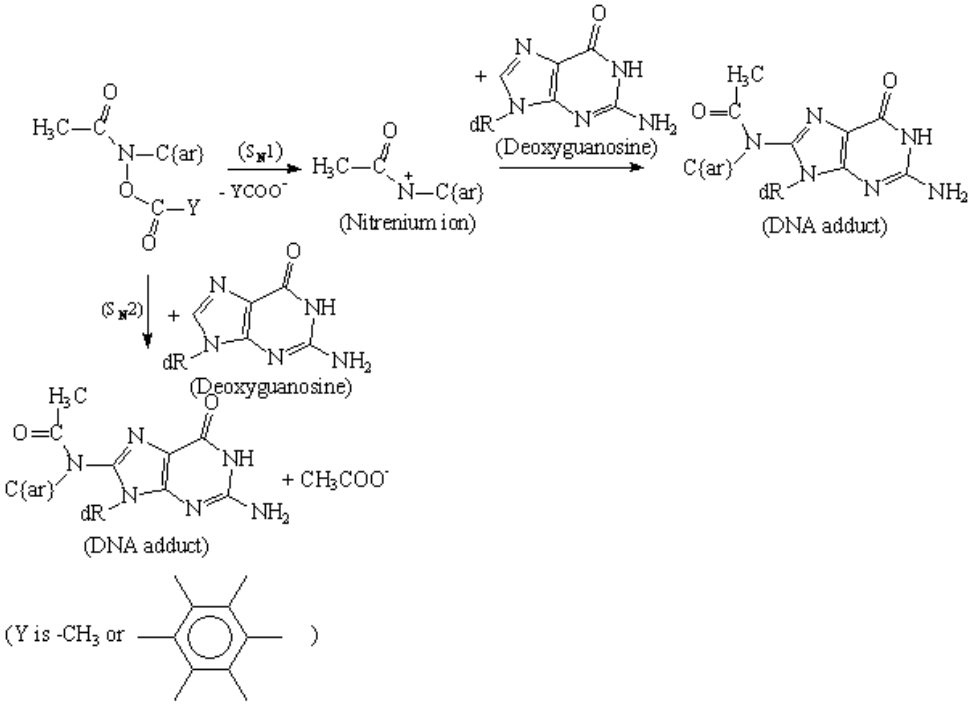
Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Glatt, 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-Aryl-N-Acetoxy(Benzoyloxy) Acetamides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N2 or S_N1 reaction at nitrogen atom bound to a good leaving group or on nitrenium ion**

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



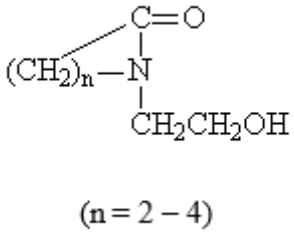
The general scheme for such interactions with DNA fragments could be outlined as follows:

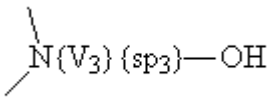
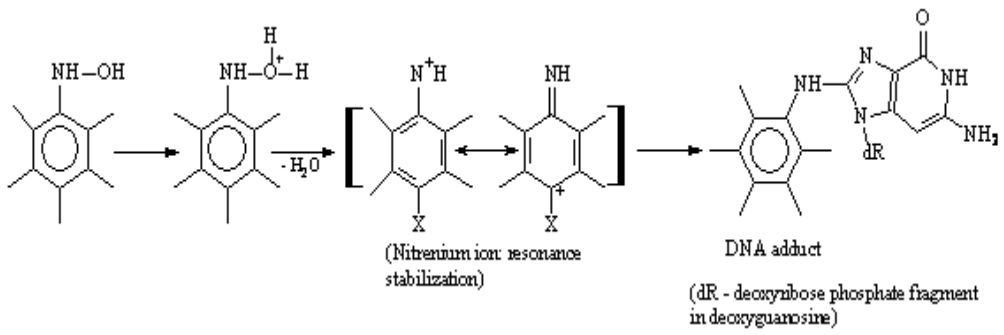


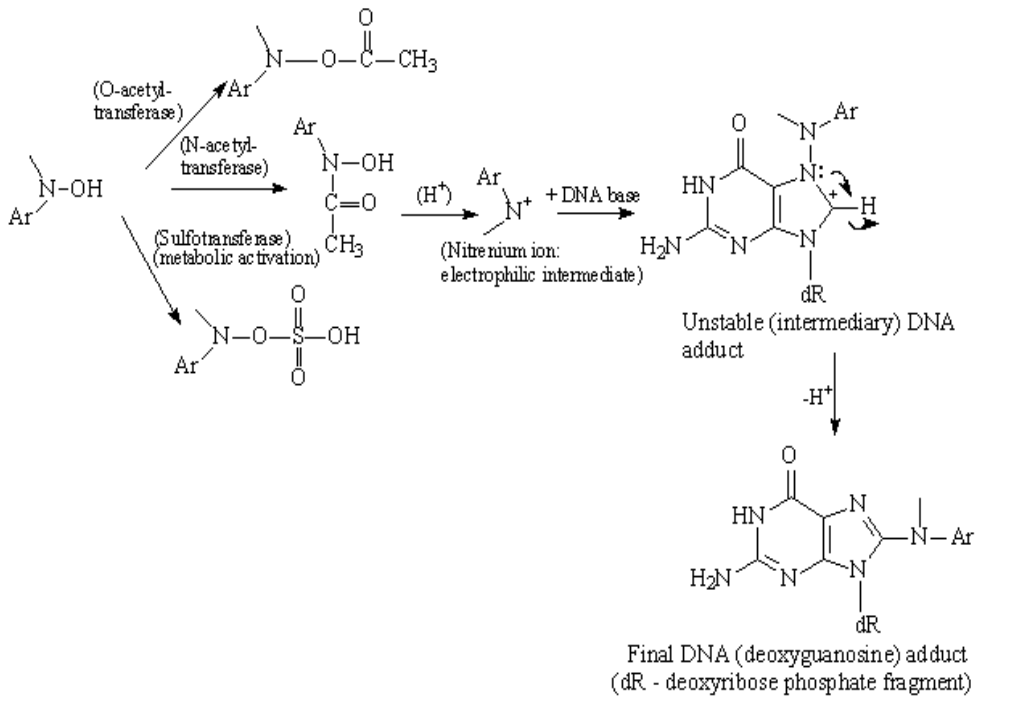
Scheme 1

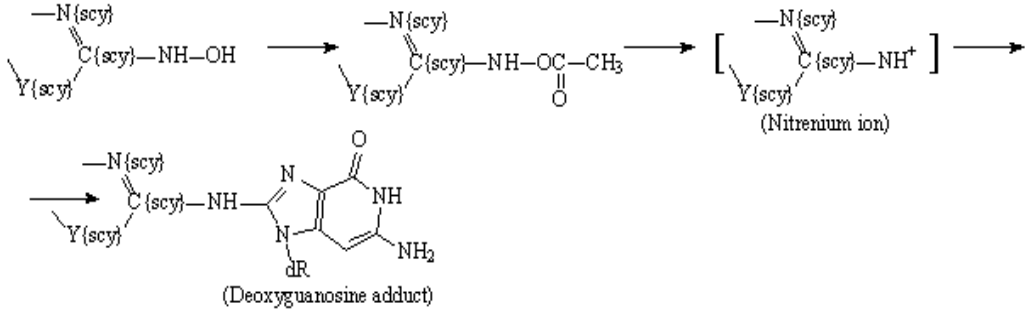
Set of chemicals used for profile development	N-Aryl-N-Acetoxy(Benzoyloxy) Acetamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references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

Individual profile/alert	
Name	N-Hydroxyethyl Lactams
Type of profile	Structural alert

Description/applicability domain	 (n = 2 – 4)
Mechanism	**Non-covalent interactions DNA intercalation**
Positive *in vitro* bacterial mutagenicity test results with *Salmonella typhimurium* strains TA100 and TA1535 were reported for 1-(2-Hydroxyethyl)-2-pyrrolidinone as parent chemical. The chemical is probably frameshift mutagen [1]. According to one publication, the oxopyrrolidine derivatives may interact with DNA as one of their possible mechanisms of action. For example, hydrogen bonds might be formed among the base pairs of DNA (adenine, guanine, cytosine and thymine), the free carbonyl group, and the nitrogen atom of oxopyrrolidine ring [2].	
Set of chemicals used for profile development	**Not appliciable** – all chemicals are private and can`t be disclosed.
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *2-Pyrrololidinone, 1- (2Hydroxyethyl)-, Full Public Report*, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), 14 February 2005; https://www.nicnas.gov.au/__data/assets/word_doc/0007/18538/STD1134FR.docx, last visited 09.2019. 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).

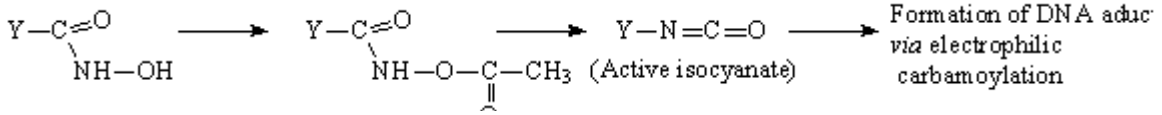
Individual profile/alert	
Name	N-Hydroxylamines
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N1 Nucleophilic attack after nitrenium ion formation, Radical ROS formation after GSH depletion (indirect), S_N2 Acylation & A_N2 Carbamoylation after isocyanate formation**
1. Aromatic and Heterocyclic N-Hydroxylamines  (Nitrenium ion: resonance stabilization) DNA adduct (dR - deoxyribose phosphate fragment in deoxyguanosine)	

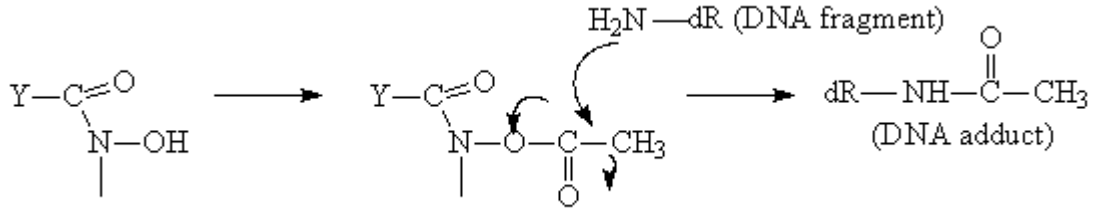




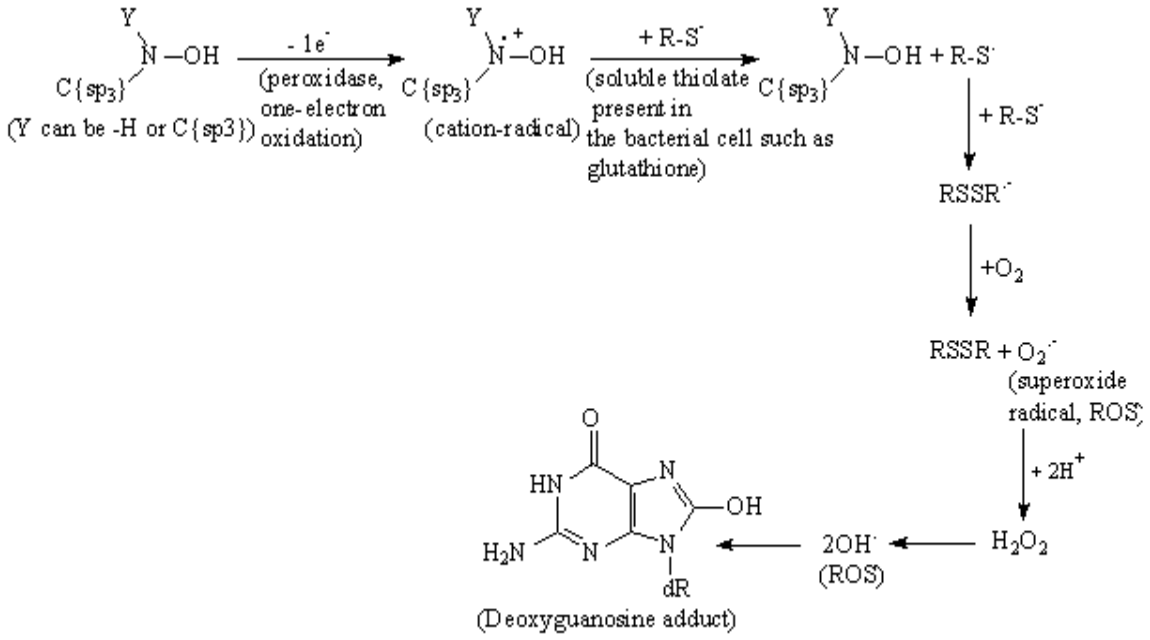
where Y{scy} can be heterocyclic N{V3} or S{V2}.

2 Hydroxamic Acids



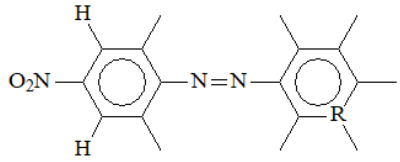
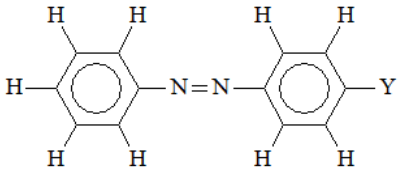


3. Other Non-Aromatic N-Hydroxylamines



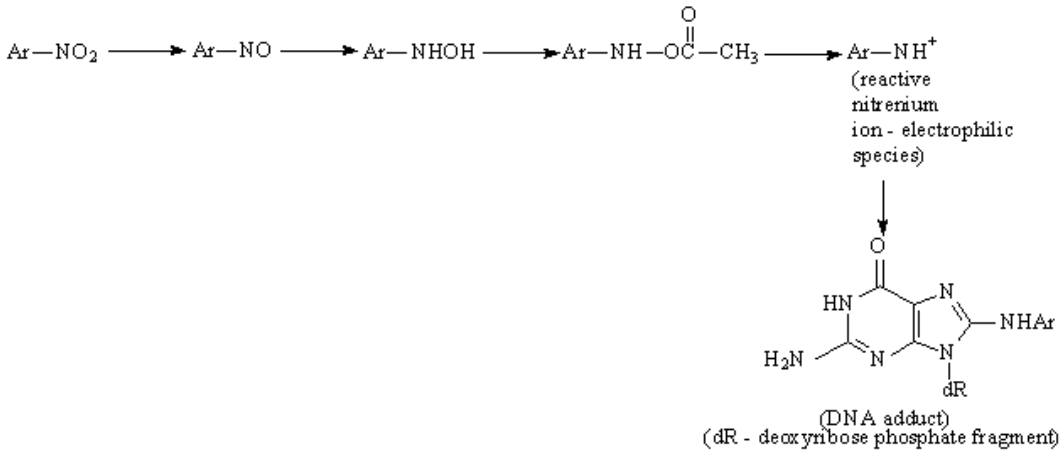
Set of chemicals used for profile development	N-Hydroxylamines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Josephy P. D., M. Novak, *Reactive electrophilic metabolites of aromatic amine and amide carcinogens*, Front. Biosci.Scholar, S5(1) 2013, 341-359, DOI: 10.2741/S376. 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)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. 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. 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 09.2019.

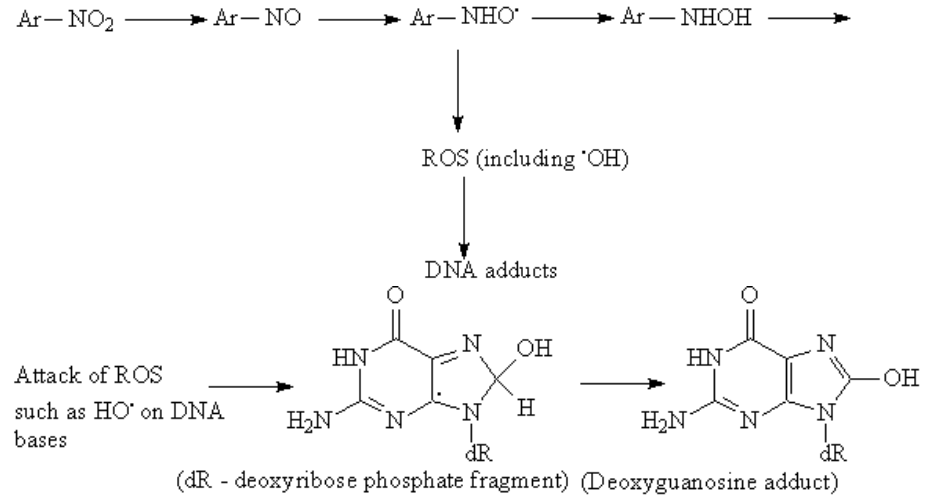
Individual profile/alert	
Name	Nitro Azoarenes and p-Monosubstituted Azobenzene Derivatives
Type of profile	Structural alert
Description/applicability domain	 R = any carbon or nitrogen, single arene ring only, no fused ring fragments in the molecular structure  Y= NH_2 or OH
Mechanism	**Heterolytic 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

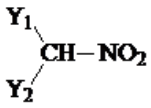
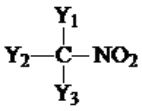


Radical (Homolytic) Mechanism

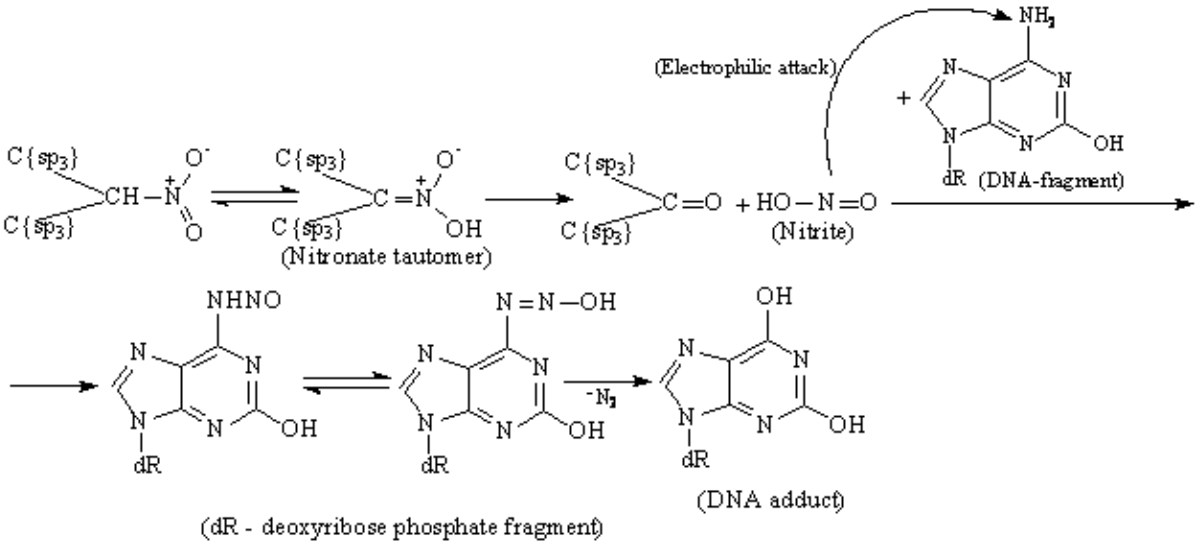


Set of chemicals used for profile development	Nitro Azoarenes and p-Monosubstituted Azobenzene Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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; http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+genetox:@term+@rn+@rel+"2491-52-3". 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). 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 17. Lang, B., Mutat. Res. **191** (1987), 139 – 143. 18. Shamovsky, I., JACS **133** (2011), 16168 – 16185.

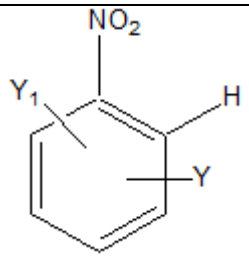
Individual profile/alert	
Name	Nitroalkanes
Type of profile	Structural alert
Description/applicability domain	**Monoalkanes**  **Y_1- Me or H** **Y_2- Me or CH_2OH or CH_2COOH** **Low Molecular weight germinal Polynitroalkanes**  **Y_1, Y_2, Y_3 can be NO_2(all) or a combination between $–CH_3$, -H,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

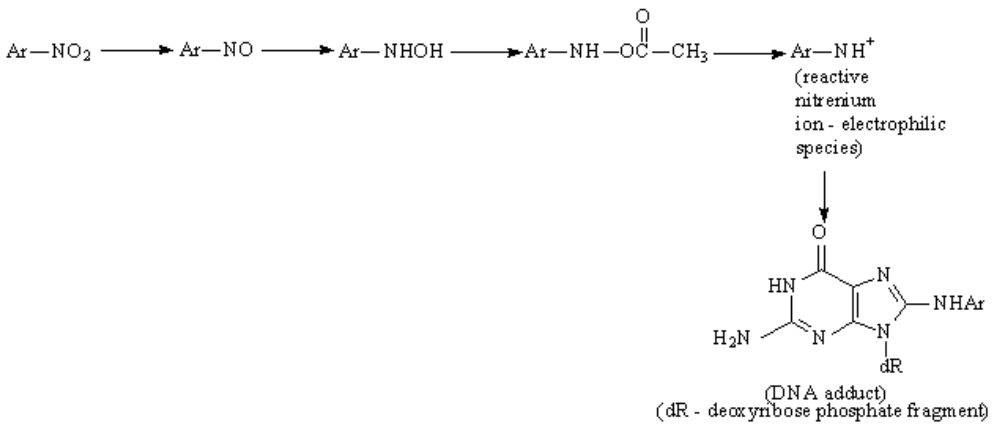


Set of chemicals used for profile development	Nitroalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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. 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. 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. 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. 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. 8. Murata, M., Chem. Res. Toxicol. **19**(10) (2006), 1379 – 1385. 9. Linhart, I., Chem.-Biol. Interact. **80** (1991), 187 – 210. 10. Sundvall, Mutat. Res. **137** (1984), 71 – 78..

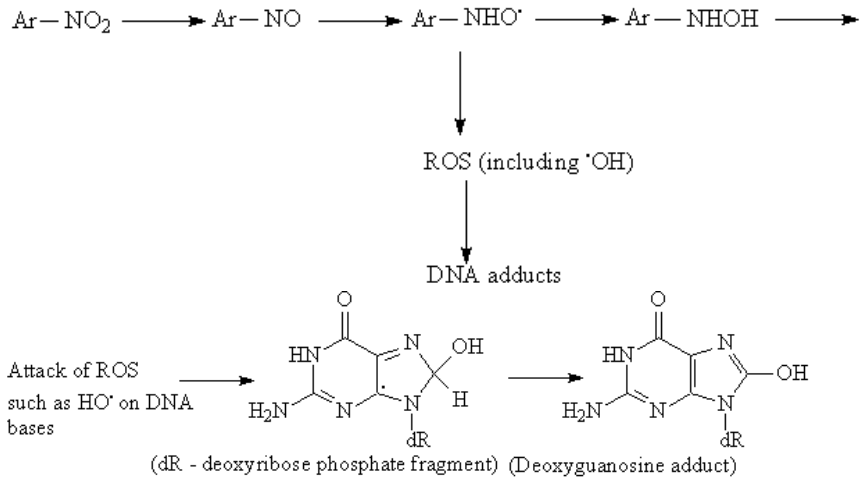
Individual profile/alert

Name	Nitroaniline Derivatives
Type of profile	Structural alert
Description/applicability domain	 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
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

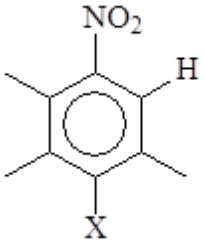
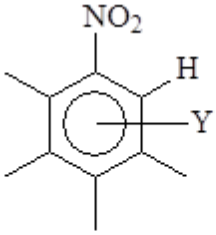
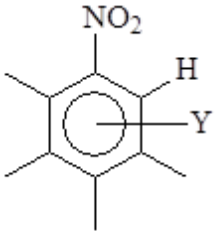


Homolytic

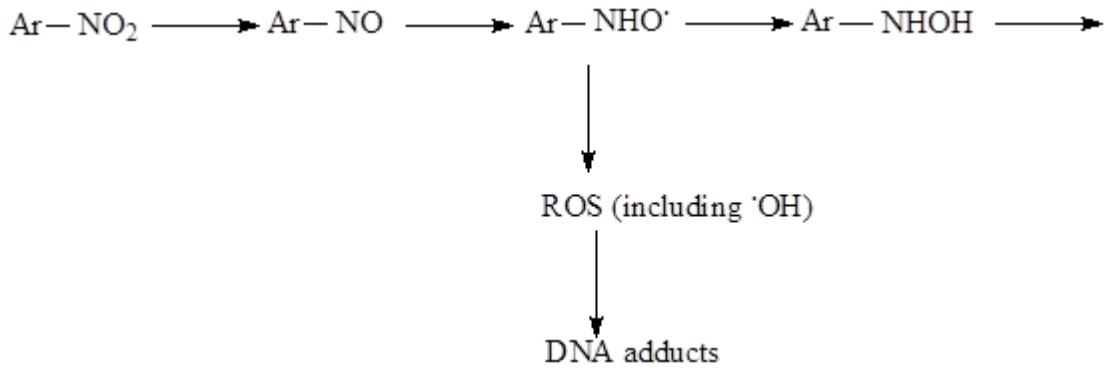


Set of chemicals used for profile development	Nitroaniline Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
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); http://agris.fao.org/agris-search/search/display.do?f=2006/KR/KR0603.xml;KR2006013346. 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.

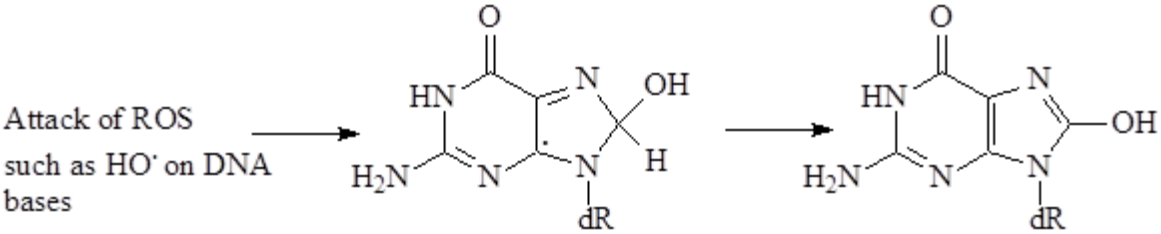
Individual profile/alert	
Name	Nitroarenes with Other Active Groups
Type of profile	Structural alert
Description/applicability domain	

	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) **Nitrophenyl Diazonium Salts and Nitrophenyl Triazenes:**  (Y can be $-N=N-N\{V_3\}\{sp_3\}$(triazene) or $-N^{+}\equiv N$ (diazonium)); totally no more than four substituents)
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	

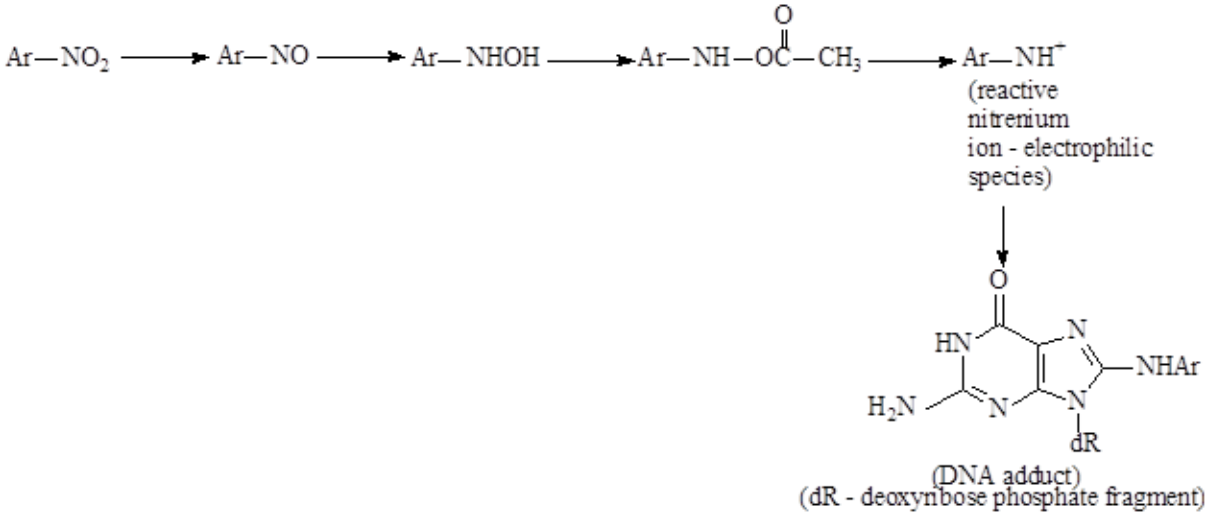
(strand breaks)**:**



As a result from the generation of reactive radical species such as ArNHO·, an additional formation of ROS such as $O_2^{\cdot-}$ 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]:



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]:

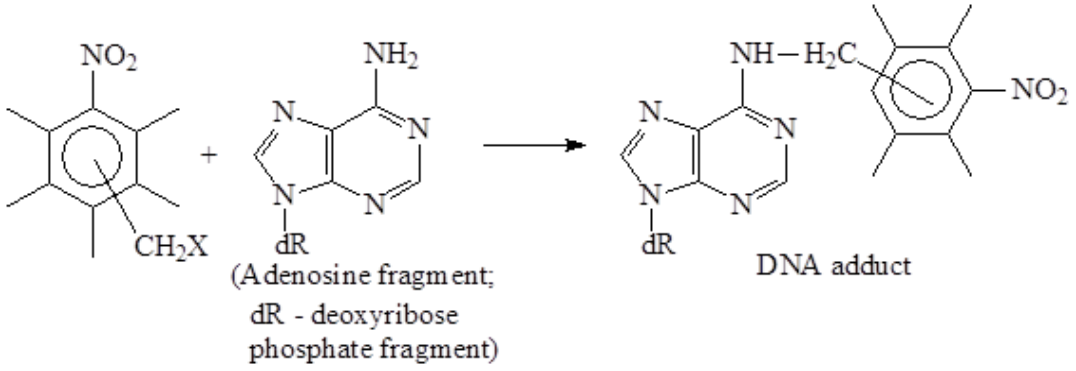


Among the isomers of chloronitrobenzenes, only *p*-chloronitrobenzene (4-chloronitrobenzene) showed mutagenicity in *Salmonella typhimurium* when tested in the presence or absence of induced rodent liver S9 [9]. This confirms the importance of *p*-position with respect to the nitro group in eliciting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

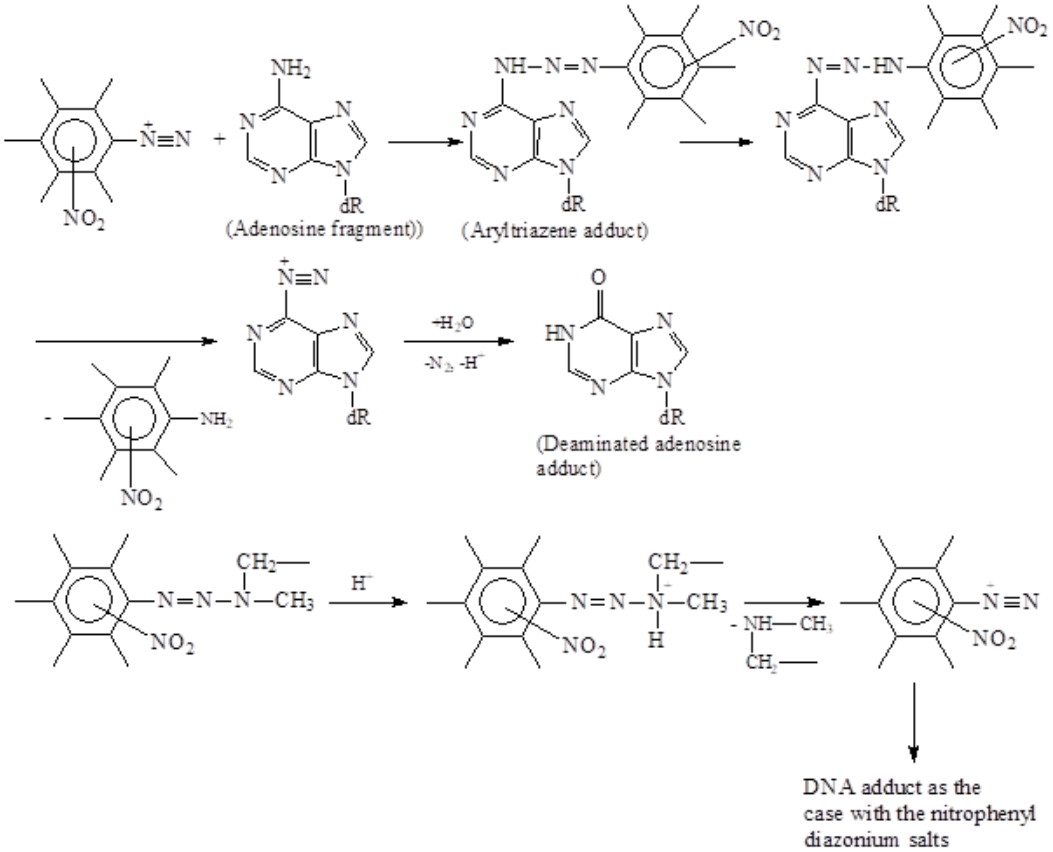
resonance structures, and reduced steric hindrance [10, 11].

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]:



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.

10. Shimizu, M., E. Yano, Mutagenicity of Mono-Nitrobenzene Derivatives in the Ames Test and Rec Assay, Mutat. Res. 170 (1986), 11 – 22.

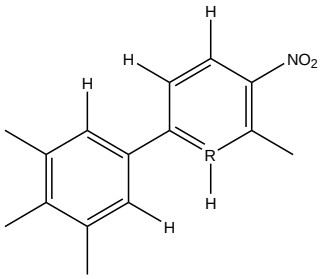
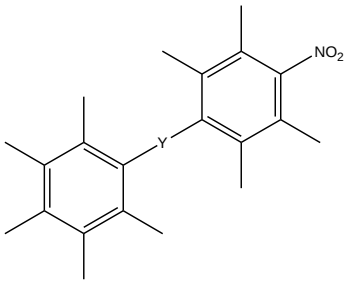
11. Chemical Carcinogenesis Research Information System, TOXNET, US National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS, last visited 09.2019.

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.

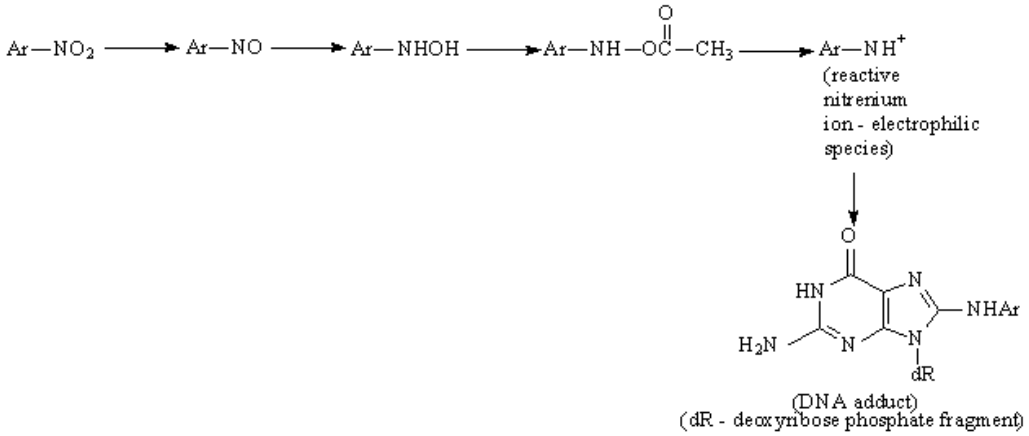
14. Lawson, T., P. M. Gannett, W. M. Yau, N. S. Dalal, B. Toth, Different Patterns of Mutagenicity of Arenediazonium Ions in V79 Cells and Salmonella typhimurium TA102: Evidence for Different

	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.

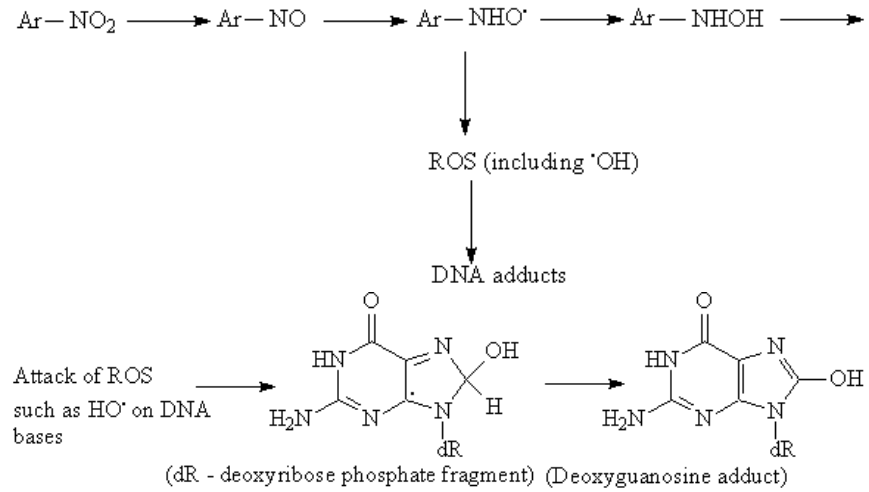
Individual profile/alert	
Name	Nitrobiphenyls and Bridged Nitrobiphenyls
Type of profile	Structural alert
Description/applicability domain	**Nitrobiphenyl**  **R= C or N(aromatic)** **o-distributed nitrobiphenyl are excluded** **Bridged Nitrobiphenyl**  **Y= O, S(V2), Ethyl, Ethene**
Mechanism	**Heterolytic 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

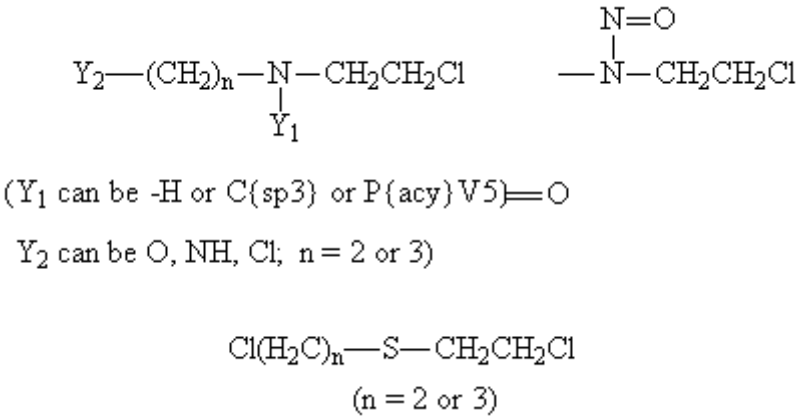
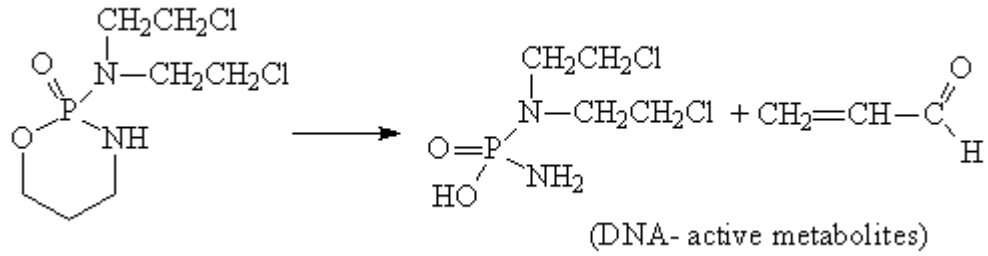


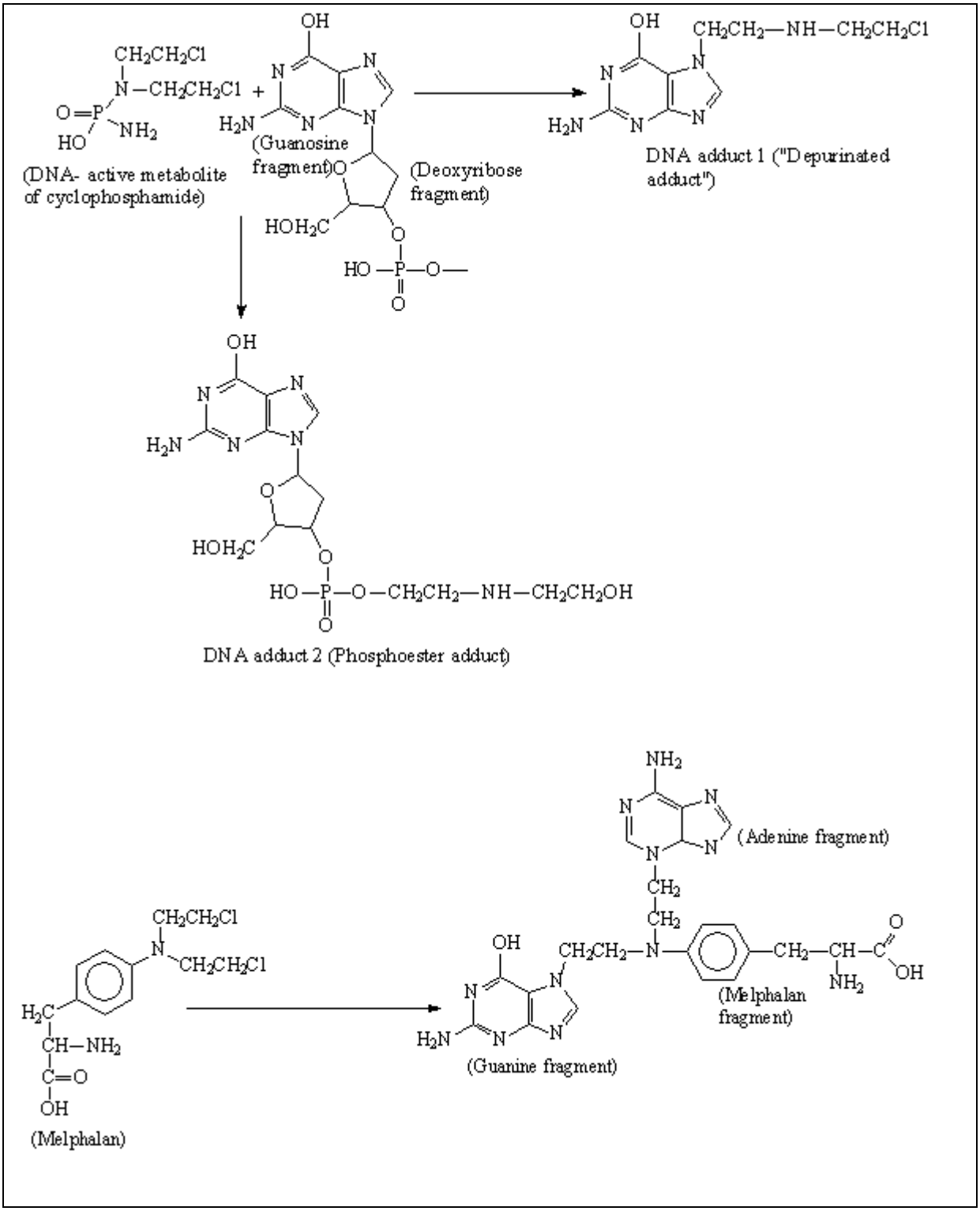
Homolytic

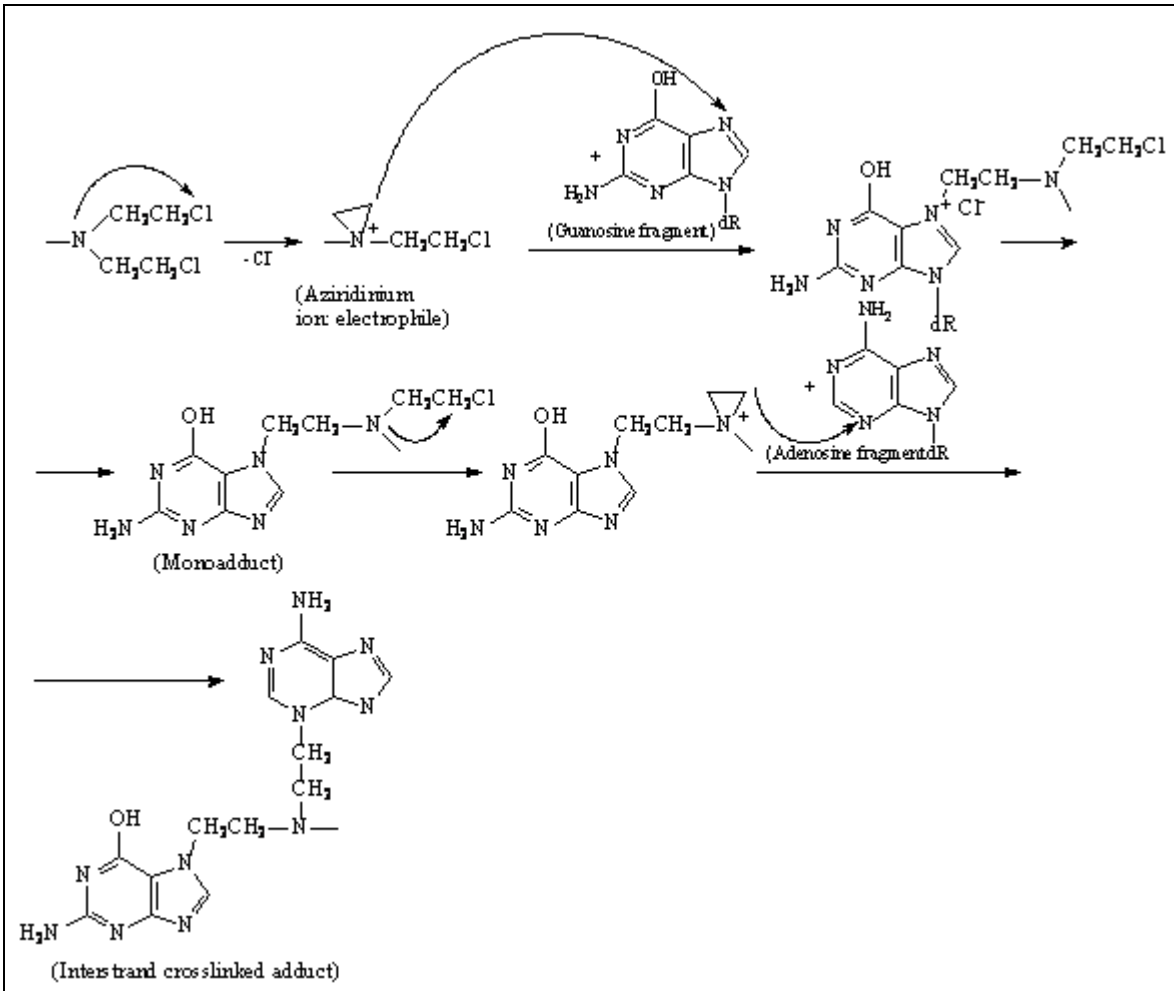


Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1.Sabbioni, 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)* http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+ccris:@term+@rn+620-88-2. 12. Juneja, Mutat. Res. **263 (**9) (1991), 13 – 19. 13. Hooberman, Mutat. Res. **341** (1994), 57 – 69.

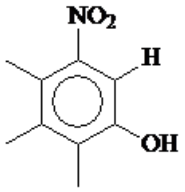
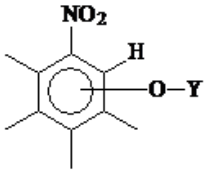
Individual profile/alert	
Name	Nitrogen and Sulfur Mustards
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N2 Alkylation, direct acting epoxides and related after cyclization**
	

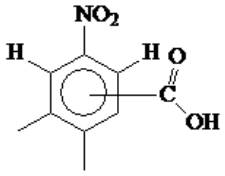
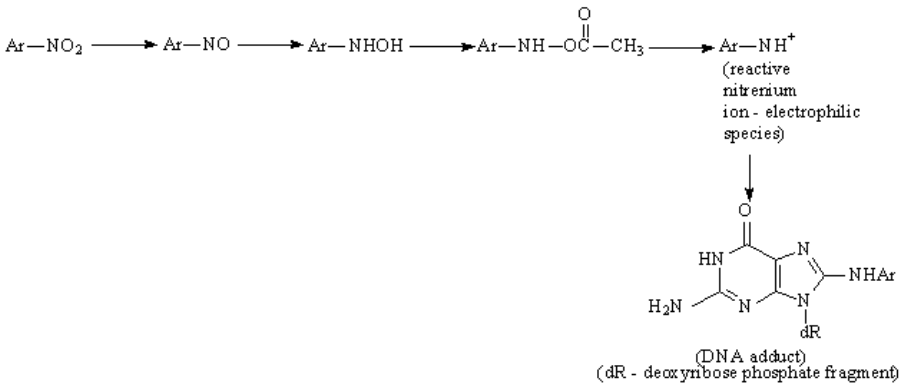

(DNA- active metabolite of cyclophosphamide)
(Guanosine fragment)
(Deoxyribose fragment)
DNA adduct 1 ("Depurinated adduct")
DNA adduct 2 (Phosphoester adduct)
(Melphalan)
(Adenine fragment)
(Melphalan fragment)
(Guanine fragment)



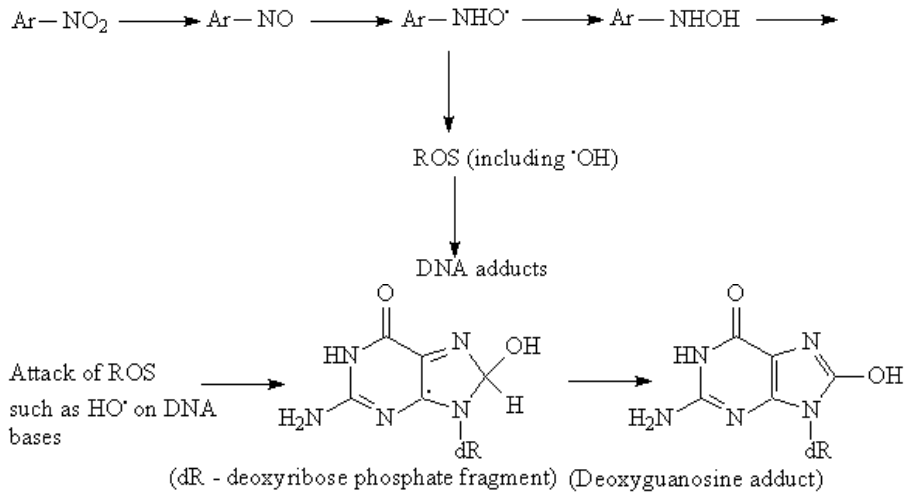
Set of chemicals used for profile development	Nitrogen and Sulfur Mustards
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kovacic, P., J. D. Jacinto, *Mechanism of Carcinogenesis: Focus on Oxidative Stress and Electron Transfer*, Current Med. Chem. **8** (2001), 773 – 796. 2. Hartley, J. A., J. P. Bingham, R. L. Souhami, DNA *Sequence Selectivity of Guanine-N7 Alkylation by Nitrogen Mustards is Preserved in Intact Cells*, Nucl. Acids Res. **20**(12), (1990), 3175 - 3178. 3. *Nitrogen Mustard*; http://en.wikipedia.org/wiki/Nitrogen_mustard. 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)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. 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) 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; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+505-60-2 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** **No -SO_3H and –COO-** **Nitrobenzyl and Nitrobenzyl Halides**  **Y= Me, Et** **No more than three substituents** **No –SO_3H and –COO-** **Nitrophenyl Diazonium Salts and Nitrophenyl**

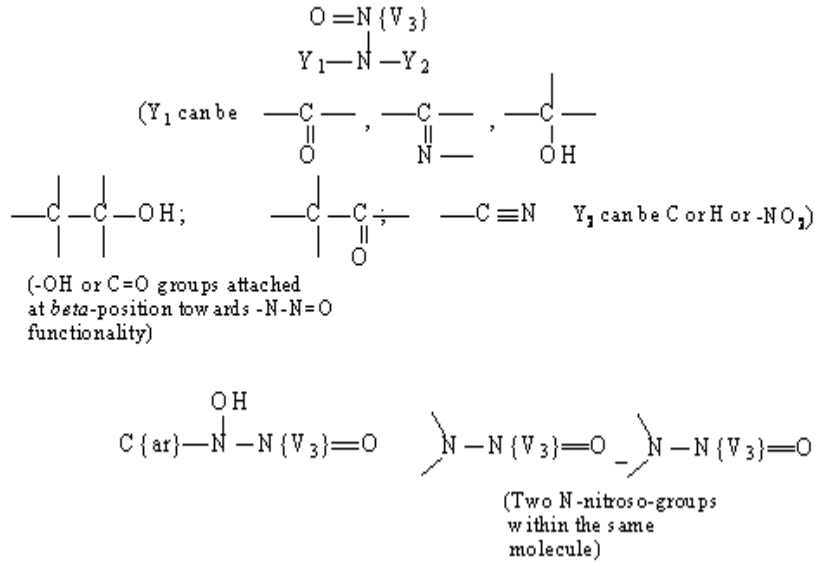
	 **No more than three substituents** **No –SO_3H and –COO-**
Mechanism	**Heterolytic Mechanism.** This is the most important mechanism, associated with the bacterial mutagenicity of nitroarenes, and, particularly, the sub-class discussed here. The DNA damage, eliciting bacterial mutagenicity results mainly 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

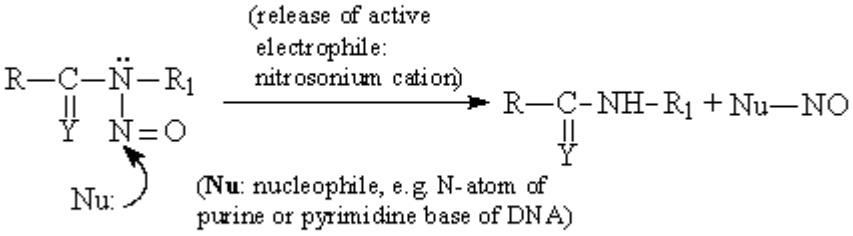


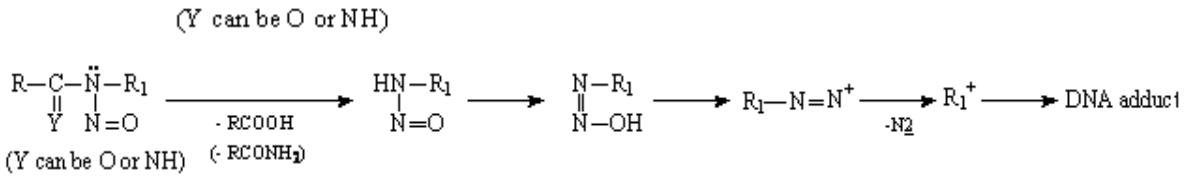
Set of chemicals used for profile development	Nitrophenols, Nitrophenyl Ethers and Nitrobenzoic Acids
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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; http://www.who.int/ipcs/publications/cicad/en/cicad20.pdf.

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

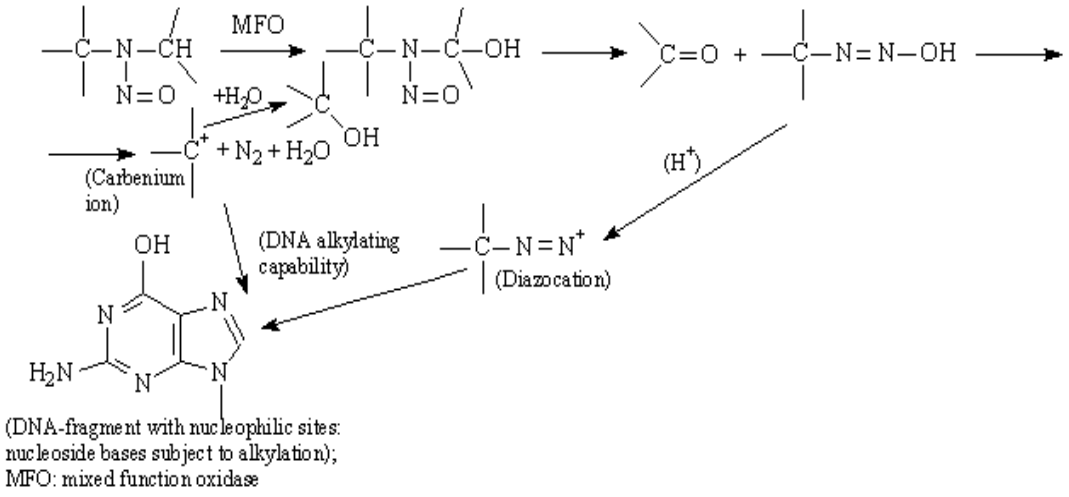
Description/applicability domain	
Mechanism	**S_N1 Nucleophilic attack after carbenium ion formation & S_N1 Nucleophilic attack after nitrosonium cation formation**

1. Mutagenicity without metabolic activation.



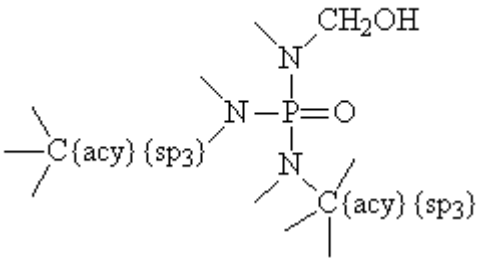


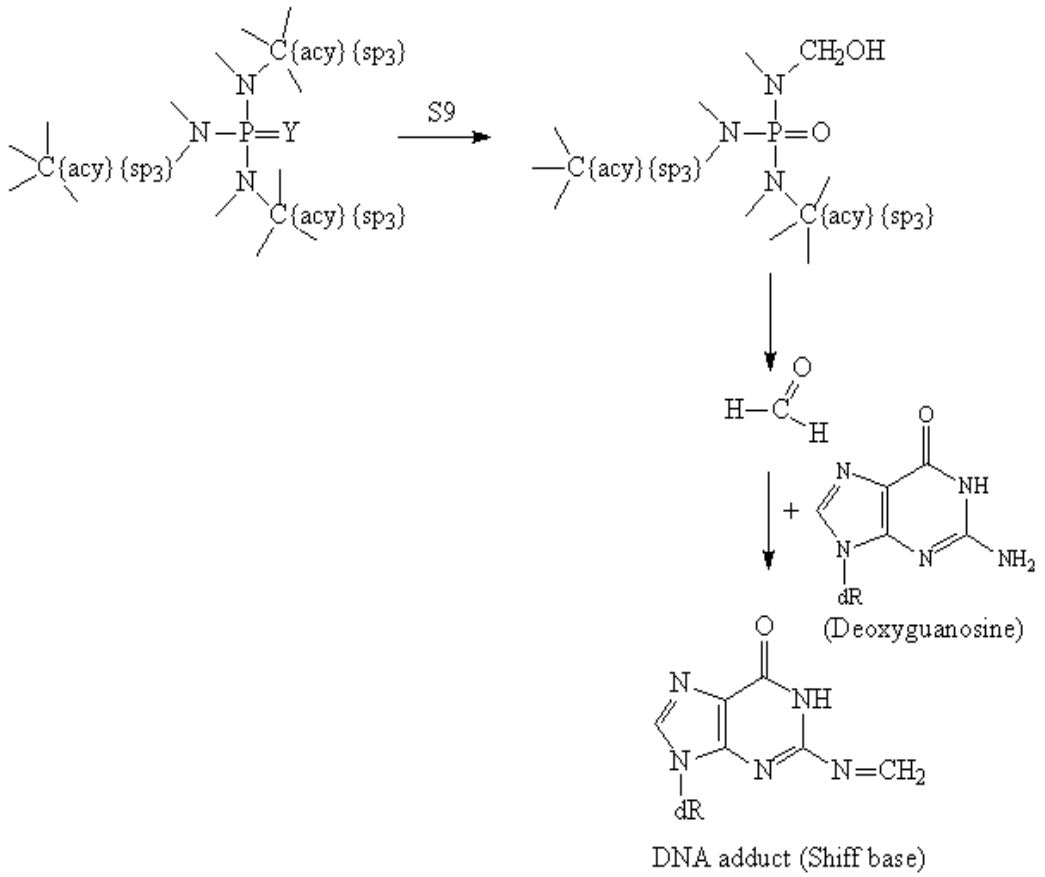
2. Mutagenicity with metabolic activation.



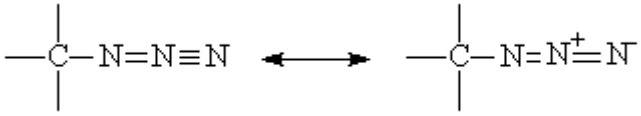
Set of chemicals used for profile development	N-Nitroso Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Toxicological Profile for N-Nitrosodiphenylamine*, US Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Sisease Registry, April 1993; http://www.atsdr.cdc.gov/ToxProfiles/tp16.pdf 2. Miura, M., Tetrahedron Lett. **41** (2000), 3637 – 3641. 3. Kovacic, P., Current Med. Chem. **8**, (2001), 773 – 796. 4. Wang, P. G., Chem. Rev. **102** (2002), 1091 – 1134. 5. Janczuk, *Nitric Oxide Donors: Chemical Activities and Biological Applications*, Chem. Rev. **102** (2002), 1091 – 1134. 6. Guttenplan, J. B., Mutat. Res. **186** (1987), 81 – 134. 7. Ethylnitrosocyanamide CASRN: 38434-77-4, GENE-TOX, Toxicology Data Network, U.S. National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+genetox:@term+@rn+@rel+38434-77-4). 8. Nakamura, S.-i., Mutat. Res. **192** (1987), 239 – 246. 9. Lee, K., Mutat. Res. **48** (1977), 131 – 138. 10. *Chemical Carcinogenesis Research Information System (CCRIS)*; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS (for bacterial mutagenicity data for chemicals such as Dinitrosopentamethylenetetramine, N-nitroso phenylhydroxylamine and N-Nitrosodiethanolamine. 11. Kushida, H., Carcinogenesis **21**(6) (2000), 1227 – 1232. 12. Maertens, L. A., Drug Metabol. Dispos. **38** (2010), 752 – 760. 13. Peterson, L. A., Canc. Res. **61** (2001), 5757 – 5763. 14. *N-Nitrosomethylethylamine, Summaries & Evaluations*, IARC, Vol. 17 (1978), p. 221; http://www.inchem.org/documents/iarc/vol17/nitrosomethylethylamine.html. 15. Farelly, J. G., Canc. Res. **42** (1982), 2106 – 2109. 16. Von Hofe, E., Canc. Res. **46** (1986), 1038 – 1042. 17. Rao, T.K., Mutat. Res. 89(1) (1981**)**, 35 – 43. 18. Rao, T.K., Mutat. Res**. 67**(1) (1979), 21 **-** 26.

	19. Padma, P.R., Cancer Lett. **46**(3) (1989), 173 - 180. 20. N-Nitroso-1,2,3,6-Tetrahydropyridine CASRN: 55556-92-8, GENE-TOX, Toxicology Data Network, U.S. National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+genetox:@term+@rn+@rel+55556-92-8.

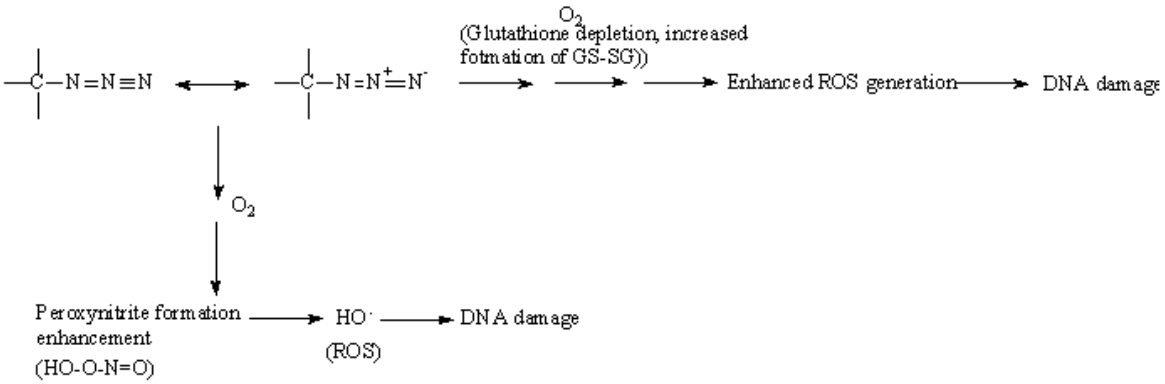
Individual profile/alert	
Name	Non-Cyclic Alkyl Phosphoramides and Thionophosphoramides
Type of profile	Structural alert
Description/applicability domain	 C(acy)(sp3) corresponds to -CH_3 or -C_2H_5 or -CH_2OH; no more than two -CH_2OH groups, should be bound to different N-atoms)
Mechanism	**A_N2 Shiff base formation (after S9 metabolic activation only)**



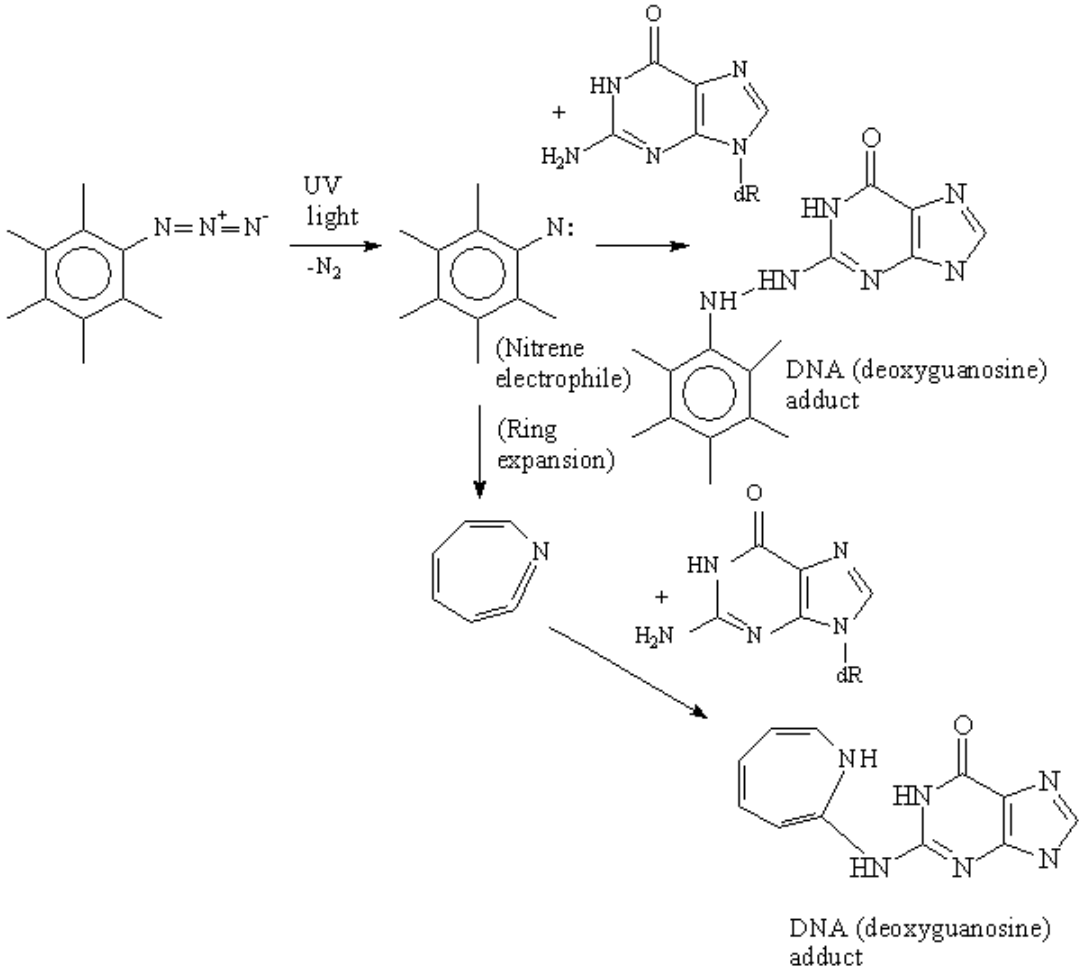
Set of chemicals used for profile development	Non-Cyclic Alkyl Phosphoramides and Thionophosphoramides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according 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; http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+680-31-9. 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	
Mechanism	**Radical ROS generation, S_N1 Nucleophilic attack after nitrene formation and Non-covalent interactions DNA intercalation**

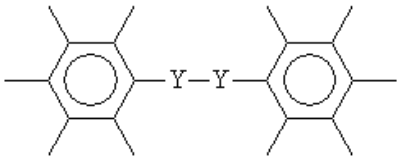
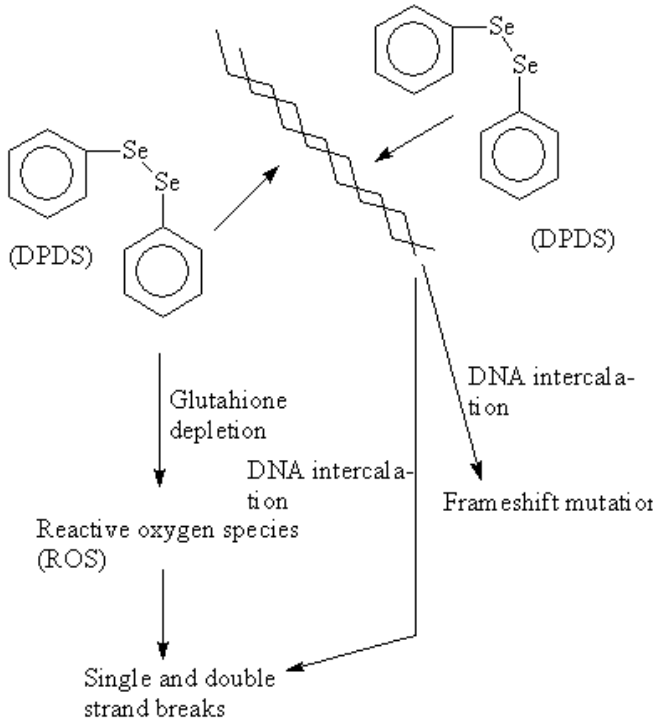
Two principal mechanisms of DNA damage, eliciting bacterial mutagenicity can be suggested. The first one is associated with the pro-oxidant properties of organic azides such as AZT, resulting in endogenous glutathione depletion and enhanced peroxynitrite and reactive oxygen species (ROS) formation [9,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



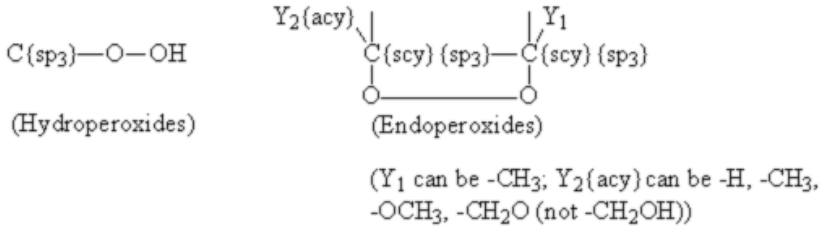
The second mechanism is mainly associated with arylazides, and the subsequent generation of electrophilic arylnitrene species, following light activation [11]. The following expertly assumed mechanistic scheme can be outlined:



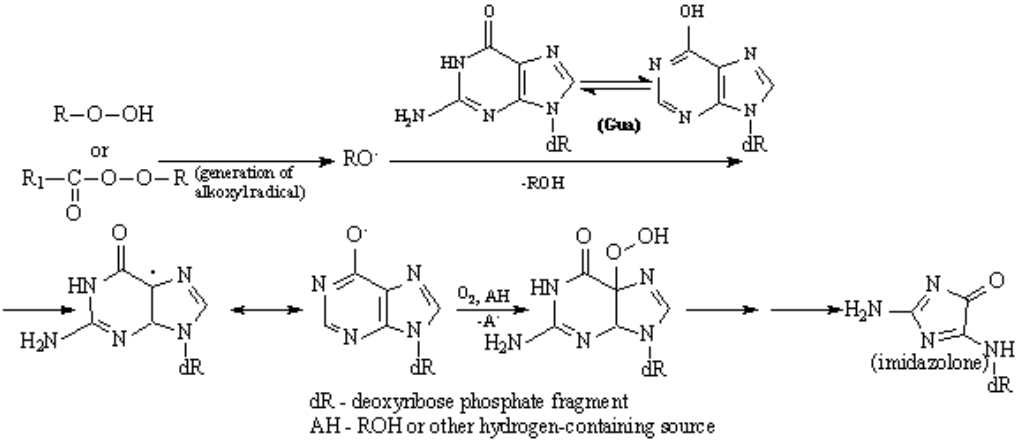
Set of chemicals used for profile development	Organic Azides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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 09.2019.

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	N/A
Data/Knowledge used for profile development	An extensive review of the literature was 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	
Mechanism	**Radical ROS generation (indirect) or direct radical attack on DNA**

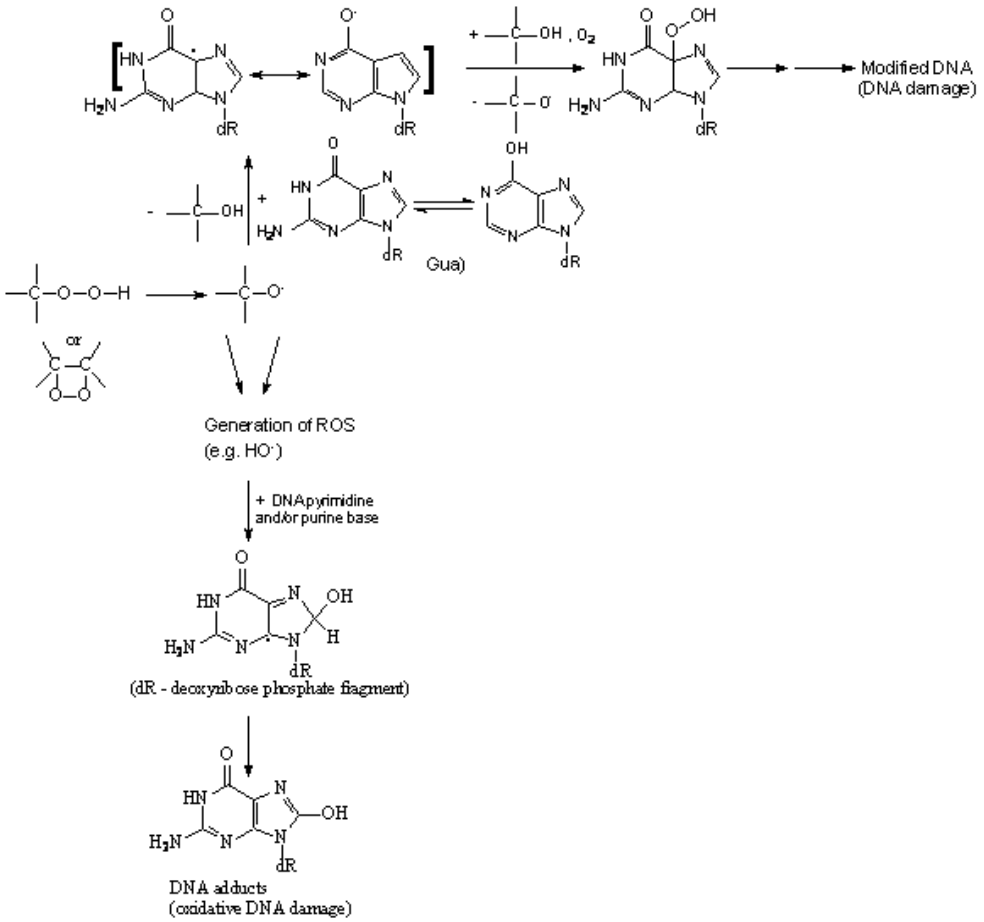
Alkoxyl radicals have been detected during the photolysis of water-soluble peroxyester, and, in the presence of DNA, oxidative damage of the latter was demonstrated *via* the formation of guanidine-releasing products by alkoxyl radicals, according to the following mechanistic scheme 1 [2]:



Scheme 1

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.

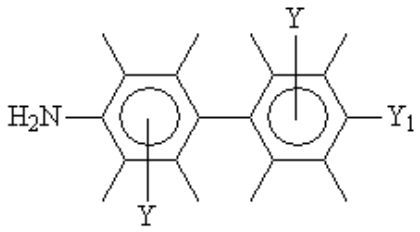
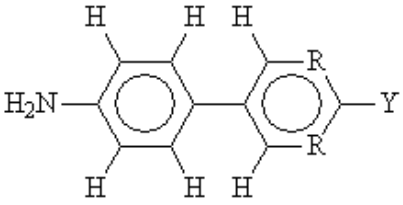
The following hypothetical mechanistic scheme for eliciting mutagenicity of hydroperoxides and endoperoxides can be assumed based on literature in Scheme 2 below.

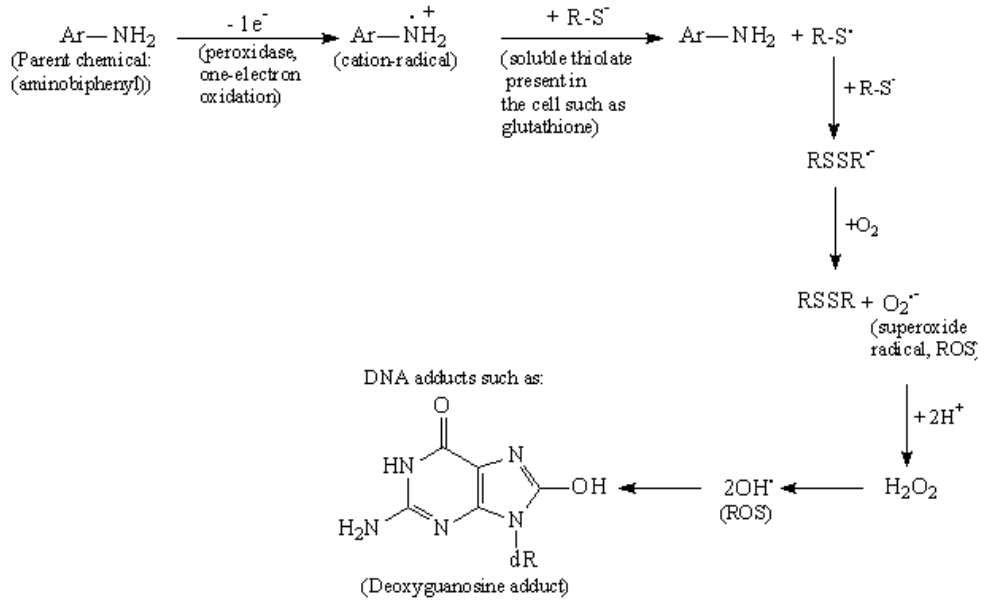


Scheme 2

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

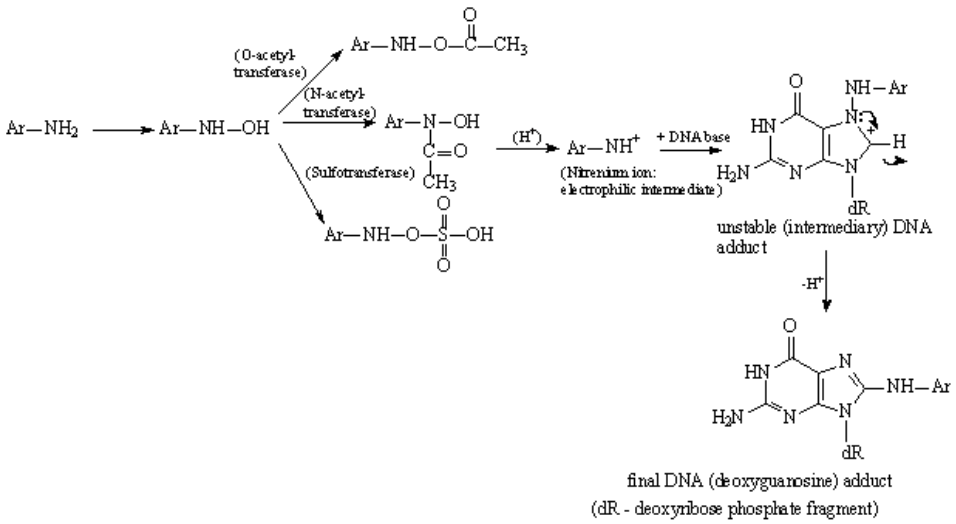
Individual profile/alert	
Name	p-Aminobiphenyl Analogs
Type of profile	Structural alert
Description/applicability domain	

	 (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:	



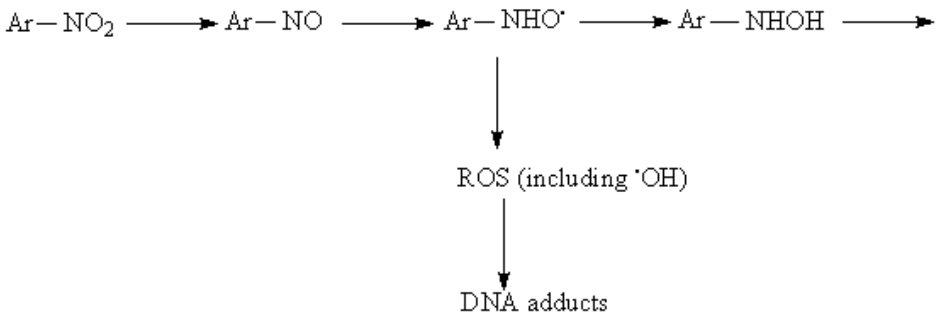
Scheme 1

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]:



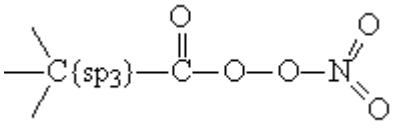
Scheme 2

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]:

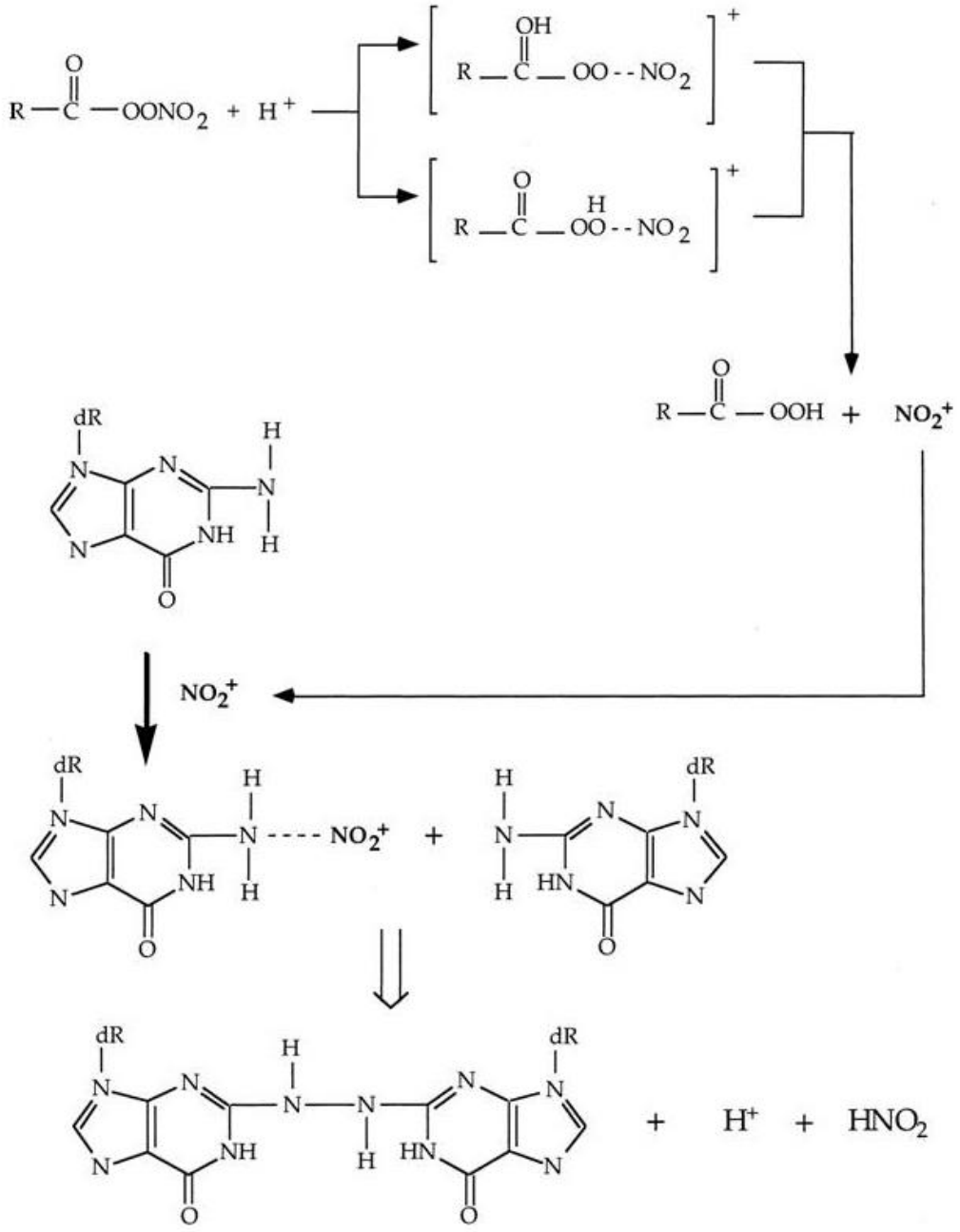


Scheme 3

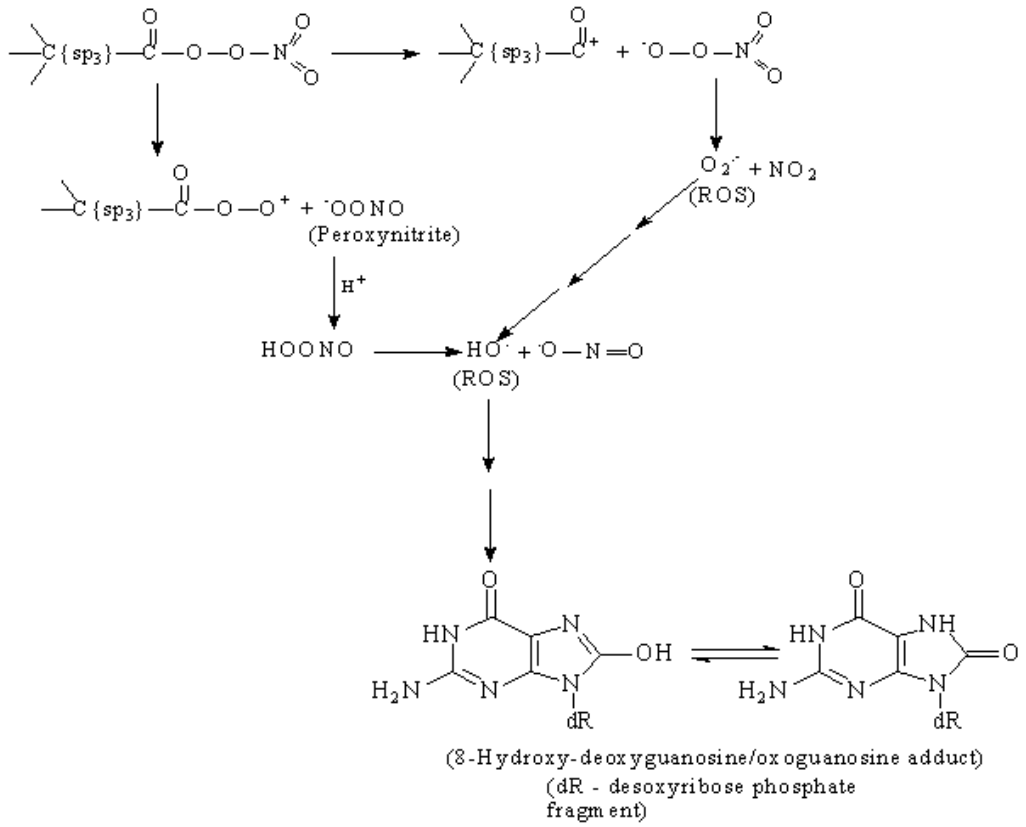
Set of chemicals used for profile development	p-Aminobiphenyl Analogs
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

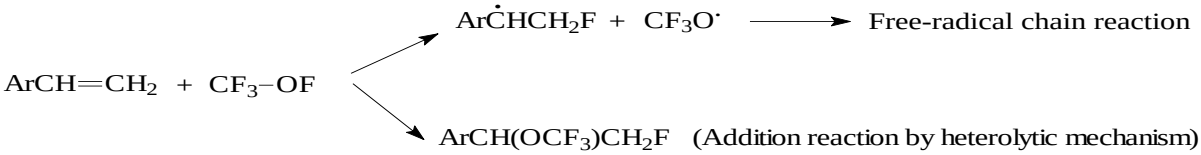


Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Kleindienst, 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. National Library of Medicine**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+2278-22-0. 5. Liu, Mol. Carcinog. **25** (1999), 196 – 206.

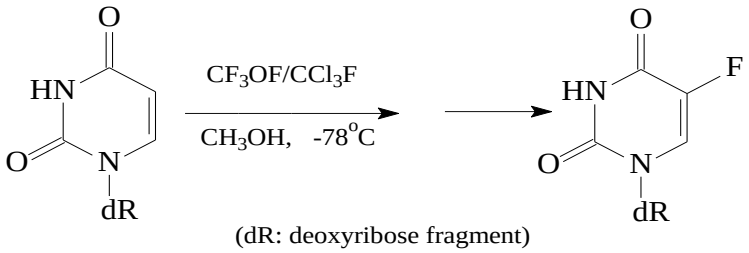
Individual profile/alert	
Name	Perfluorinated Hypofluorites
Type of profile	Structural alert
Description/applicability domain	$R_F—O—F$ $R—C(=O)—O—F$ (R_F is C_nF_{2n+1} (perfluorinated alkyl chain); R is C_nH_{2n+1} or C_nF_{2n+1}, n = 1 - 5)

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]:

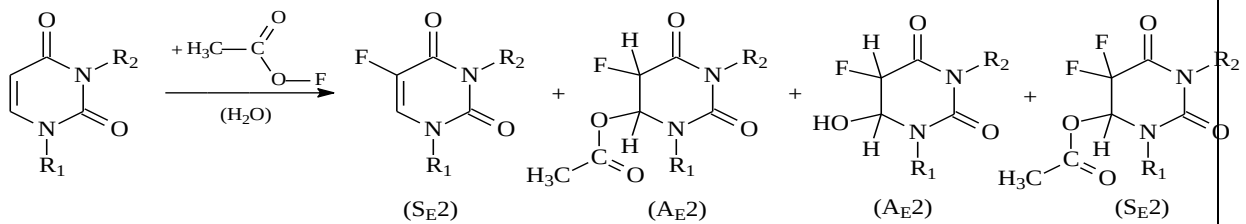


(dR: deoxyribose fragment)

(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]:

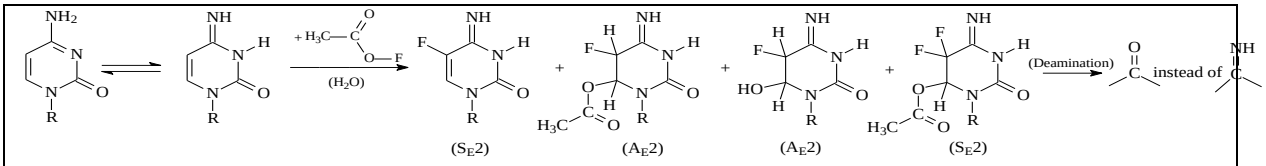
Uracil and Its Derivatives (Scheme 2):



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

(Scheme 2)

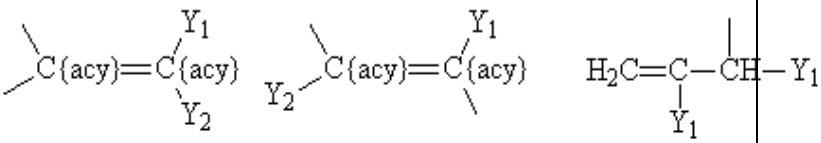
Cytosine and Its Derivatives (Scheme 3):



(Scheme 3)

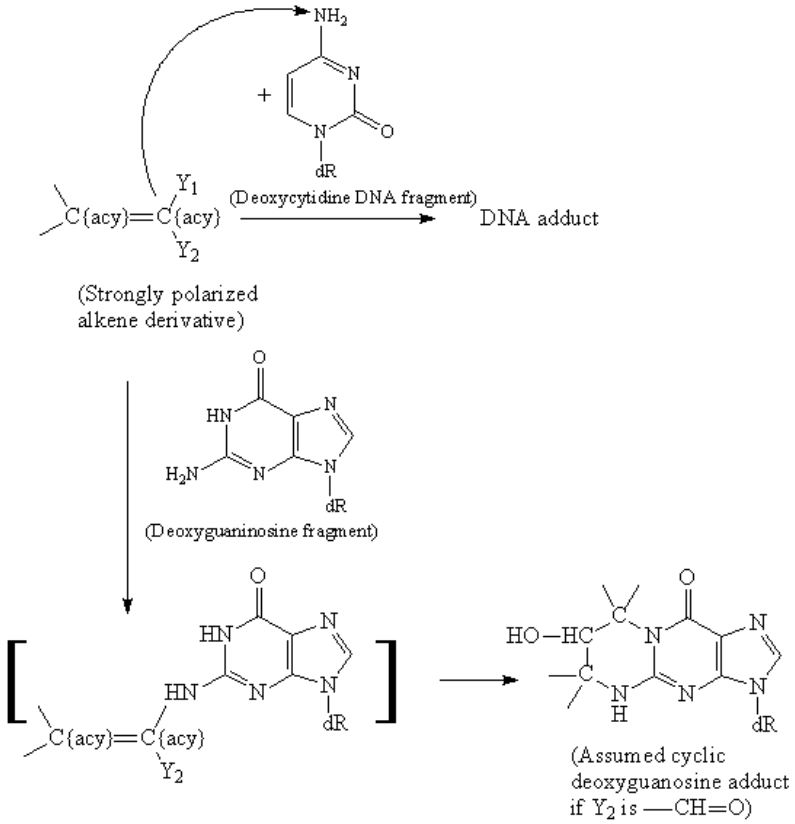
Conclusion: Chemicals from the sub-class discussed above are assumed to be DNA-reactive and, despite of lack of any relevant data, are likely to exert positive in vitro genotoxicity effects.

Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was 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	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**

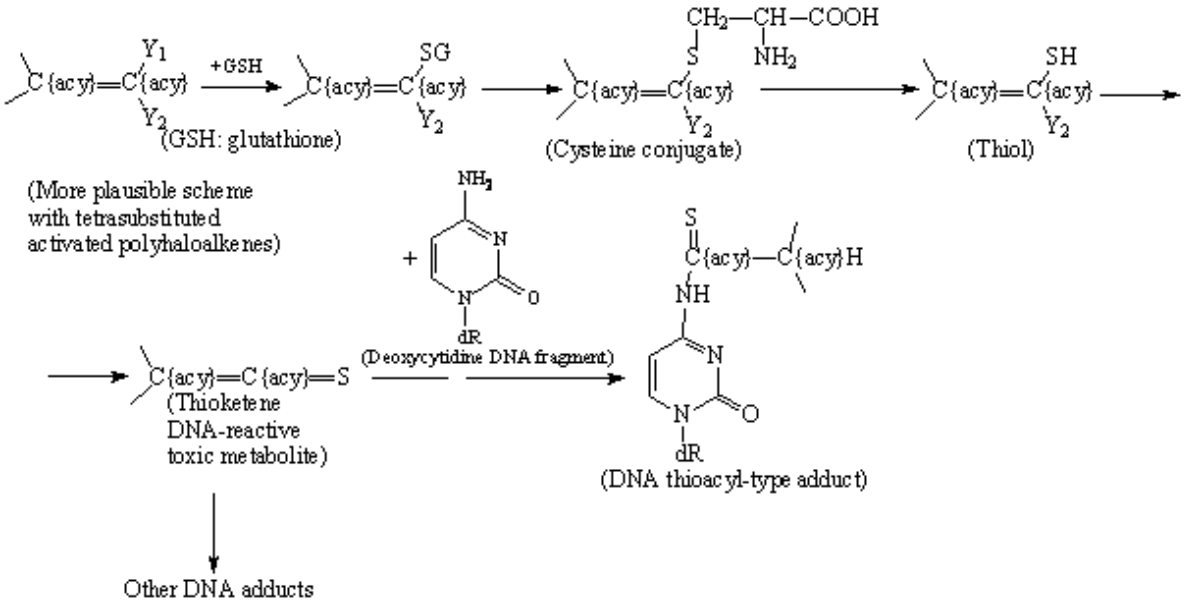
	activated sp2 carbon atom, A_N2 Thioacylation *via* nucleophilic addition after thioketene formation and A_N2 Shiff base formation

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:



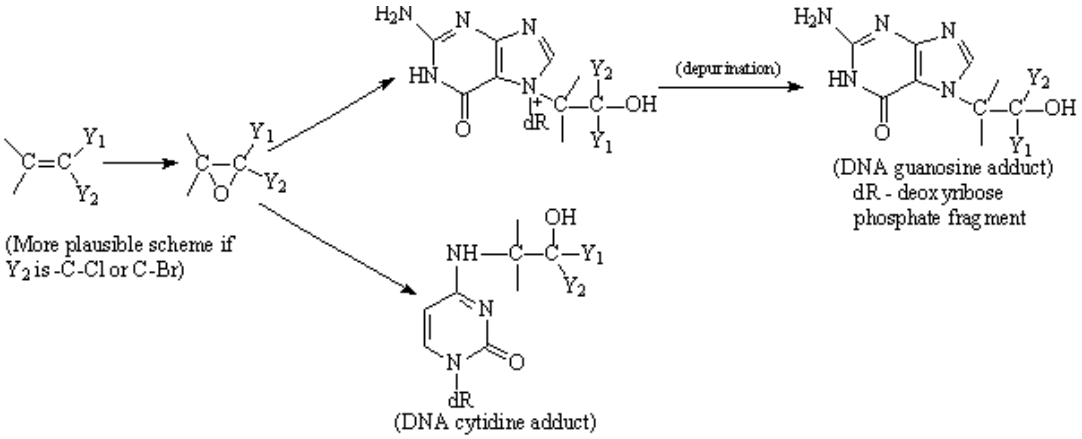
Scheme 1

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:



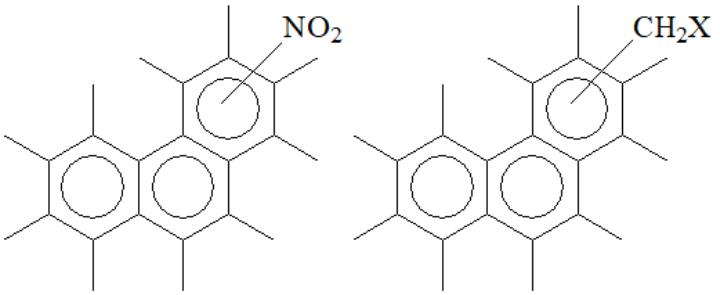
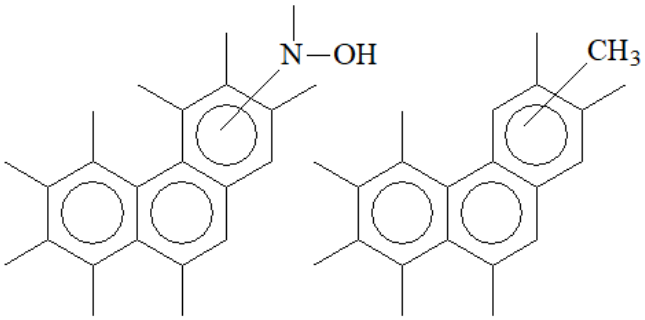
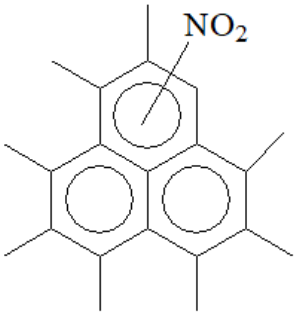
Scheme 2

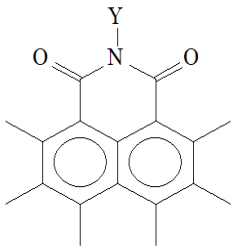
Scheme III: Metabolic activation *via* epoxidation shown in Scheme 3:

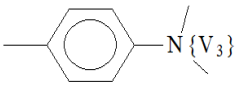


Scheme 3

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.

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

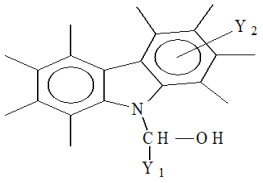


(Y is —(CH2)n—N{V3}— or 

(n = 2 or 3)

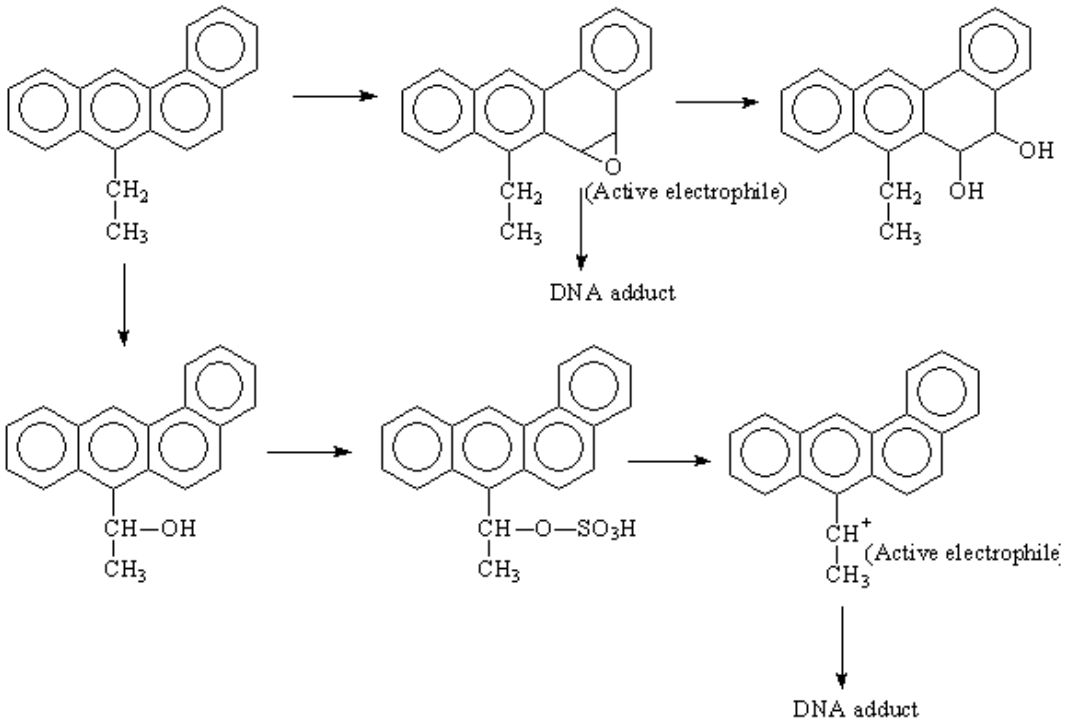
No more than two fused benzene rings;
No -C(O)O-, -C(O)NH- or -SO3H groups attached

Naphthaleneimide derivatives



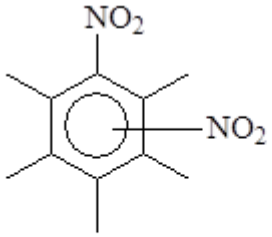
(Y1 is –H or –CH3;
Y2 is –H or –CH3 (number of –CH3 groups 1 or 2, can be attached anywhere); or –H (all);
No other substituents)

Carbazole derivatives

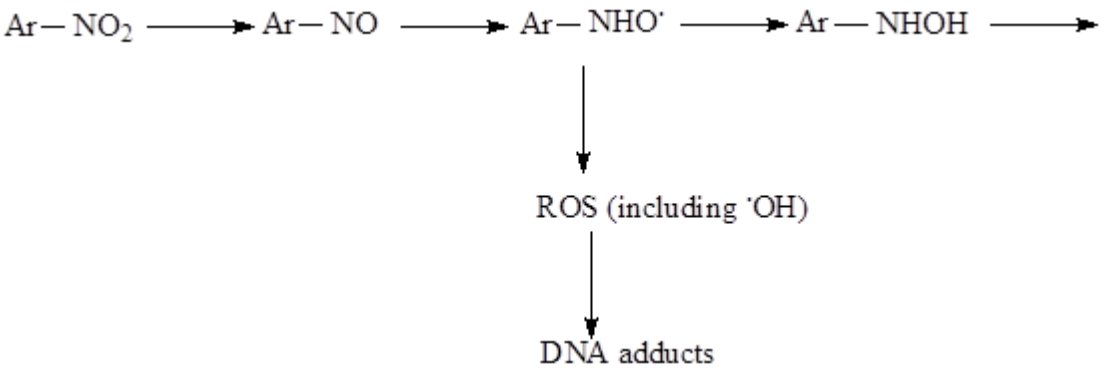
Mechanism	**SN2 Alkylation, direct acting epoxides and related after P450-mediated metabolic activation, SN1Alkylation after metabolically formed carbenium ion species and Non-covalent interactions DNA intercalation**
	
Set of chemicals used for profile development	Polycyclic Aromatic Hydrocarbon, Naphthaleneimide 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/ 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.

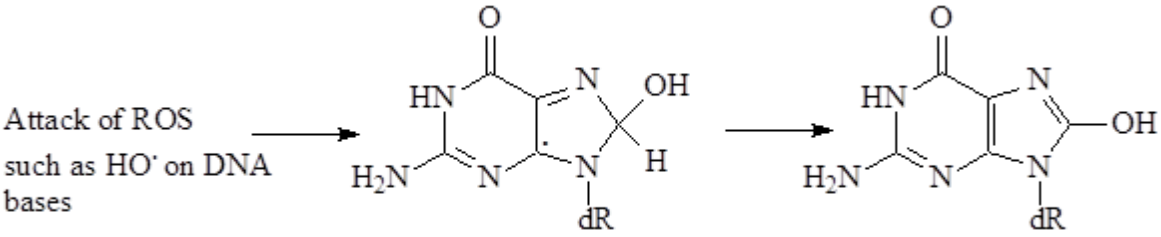
Individual profile/alert	
Name	Polynitroarenes
Type of profile	Structural alert

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)**:**

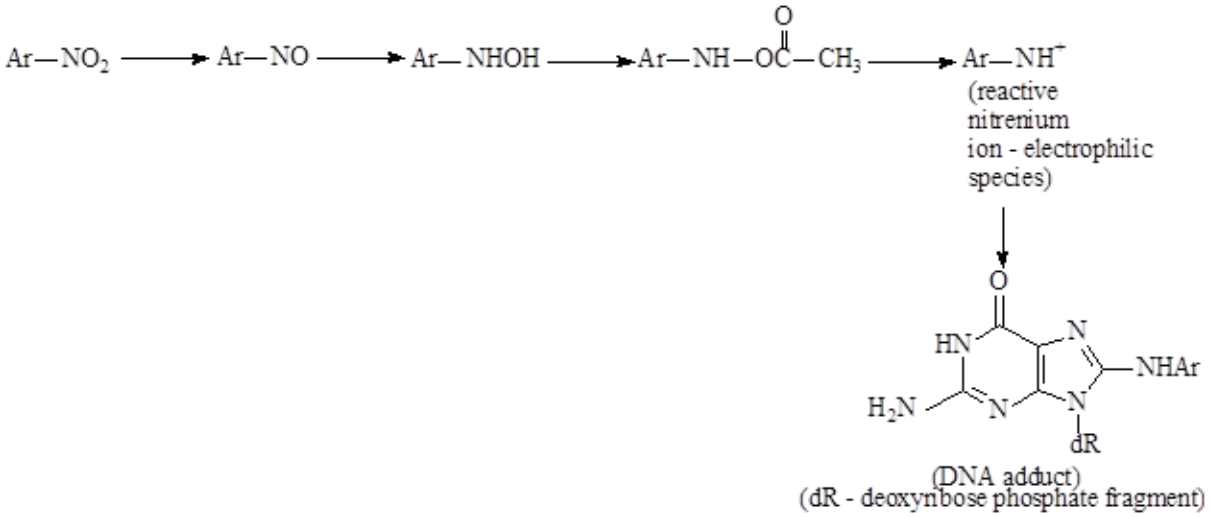


As a result from the generation of reactive radical species such as ArNHO·, an additional formation of ROS such as $O_2^{\cdot-}$ and/or HO· occurs. The hydroxyl radical, for example, is DNA-reactive and adducts, involving pyrimidine and purine nucleoside bases can be formed. The 8-hydroxyguanine adduct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



(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]:



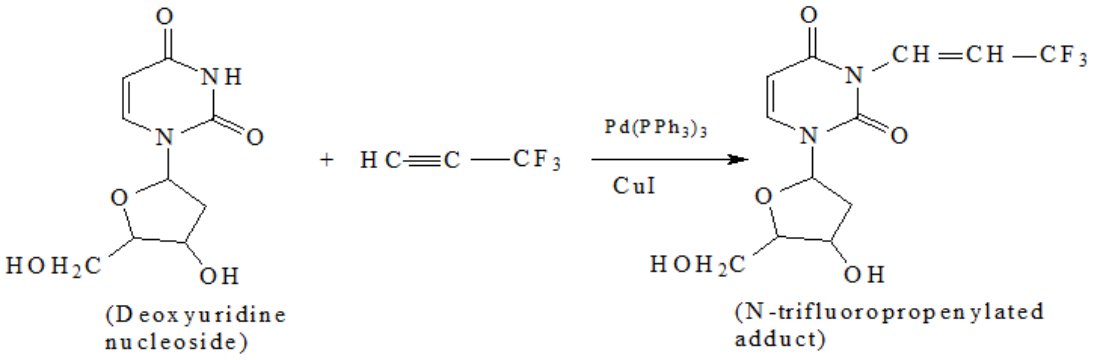
Chemicals such as 2,6-dinitrotoluene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene, etc., containing more than one nitro group were found to be bacterial mutagens both in the presence and the absence of S9 mix [4].

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.

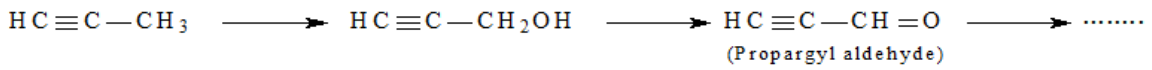
Individual profile/alert	
Name	Propyne Derivatives
Type of profile	Structural alert
Description/applicability domain	$HC{\equiv}C{-}Y$ (Y are electron-withdrawing groups such as $-CF_3$, $-CHF_2$, $-CH_2F$, $-CH_2Cl$, $-CH_2Br$ or –CH=O)
Mechanism	SN_2: Alkylation, nucleophilic substitution at sp3-carbon 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)

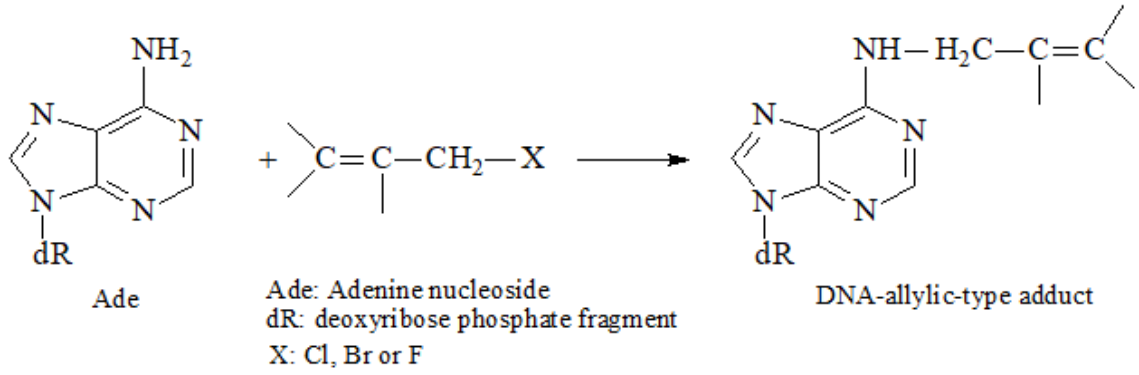
In some separate experiments, however, it was shown that the catalyst was not required for the adduct formation. The mechanism of N-trifluoropropenylation was considered to be similar to the Michael-type addition. Here the N3 atom of pyrimidine fragment adds as a nucleophile to the terminal carbon atom 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:



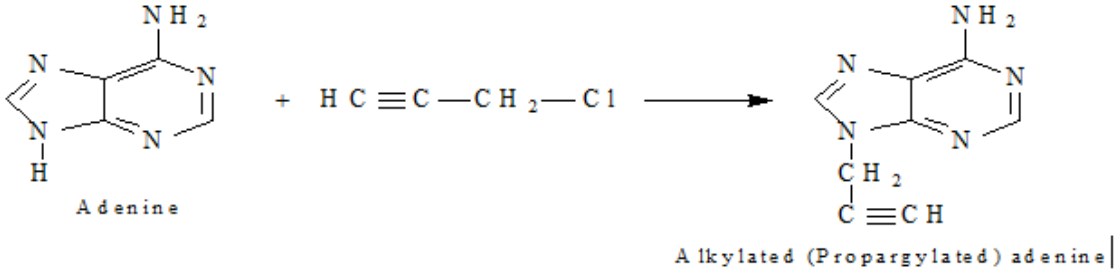
(Scheme 2)

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)

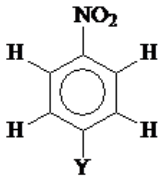


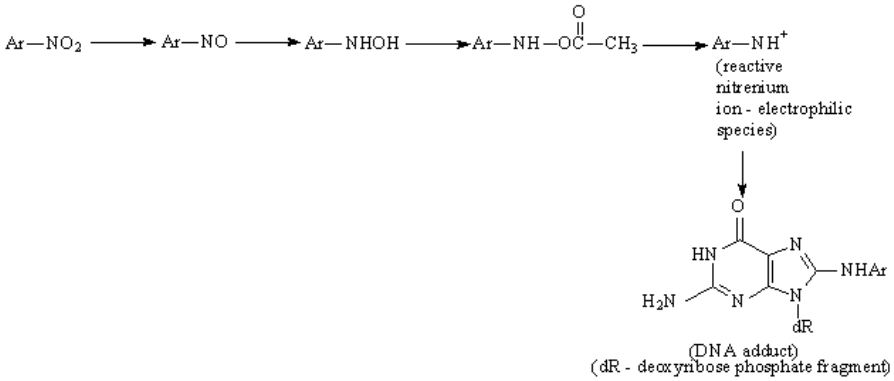
(Scheme 4)

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

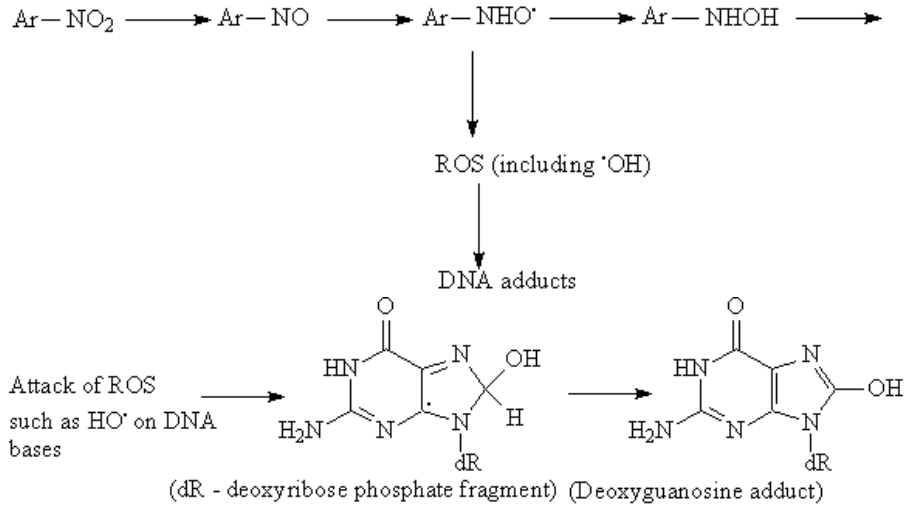
Set of chemicals used for profile development	Propyne Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	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= Any Carbon(sp^3) or Aliphatic Carbon(sp^2)**
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	

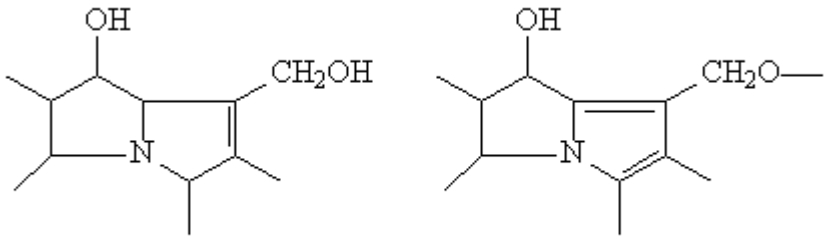


Homolytic

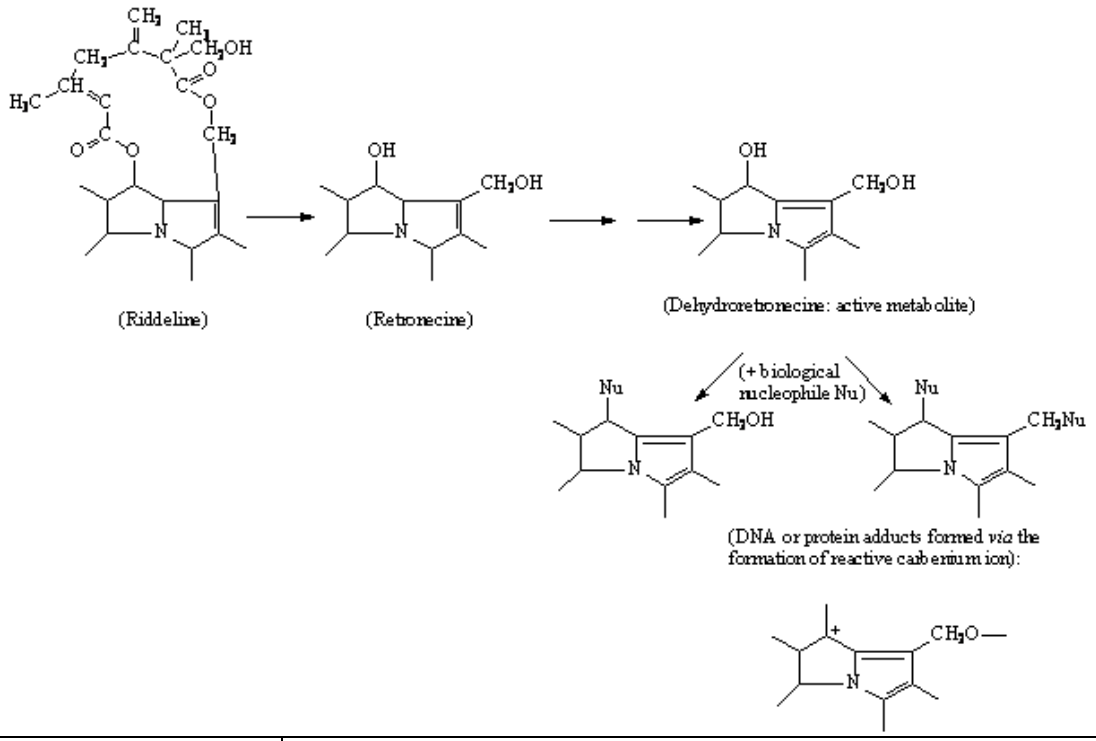


Set of chemicals used for profile development	p-Substituted Mononitrobenzenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS.

Individual profile/alert

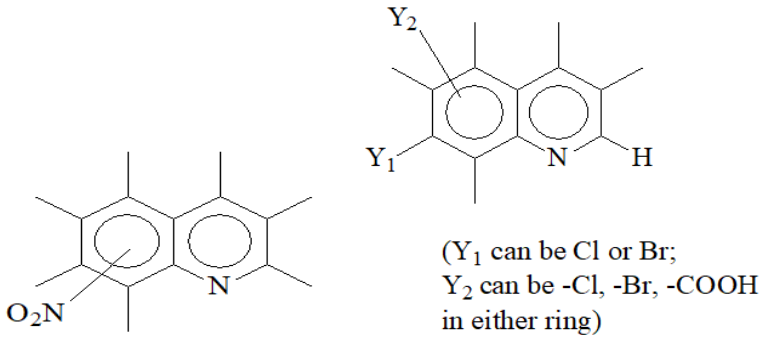
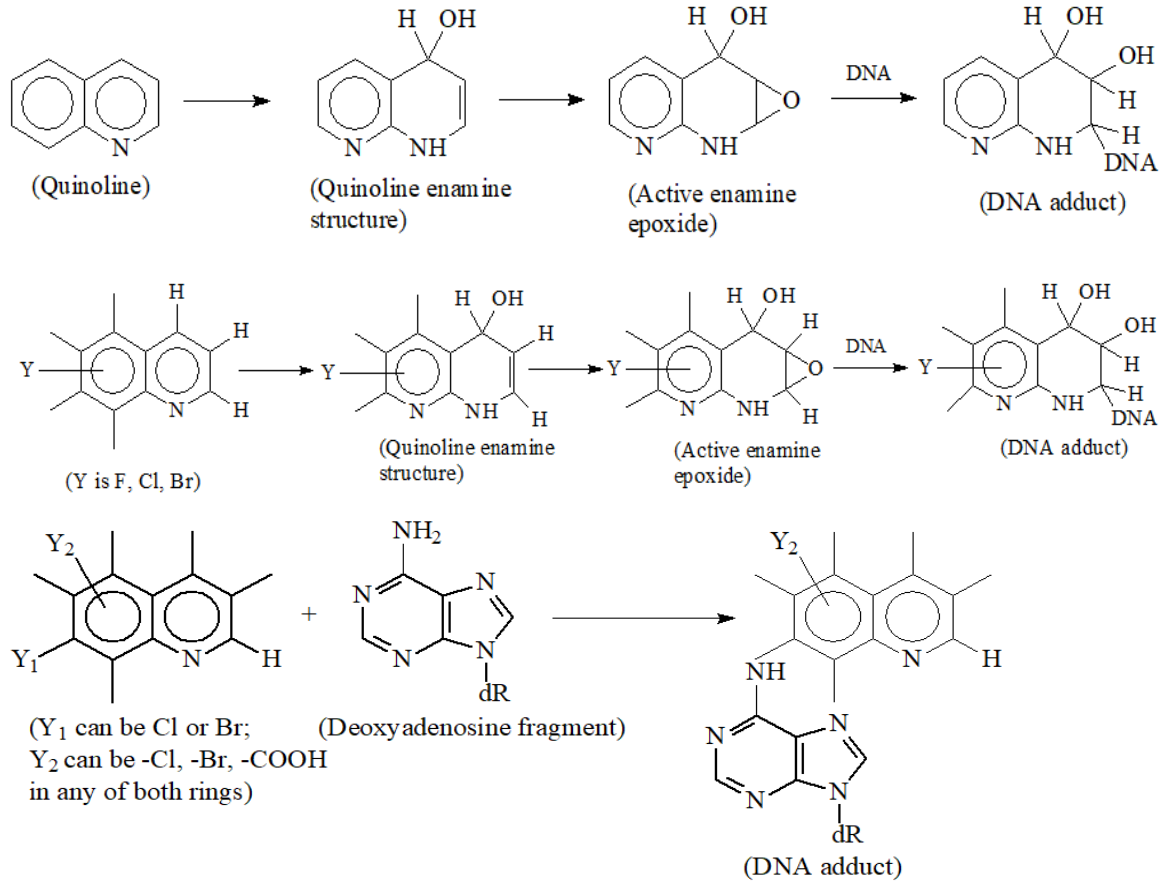
Name	Pyrrolizidine Derivatives
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N1 Nucleophilic attack after carbenium ion formation**

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

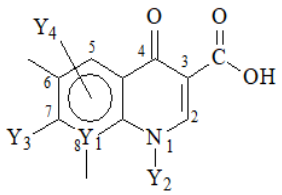
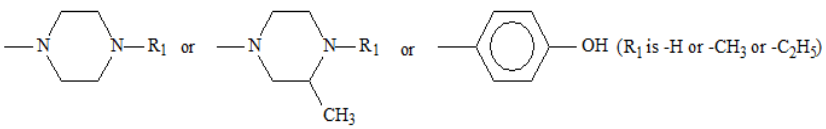


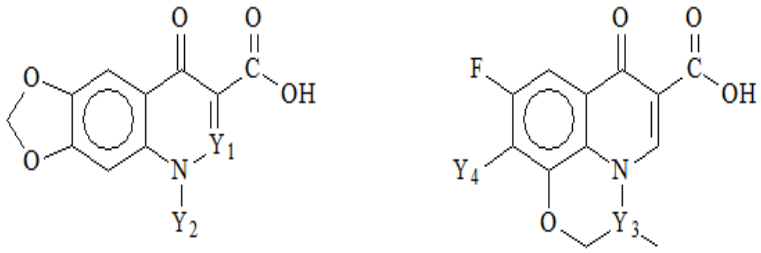
Set of chemicals used for profile development	Pyrrolizidine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
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	
Mechanism	**S_N2 Direct acting epoxides formed after metabolic activation & S_N2 at an activated carbon atom**
	
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://www.epa.gov/iris, last visited 10.2019

	2. 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. 3. *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. 4. Ferguson, L. R., W. A. Denny, Genotoxicity of Non-Covalent Interactions: DNA Intercalators (Review), Mutat. Res. **623** (2007), 14 – 23. 5. Snyder, R. D., Possible Structural and Functional Determinants Contributing to the Clastogenicity of Pharmaceuticals, Environ. Molec. Mutag. **51** (2010), 800 – 814. 6. 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. 7. 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

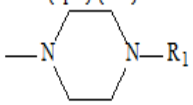
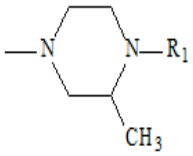


(Structures types 2 and 3: Tricyclic fused-ring systems)

Y_1 can be C or N{V3};

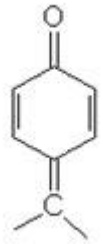
Y_2 can be  or $-CH_3$ or $-CH_2CH_3$;

Y_3 can be CH or N{sp3}{V3}

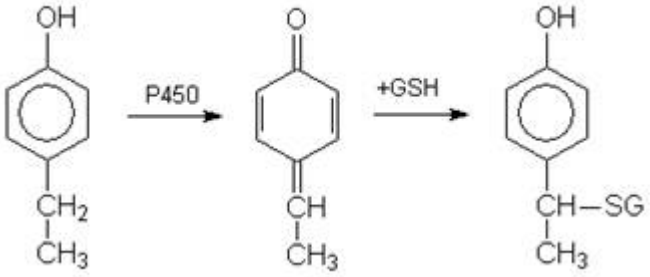
Y_4 can be  or  (R_1 is -H or $-CH_3$ or $-C_2H_5$)

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.

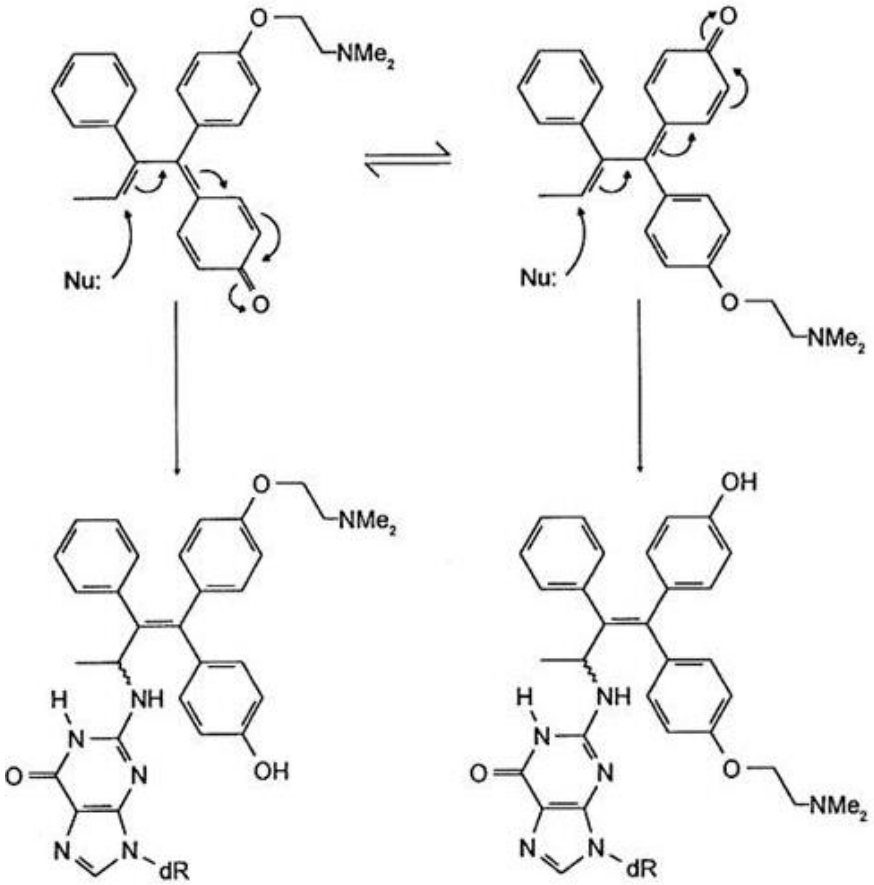
Individual profile/alert

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

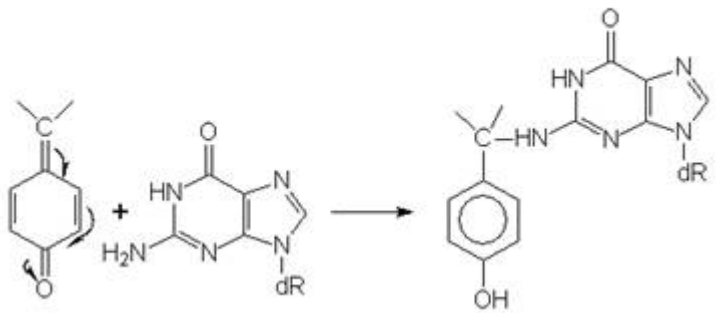
Results have demonstrated that a series of simple, sterically-unhindered alkylphenols are metabolized to reactive quinone methide intermediates by mammalian liver enzymes. This oxidation mechanism is regarded as common for an increasing number of *p*-alkylphenols and appears to play a significant role in their reported cytotoxic effects, mostly, by glutathione depletion. The following scheme of the formation of glutathione conjugates from 4-ethylphenol *via* quinone methide intermediate was suggested by these authors [3]:



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



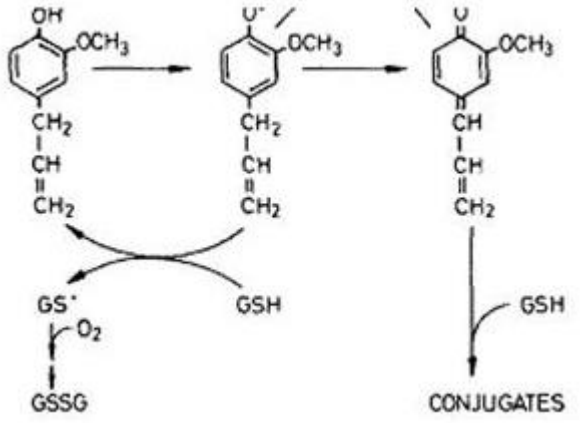
Therefore, based on the above data, the following general scheme of DNA reactivity, and the resulting mutagenicity effects of quinone methide structural fragments can be assumed:



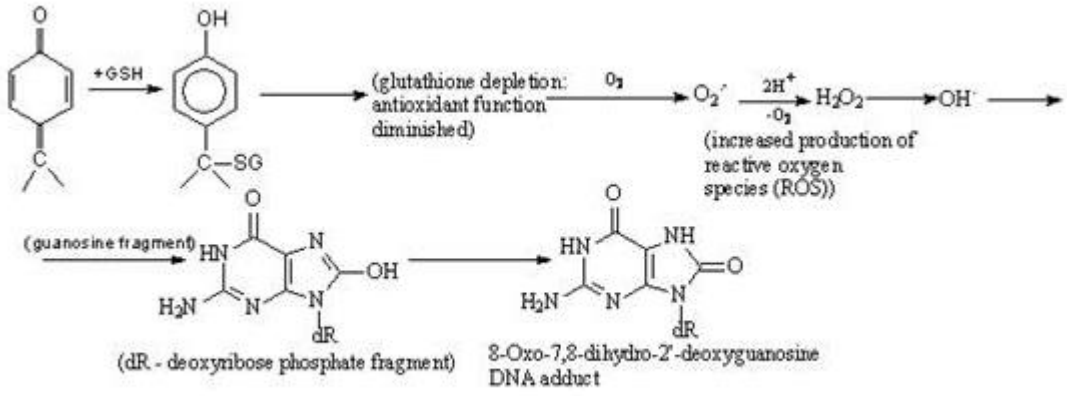
where dR represents desoxyribose fragment.

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

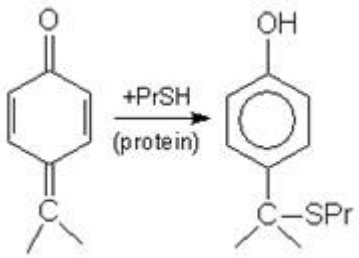
scheme [6]:



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

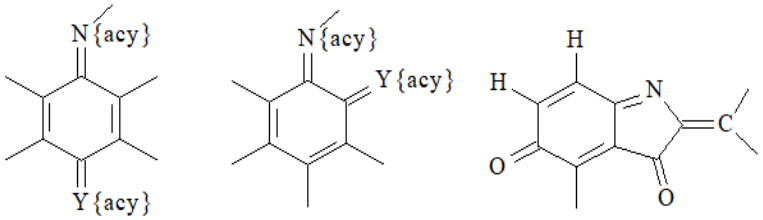
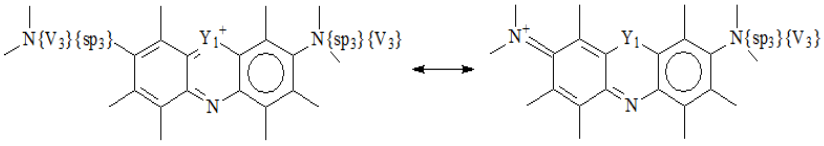


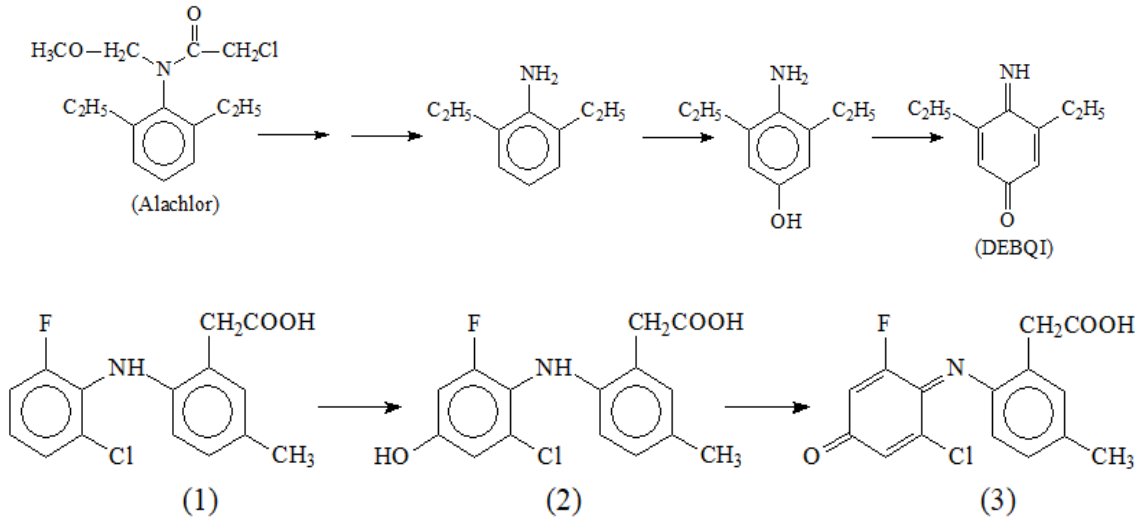
Formation of topoisomerase II inhibition complex, contributing to the chromosomal aberration *via* attack of quinone methide metabolite on the thiol functional groups of cysteine fragments in a protein (enzyme) in a similar mode as that of glutathione conjugation showed above cannot be excluded [7]:



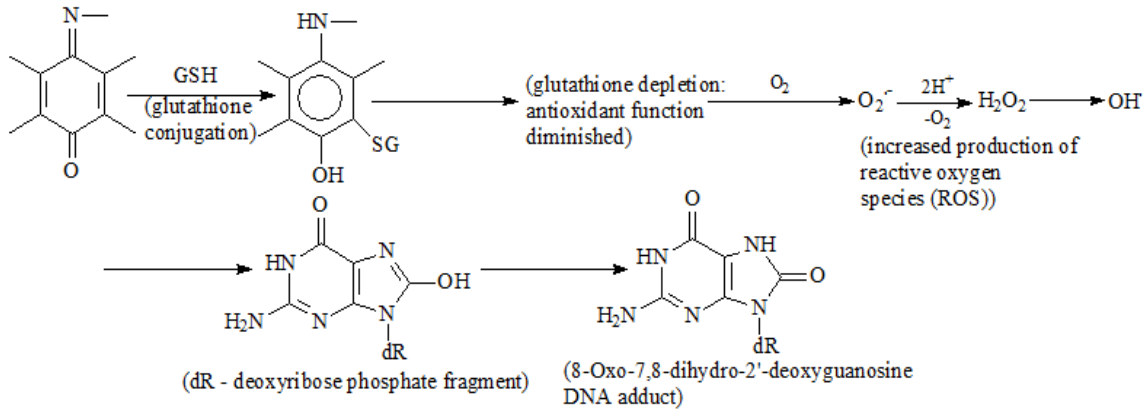
Consequently, it can be assumed that quinone methide intermediates formed during the metabolism of

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

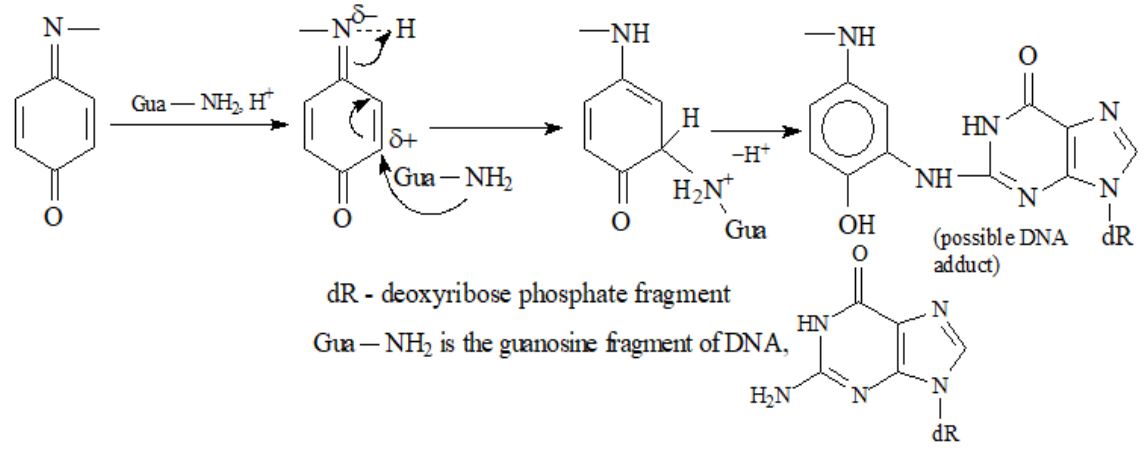
Individual profile/alert	
Name	Quinoneimine, Thione and Phenoxazinium Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is O or N{V3}); {acy}: acyclic atom (No more than one *additional* substituent on the six-membered ring; (in case of $–CH_3$ and/or $–C_2H_5$ the number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



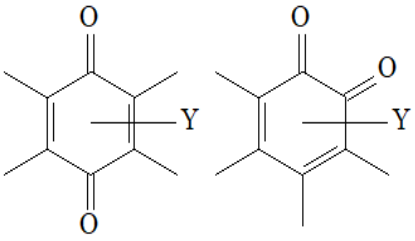
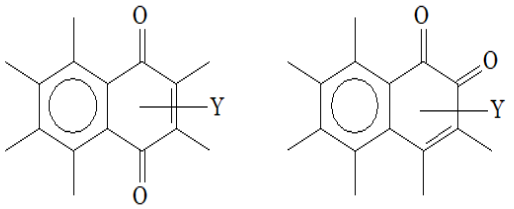
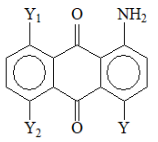
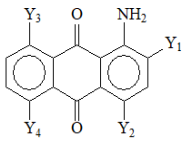
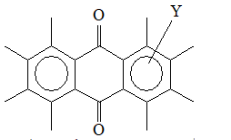
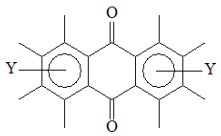
II. **<u>Michael-type addition mechanism</u>.** Such a scheme is regarded as less plausible, since it is based on the direct interaction of "soft" electrophile (quinoneimine derivative) with "hard" nucleophile (DNA base):



III. **<u>DNA intercalation between DNA base pairs</u>:** 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 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 11. Hesbert, A., Toxicol. Lett. **21**(1) (1984), 119 – 125 12. CCRIS: Indigo, Toxicology Data Network, U.S. National Library of Medicine; http://chem.sis.nlm.nih.gov/chemidplus/rn/482-89-3 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 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://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+581-64-6. 18. Methylene Blue, CCRIS, Toxicology Data Network, U.S. National Library of Medicine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+61-73-4. 19. Basic Blue 3, Toxicology Data Network, U.S. National Library of Medicine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+33203-82-6 20. Hossain, M., Mol. BioSyst **5** (2009), 1311 – 1322. 21. Hecht, Chr., *J. Phys. Chem. B* 108(29), (**2004)**, 10241 – 10244.

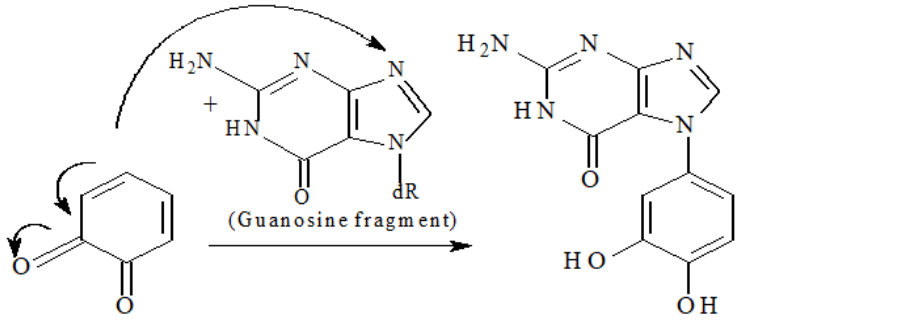
Individual profile/alert	
Name	Quinones and Trihydroxybenzenes
Type of profile	Structural alert

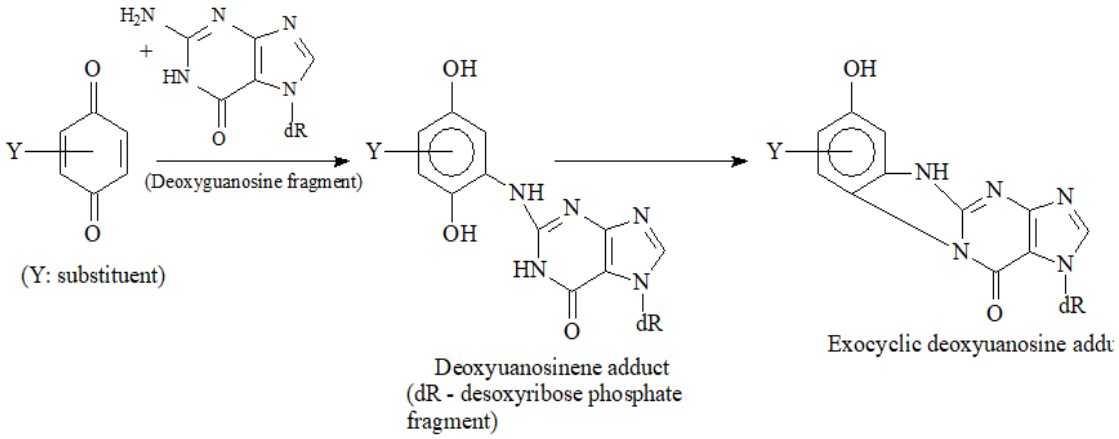
Description/applicability domain	 (Y can be Cl, Br (more than one); -CN, $-NO_2$, -C=O, -CHOH or H or C{ar}or N{acy}{V3}or $-CH(CH_3)_2$ or $-C(CH_3)_3$ or combinations with -H), $-CH_2$-NH- no other substituents; for catechol quinones Y = -OH should be added  1,4-Naphthoquinones 1,2-Naphthoquinones 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  (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)

(Other possible substituents: -H, -CH_3,-OCH_3, -NH_2;
No substituents other than these)

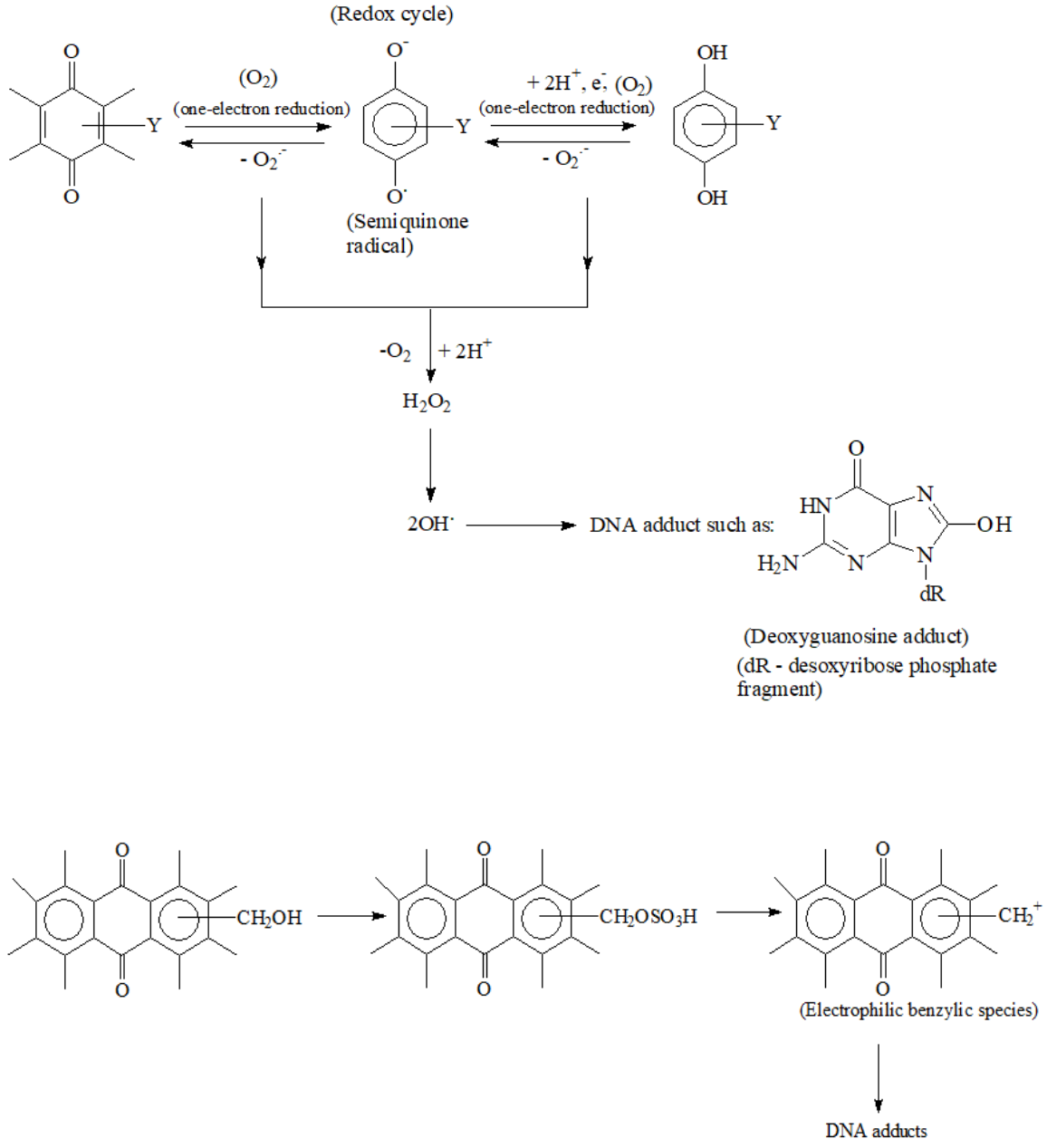
Mechanism	**A_N2 Michael-type addition, quinoid structures , Radical ROS generation (indirect) & Non-covalent interactions DNA intercalation**

1. Electrophilic mechanism for simple quinones and naphthoquinones:





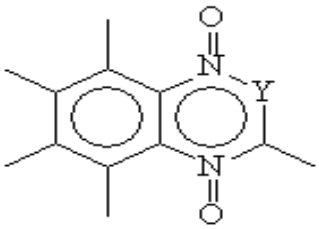
2. Radical mechanism for simple quinones, naphthoquinones, anthraquinone derivatives and trihydroxybenzenes



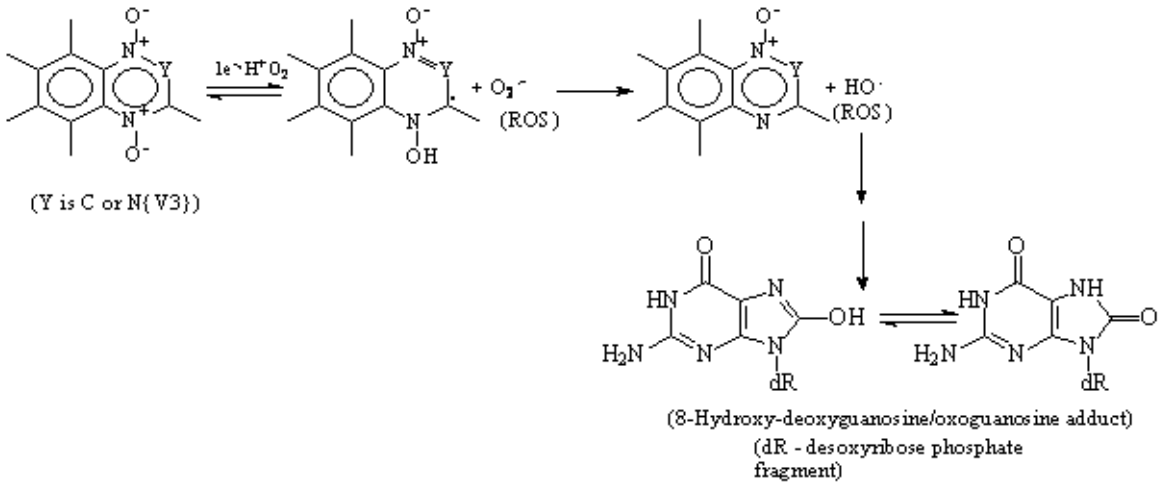
Set of chemicals used for profile development	Quinones and Trihydroxybenzenes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed 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 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; https://echa.europa.eu/documents/10162/de28d339-f99c-e6d7-564a-35a66a4319bc, last visited 10.2019. 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**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+20830-81-3); 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**;** http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+23214-92-8. 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

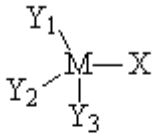
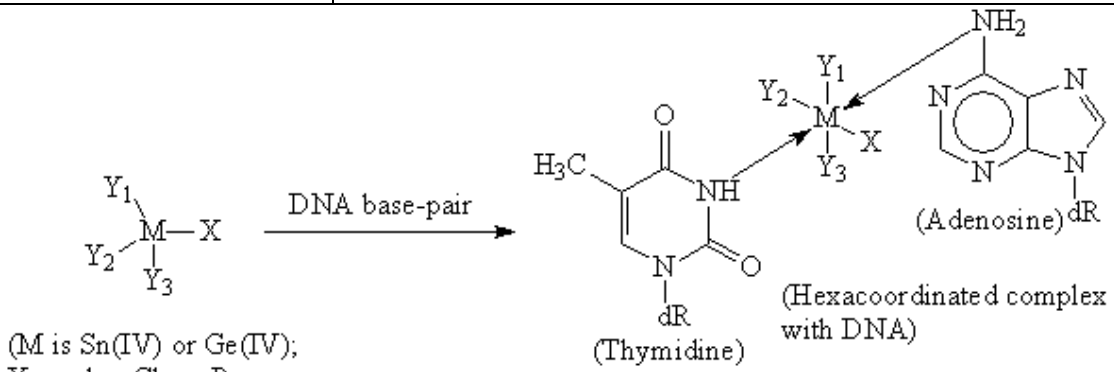
Description/applicability domain	 (Y is C or N{V3})
Mechanism	**Radical ROS generation**

The following scheme for generation of ROS and formation of DNA adducts can be assumed [5]:

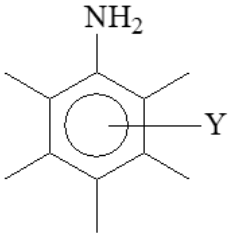


Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Yoshimura,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

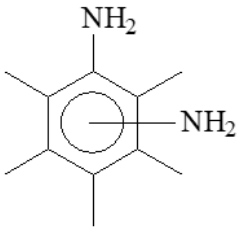
Individual profile/alert	
Name	Short-Chain Alkytin and Alkylgermanium Halides
Type of profile	Structural alert

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	N/A
Data/Knowledge used for profile development	An extensive review of the literature was 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	

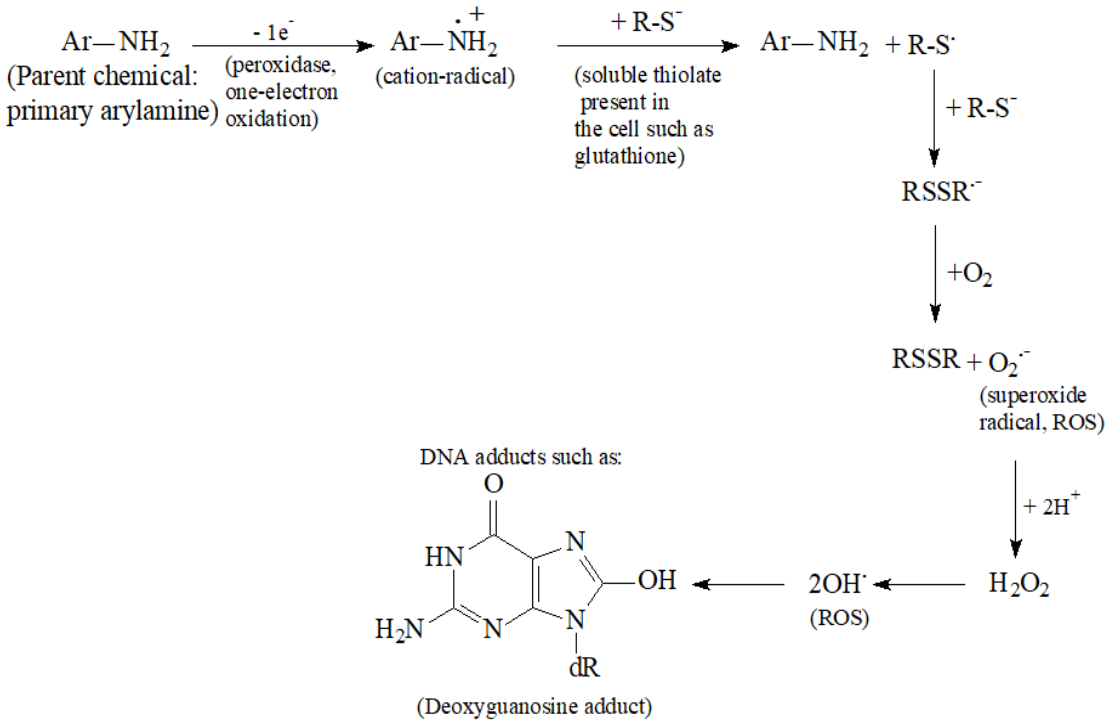


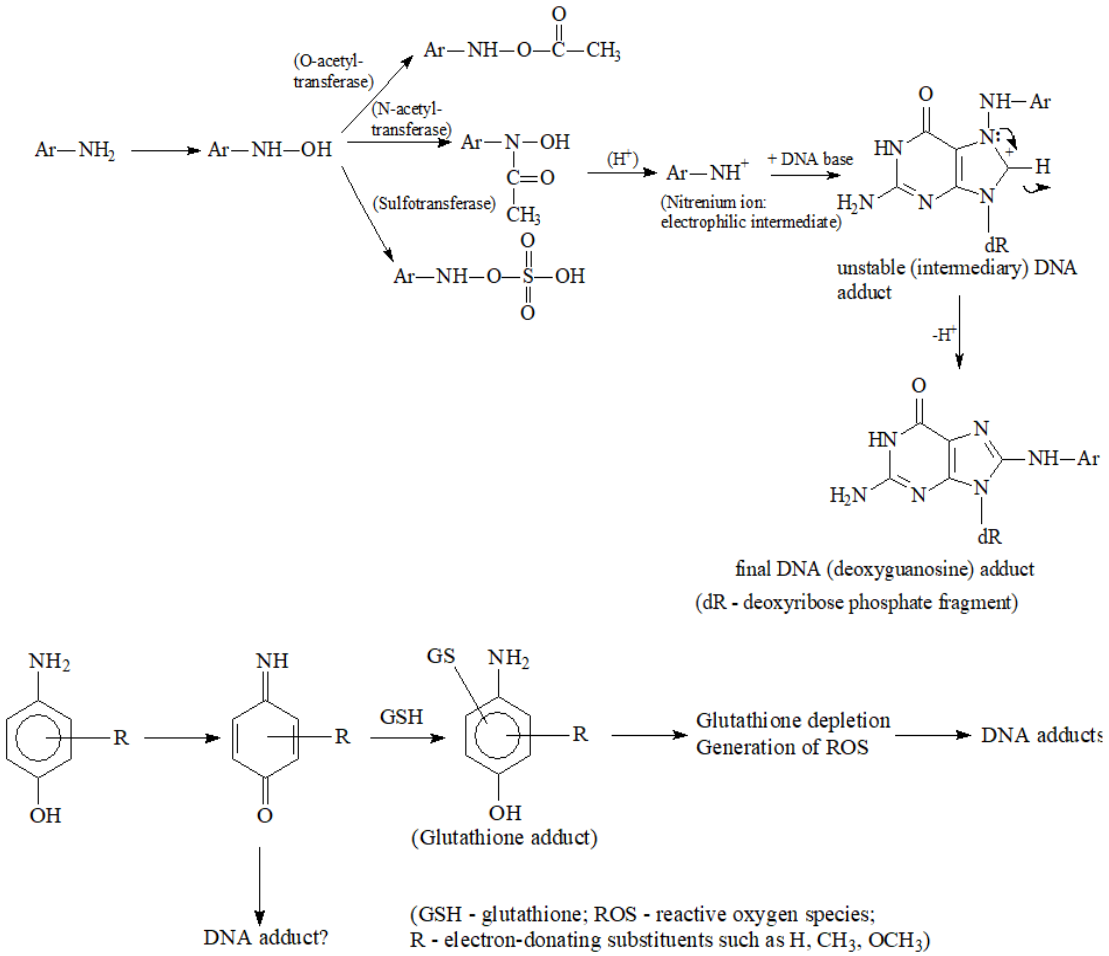
(Y can be N{V3}, C{sp3}, O-C{sp3});
No more than four substituents;
Single-ring aromatic system;
Total "masks": -SO_3H and aniline $C_6H_5NH_2$



(No more than two -NH_2 groups)

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





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*

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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 Ions*. Kerdar, R. S., D. Dehner, D. Wild, Reactivity and Genotoxicity of Arylnitrenium Ions in Bacterial and Mammalian Cells, Toxicol. Lett. 67(1-3) (1993), 73 – 85
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.
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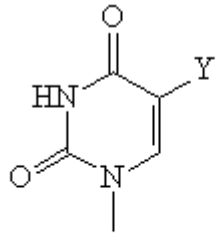
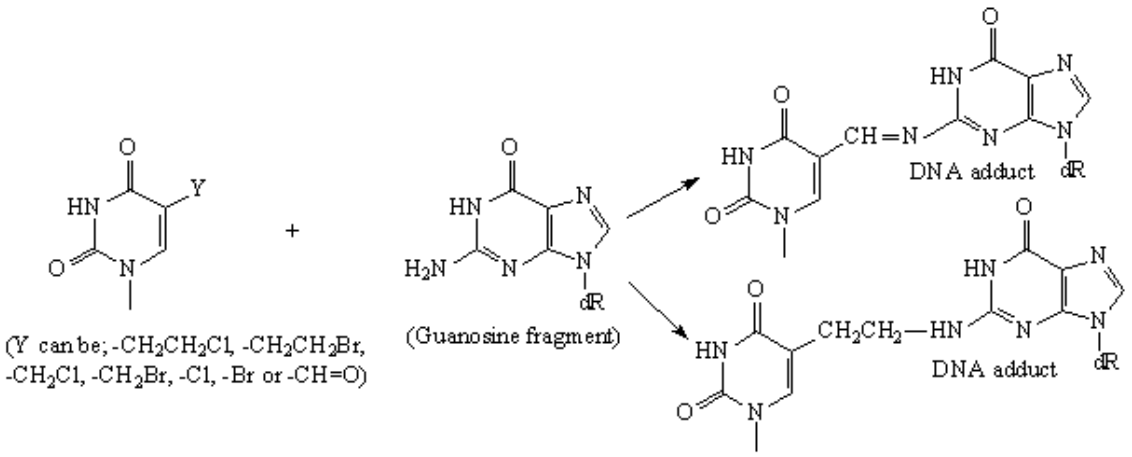
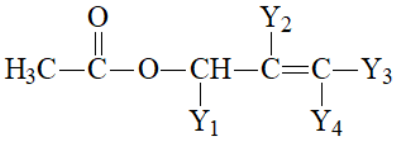
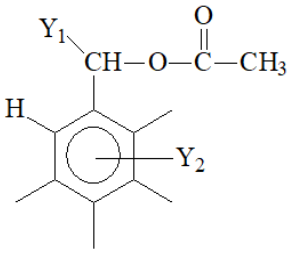
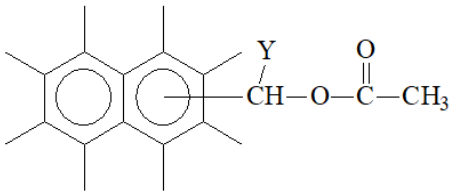
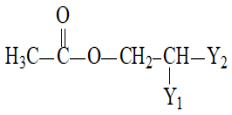
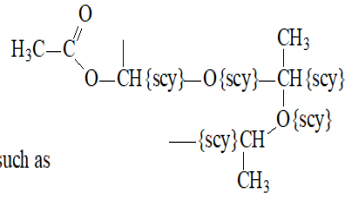
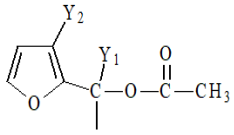
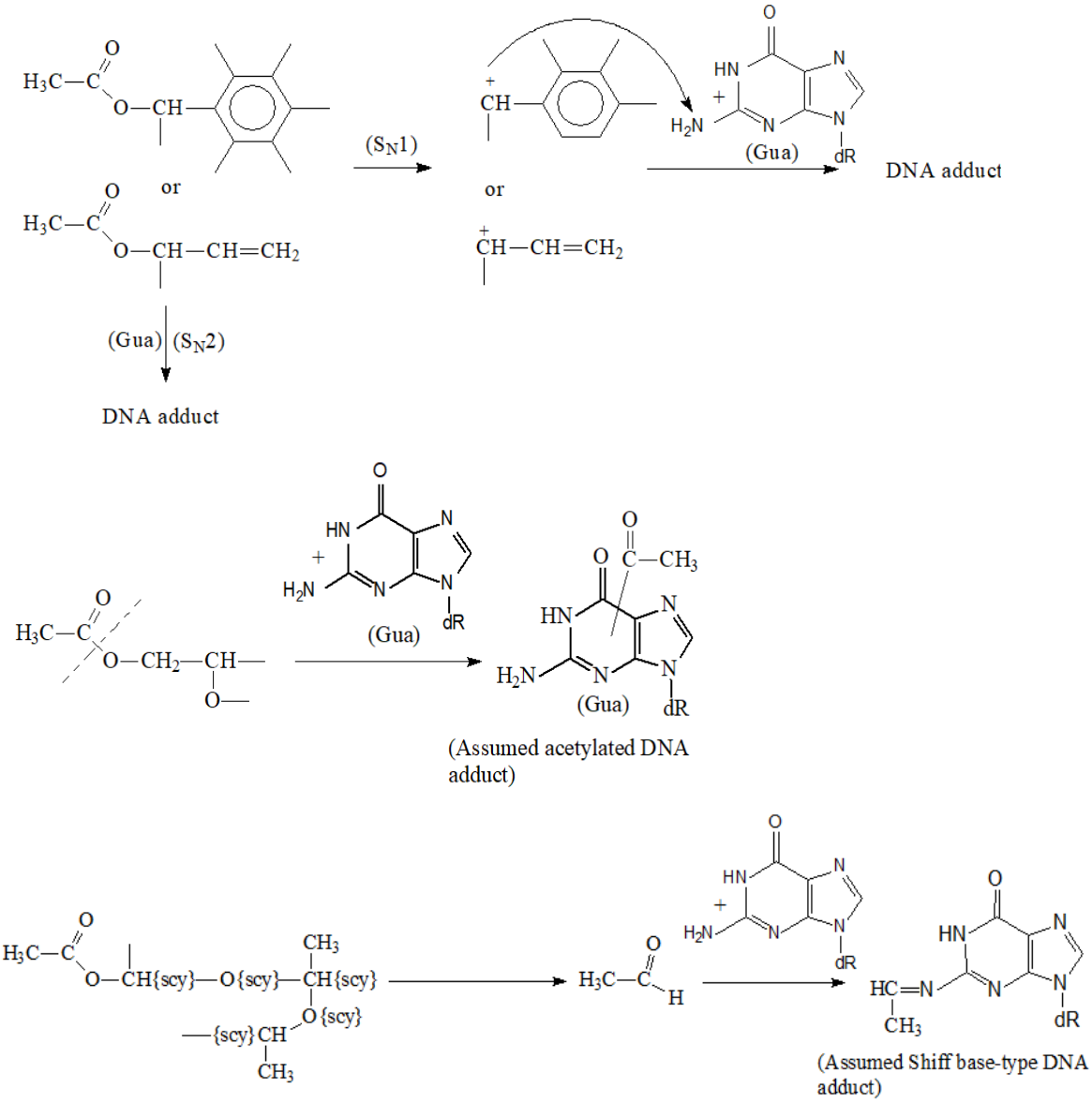
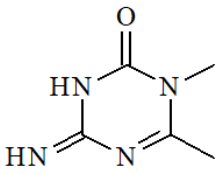
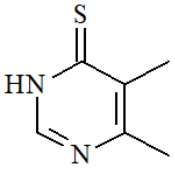
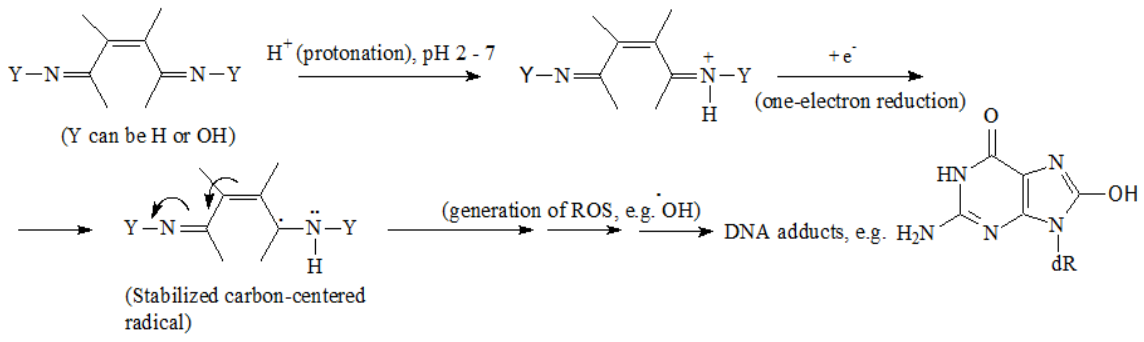
Individual profile/alert	
Name	Specific 5-Substituted Uracil Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y can be; $-CH_2CH_2Cl$, $-CH_2CH_2Br$, $-CH_2Cl$, $-CH_2Br$, -Cl, -Br or -CH=O)
Mechanism	**A_N2 Shiff base formation, S_N2 Alkylation, nucleophilic substitution 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:  (Y can be; $-CH_2CH_2Cl$, $-CH_2CH_2Br$, $-CH_2Cl$, $-CH_2Br$, -Cl, -Br or -CH=O) (Guanosine fragment) DNA adduct DNA adduct	
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.

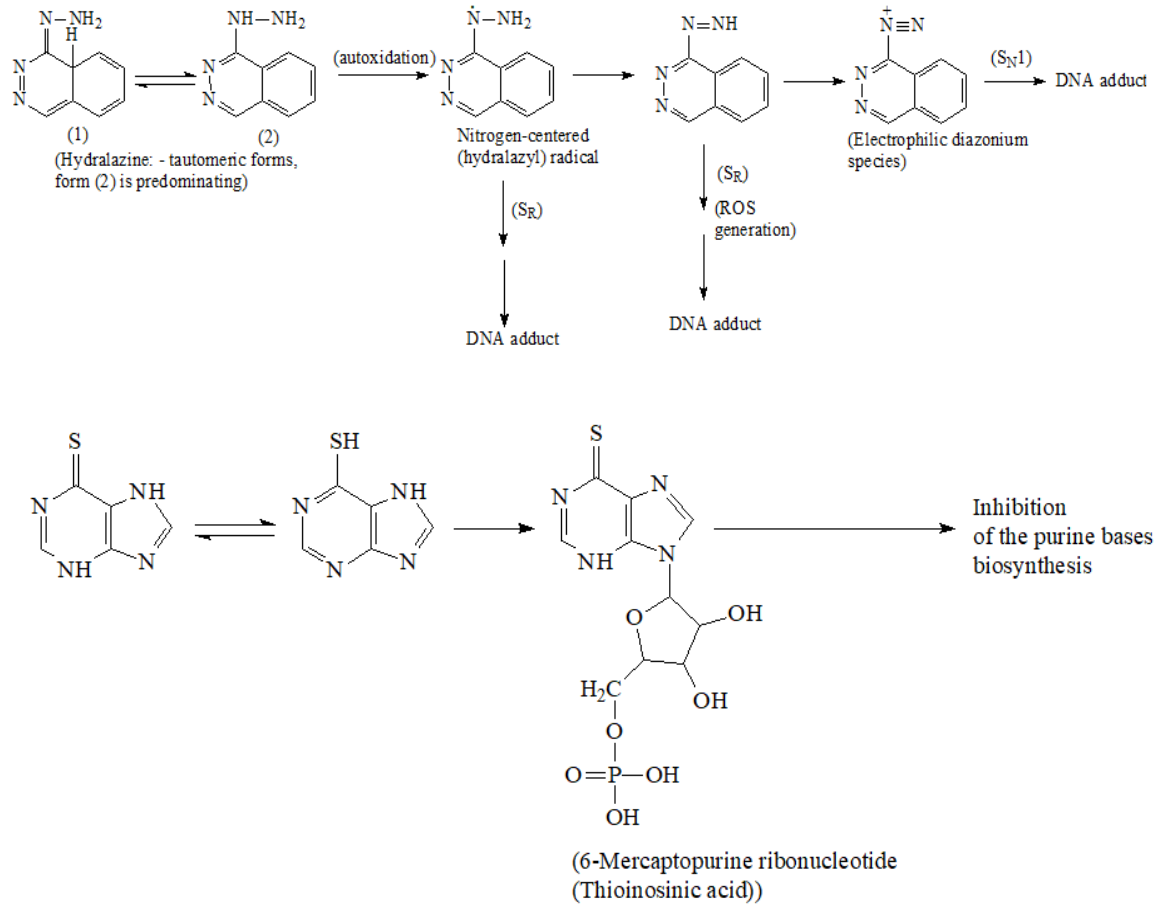
Individual profile/alert	
Name	Specific Acetate Esters
Type of profile	Structural alert
Description/applicability domain	 (Y_1: -H or C{ar}; Y_2, Y_3: -H or electron-withdrawing substituents such as -O-, -NO_2, -CN, -C(O)-, -CHO capable of conjugation); Y_4: -H or -C: number of C-atoms in Y_4 0 - 2)  (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)  (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)   (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 09.2019. 8. *Acetin, Chemical Carcinogenesis Research Information System*

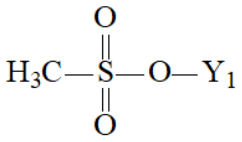
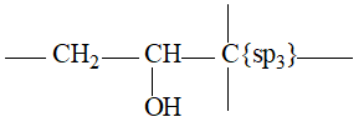
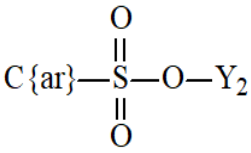
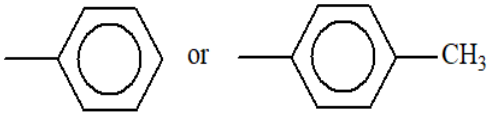
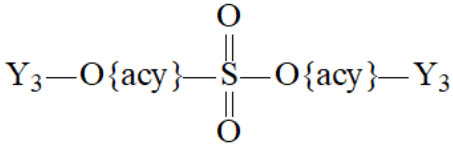
	(CCRIS); https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+26446-35-5

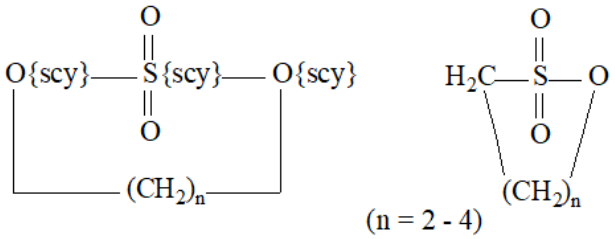
Individual profile/alert	
Name	Specific Imine and Thione Derivatives
Type of profile	Structural alert
Description/applicability domain	(1) —C{scy}=C{scy}—C{scy}=N{acy}{V_3}— (2) —C{scy}=N{scy}{V_3}—C{scy}=S (3) —N{scy}{V_3}=N{scy}{V_3}—C{scy}=N{acy}{V_3}— {scy} - cyclic atom; {acy}: acyclic atom; V - 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**
 (Y can be H or OH) (Stabilized carbon-centered radical) (generation of ROS, e.g. $\cdot OH$) (one-electron reduction) DNA adducts, e.g.	



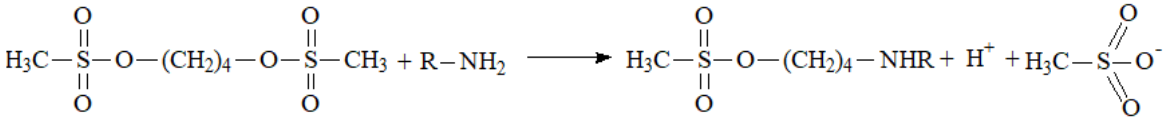
Set of chemicals used for profile development	Specific Imine and Thione Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references 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. 10. Christman, J. K., Oncogene **21** (2002), 5483 – 5495. 11. Kelecsenyi, Z., Mutag. **15**(1) (2000), 25 – 31.

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

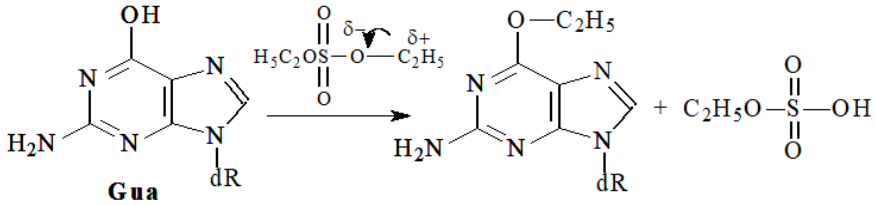
Description/applicability domain	 (Y_1 is $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$ $-CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_3$ $-(CH_2)_n$-O-S{V6}, n = 1 - 4), $-CH_2CH_2CH(CH_3)_2$   (Y_2 is $-CH_3$, $-CH_2CH_3$ $-CH(CH_3)_2$, $-CH_2CH_2CH_3$, $-CH_2CH(CH_3)_2$ $-CH_2CH_2Y$ (Y is Cl, Br, O, N, C{ar})) C{ar} is carbon atom in benzenoid aromatic nucleus such as :   (Y_3 is $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$ or combinations)

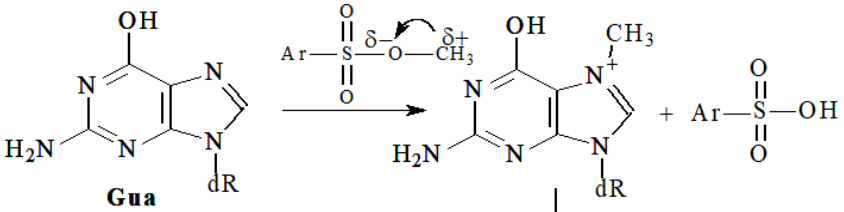


Mechanism	**S_N2 at sp3-carbon atom (alkylation)**



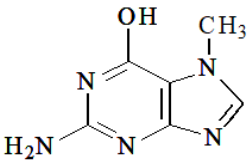
(R-NH_2: biological macromolecule (e.g., adenine or guanine fragment in DNA)

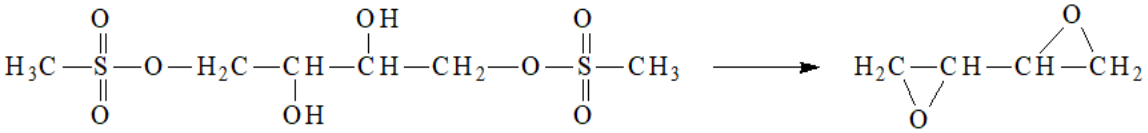




(dR - deoxyribose phosphate fragment)

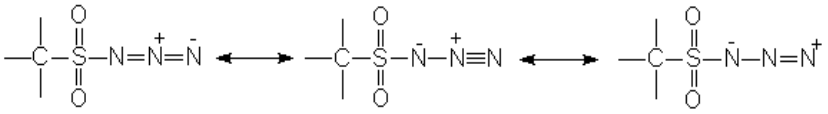
("depurination")



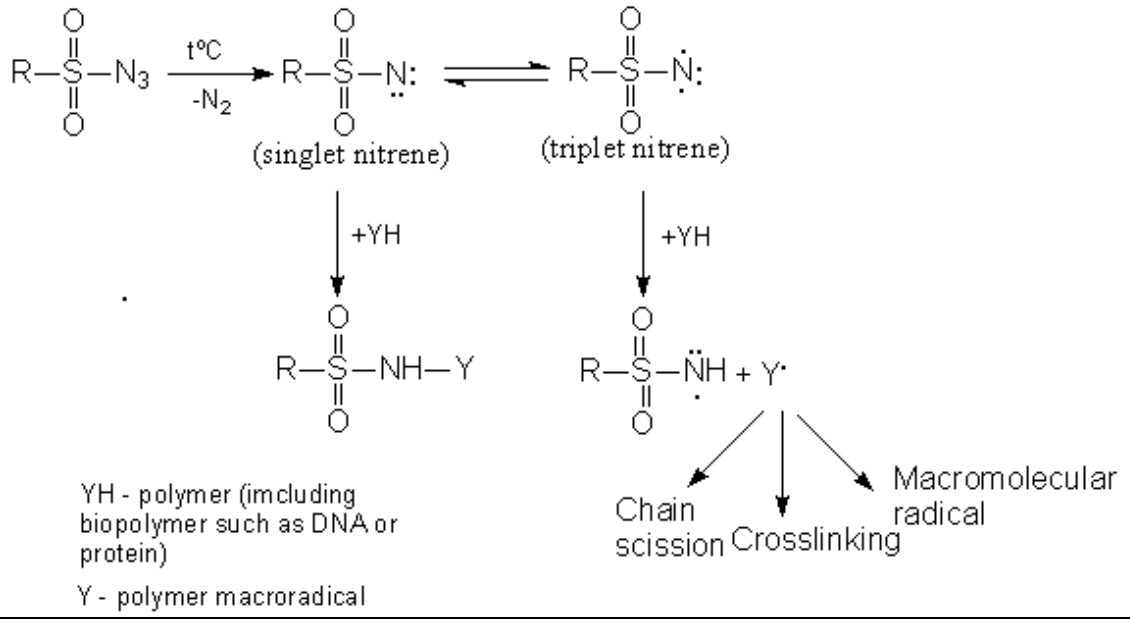


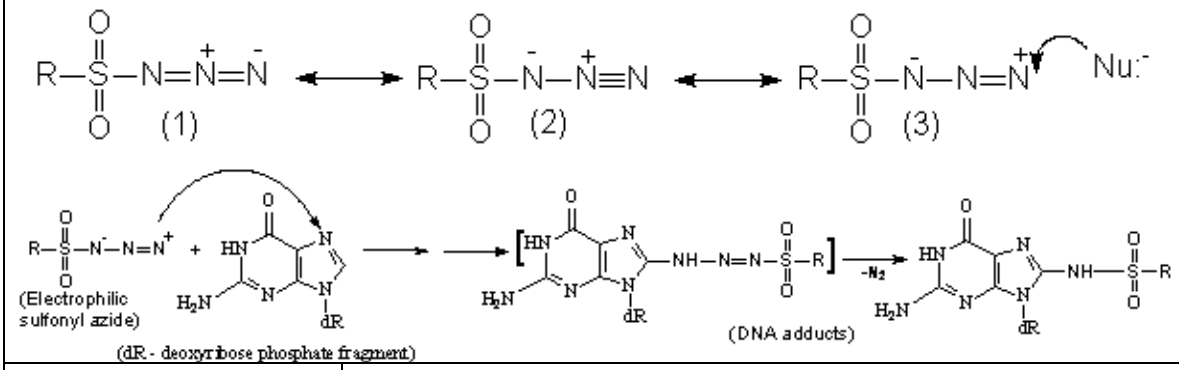
Set of chemicals used for profile develochain heter pment	Sulfonates and Sulfates
Data/Knowledge used	An extensive review of the literature was performed enabling the

for profile development	chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. 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. 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.

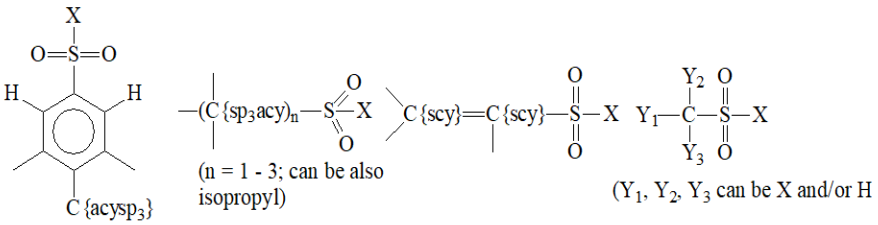
Individual profile/alert	
Name	Sulfonyl Azides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N1 Nitrenium ion formation**

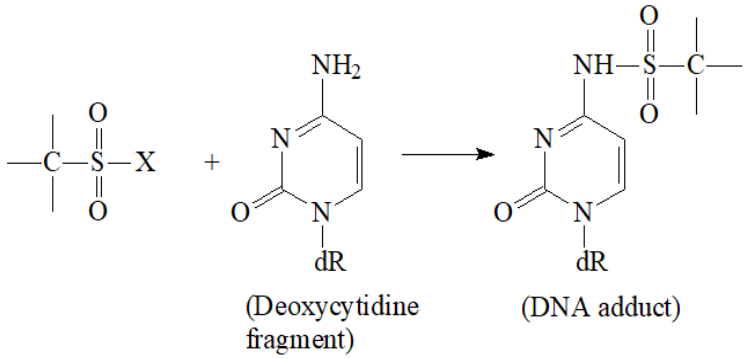
The following mechanistic schemes can be expertly outlined:



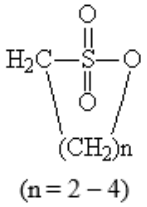
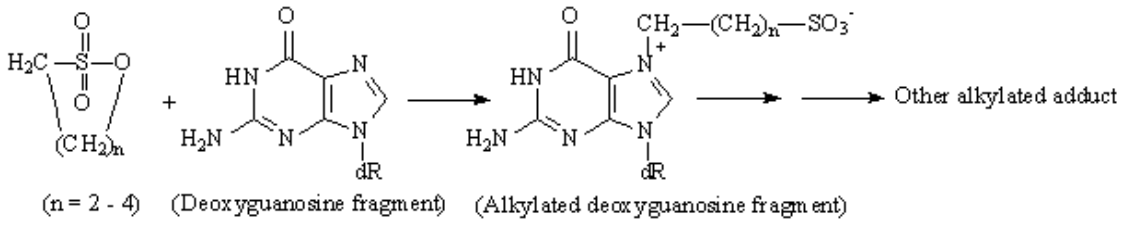


Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Clarke, J. Biol. Chem. **261**(22), 1986, 10063 – 10072. 2. *22778 4,4'-Oxydibenzenesulfonyl Azide*, Opinion of the Scientific Committee on Food on the 11th Additional List of Monomers and Additives for Food Contact Materials, Scientific Committee on Food, European Commission, Health&Consumer Protection Directorate General, 13 November 2000; https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out76_en.pdf, last visited 09.2019. 3. Li, IPR 28th Annual Symposium Waterloo, May 16, 2006; http://www.ipruw.com/publications/2006/oral_pres/BobLi.pdf. 4. Merkx, R., *Application of Azides in Chemoselective Amidation Reactions*, PhD Thesis; ISBN: 978-90-393-5025-6. 5. Brase, Angew. Chem. Int. Ed. **44** (2005), 5188 – 5240.

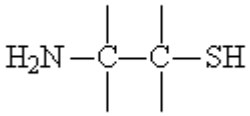
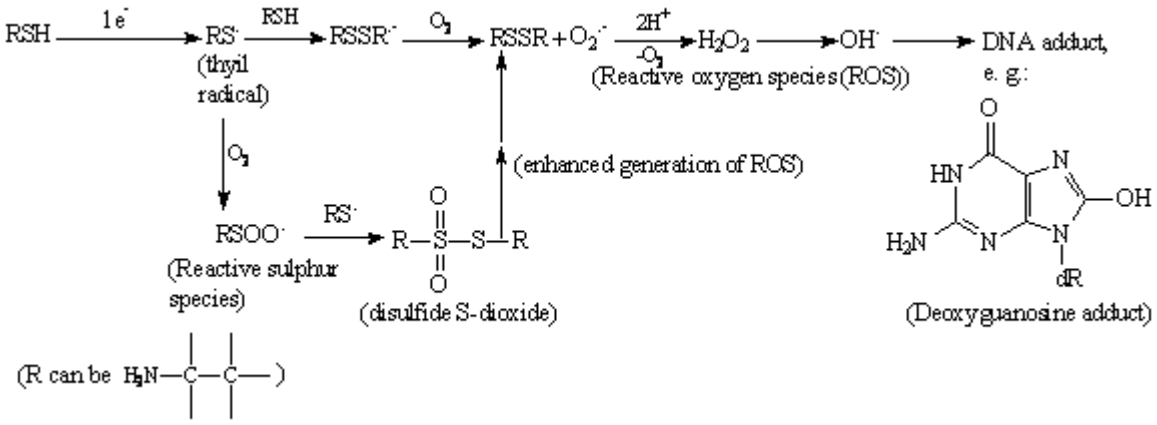
Individual profile/alert	
Name	Sulfonyl Halides
Type of profile	Structural alert
Description/applicability domain	
Mechanism	**S_N2 attack on sulfur atom**

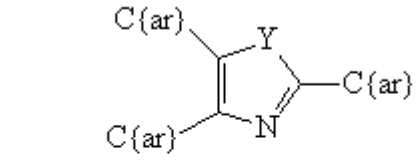


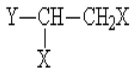
Set of chemicals used for profile development	N/A
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Sawatari, 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; http://www.eeaa.gov.eg/cmuic/cmuic_pdfs/generalpub/4-Methylbenzenesulfonyl%20chloride.pdf, last visited 10.2019. 4. Tsuchiya, Y., Water Sci & Technol. **25**(2) (1992), 123 – 130 (Abstract); https://iwaponline.com/wst/article/25/2/123-130/24352, last visited 10.2019.

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	Sultones

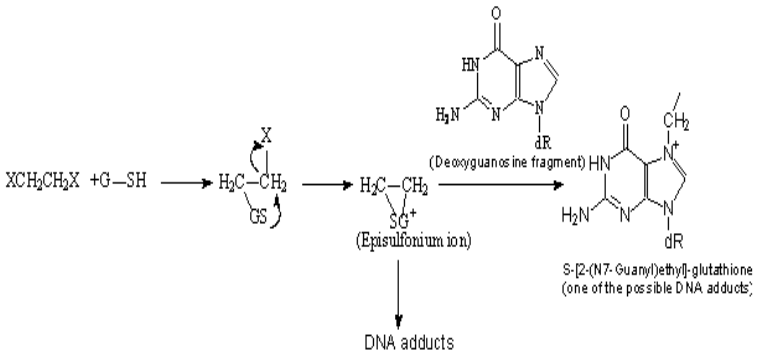
profile development	
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *1,3-Propane Sultone, Exposure Data*, 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	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 10.2019.. 6. Giles, G. I., Biochem. J. 364 (2002), 579 – 585.

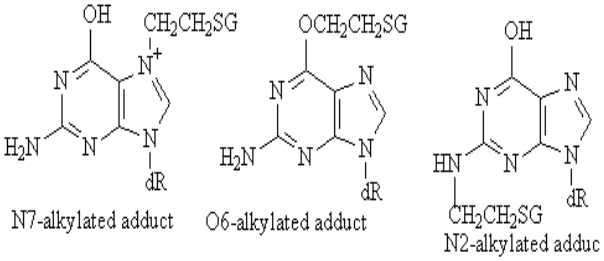
Individual profile/alert	
Name	Triarylimidazole and Structurally Related DNA Intercalators
Type of profile	Structural alert
Description/applicability domain	 (Y can be N{V3}{sp3}, -S-{V2}, -O-) (C{ar}: carbon atom as part of arene ring)
Mechanism	**Non-covalent interactions DNA intercalation**
The chemical 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	N/A
Data/Knowledge used for profile development	An extensive review of the literature was 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.

Individual profile/alert	
Name	Vicinal Dihaloakanes
Type of profile	Structural alert
Description/applicability domain	 (Y is –H, -(CH2)nH (n = 1, 2), -O(CH2)nH (n = 0 -2), -CH2-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]:

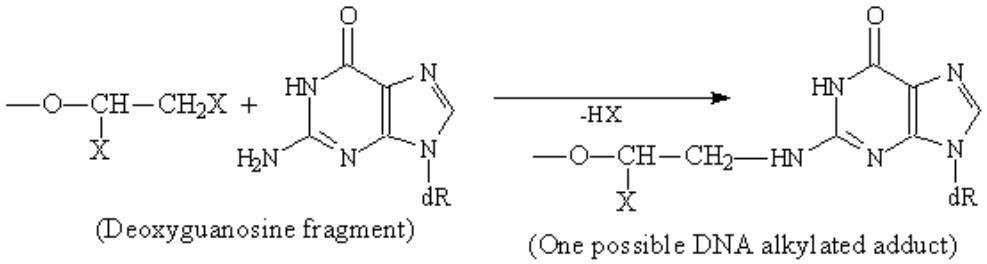


The major product of this reaction is *S*-[2-(N^7-guanyl)ethyl]glutathione, but N^2- and O^6-guanyl adducts are also formed, and all three adducts are potentially mutagenic [3]:

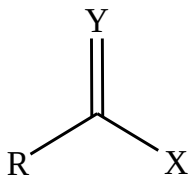


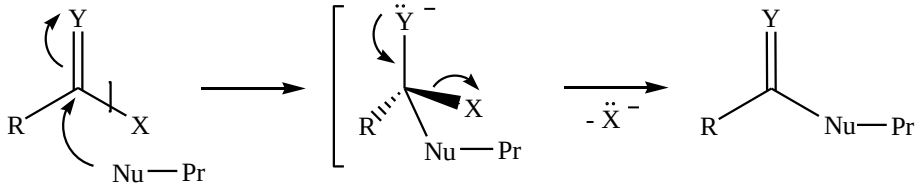
Similar mechanism of *in vitro* metabolic activation by forming episulfonium cation as reactive intermediate has also been suggested for structurally similar short-chain compounds such as 1,2-dibromo-3-chloropropane [5].

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:

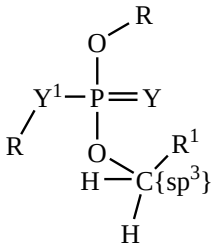


Set of chemicals used for profile development	Vicinal Dihaloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

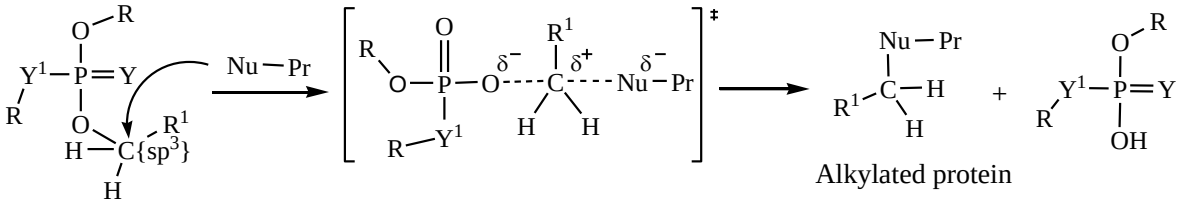
Individual profile/alert	
Name	(Thio)Acyl and (thio)carbamoyl halides, cyanides, azides, etc.
Type of profile	Structural alert
Description/applicability domain	 **Y** = O, S; **X** = F, Cl, Br, I, C≡N, N_3; **R** = C{sp^3}, N{v3}; O-C{sp3}; **Classification:** a) (Thio)Acyl halides: Y = O, S; X = F,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

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

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

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

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

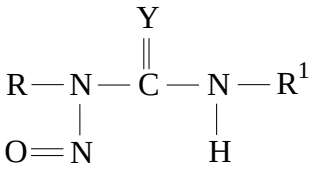


Y = O, S; Y^1 = O, S;
R = any C; R^1 = H, CH_3
Nu = -SH, $-NH_2$

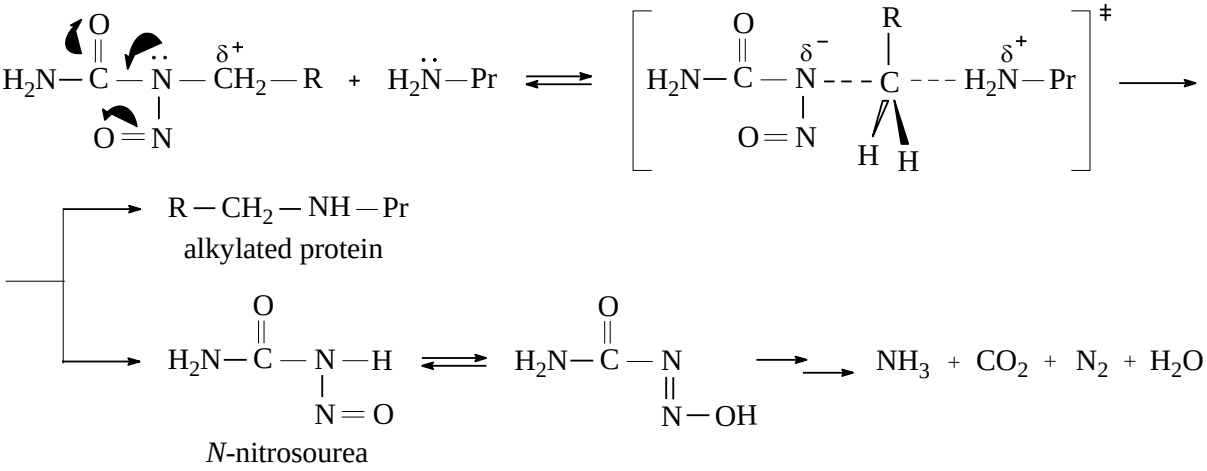
Fig. 1. Nucleophilic substitution at sp3 carbon atom for (thio)phosphonates with Cys and Lys protein nucleophiles

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

	analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 5. Trimethyl phosphate, CAS No 512-56-1, NTP, Genetic Toxicology - Mammalian Cell Cytogenetics, Study ID 445223_CA. https://manticore.niehs.nih.gov/cebssearch/genetox/002-02970-0002-0000-2/ 6. Woo, Y-T., Arcos, J.C., Argus, M.F., Phosphorous containing alkylating agents. Carcinogenicity and structure-activity relationships. Other biological properties. Activating metabolism. Environmental significance. United States Environmental Protection Agency, Chemical Hazard Identification Branch, **1982**, pp 484-485.

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

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



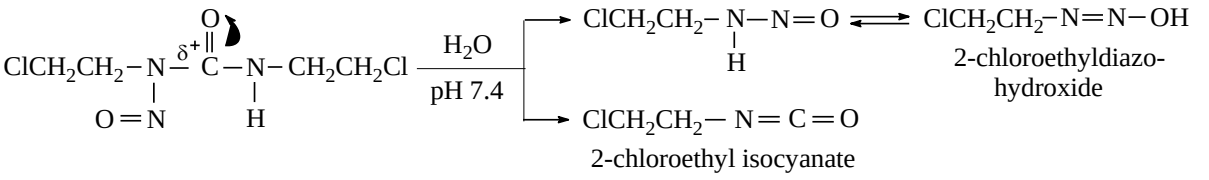
N-nitrosourea

DNA and protein alkylation via the formation of alkyldiazonium ion

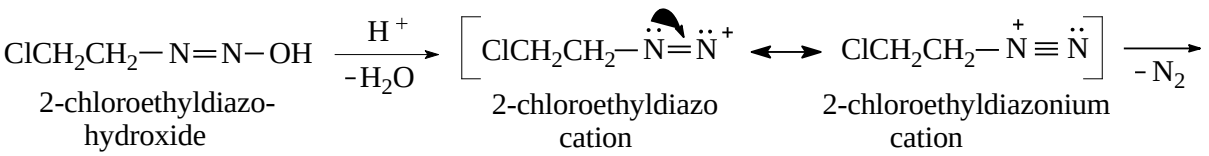
It is well known that alkylnitrosoureas may undergo non-enzymatic decomposition (i.e. hydrolysis) under physiological conditions yielding alkyldiazohydroxide and subsequently alkyldiazonium ion [6,10]. For example, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) decomposes spontaneously in aqueous media at pH 7.4, yielding 2-chloroethyldiazohydroxide and 2-chloroethyl isocyanate (Scheme 2a). 2-Chloroethyldiazohydroxide gives rise to the reactive 2-chloroethyl carbenium ion that can alkylate proteins and DNA bases mainly in the O^6- and *N*7-positions of deoxyguanine (Scheme 2b). Modification of proteins (mainly lysine amino groups) is thought to be due to the carbamoylating activity of 2-chloroethyl isocyanate (Scheme 2c) [10]. Moreover, it is tentatively suggested that the cytocidal effect of BCNU, generally attributed to its alkylating activity, may be potentiated by one of its metabolites, 2-chloroethyl isocyanate, through an inhibition of the repair of damaged DNA [11].

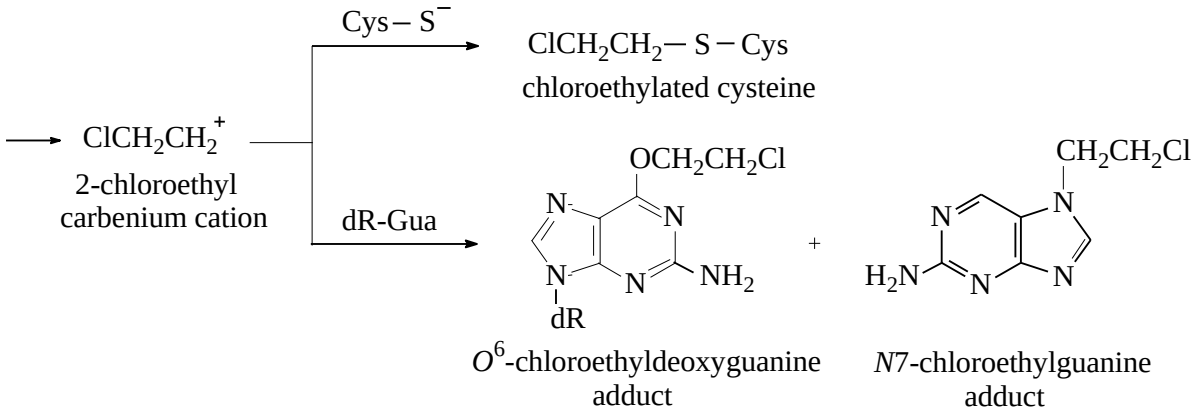
Scheme 2

a)

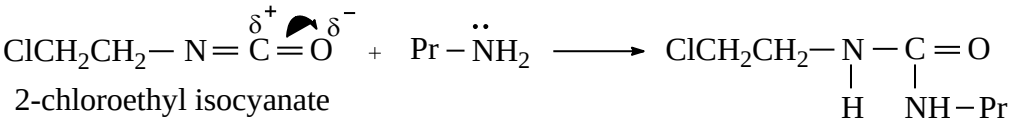


b)





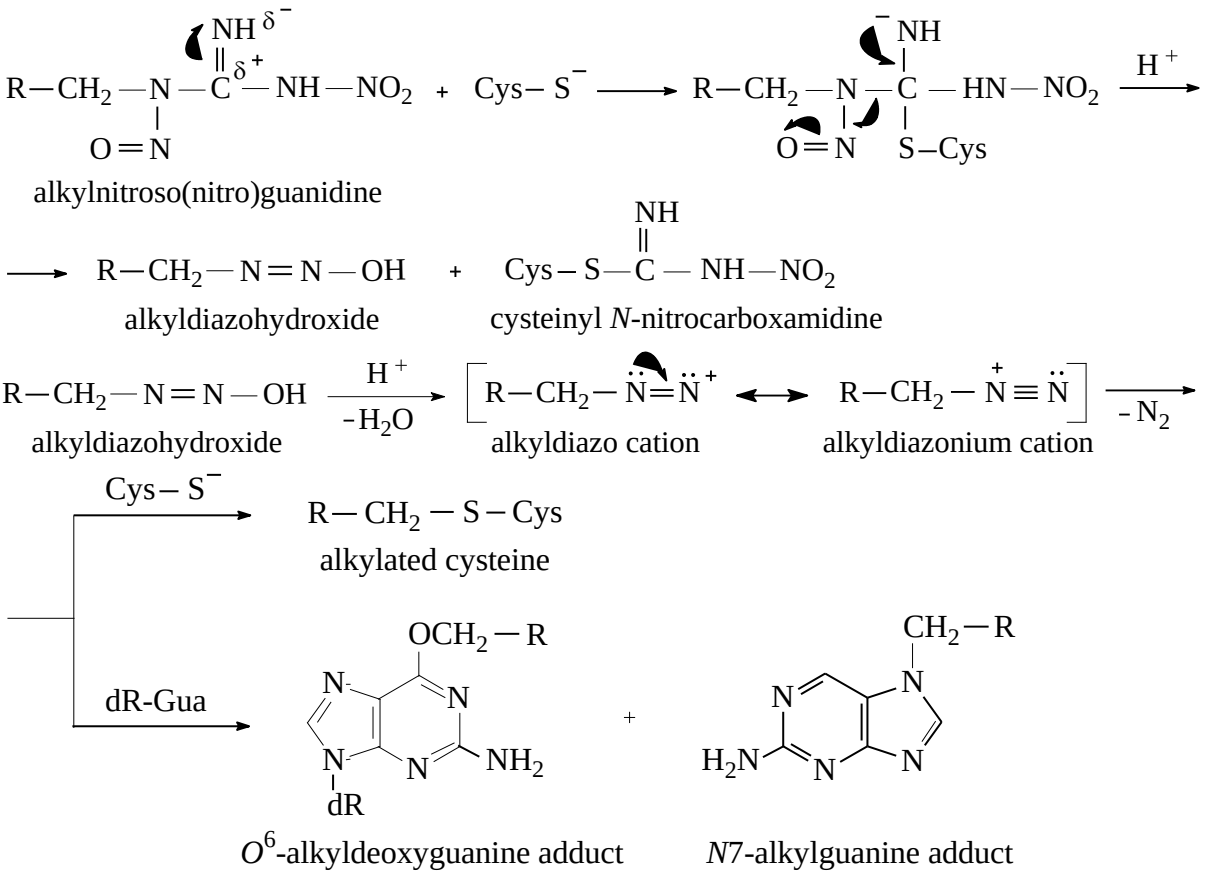
c)



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

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

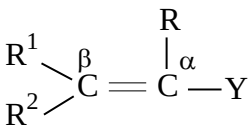
Scheme 3



Set of chemicals used for profile development	Alkylated nitrosoureas and nitrosoguanidines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Cai, T., Janczuk, A.J., Nitric oxide donors: chemical activities and biological applications. Chem. Rev., 2002, 102(4), 1091-1134. 2. Wilhelm, D., Bender, K., Knebel A., Angel, P., The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. Mol. Cell Biol., 1997, 17(8), 4792-4800. 3. Ishidate, M. Jr., Odashima, S., Chromosome tests with 134 compounds on Chinese hamster cells in vitro - a screening for chemical carcinogens. Mutat. Res., 1977, 48(3-4), 337-353. 4. Ishidate, M. Jr., Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutat. Res.,

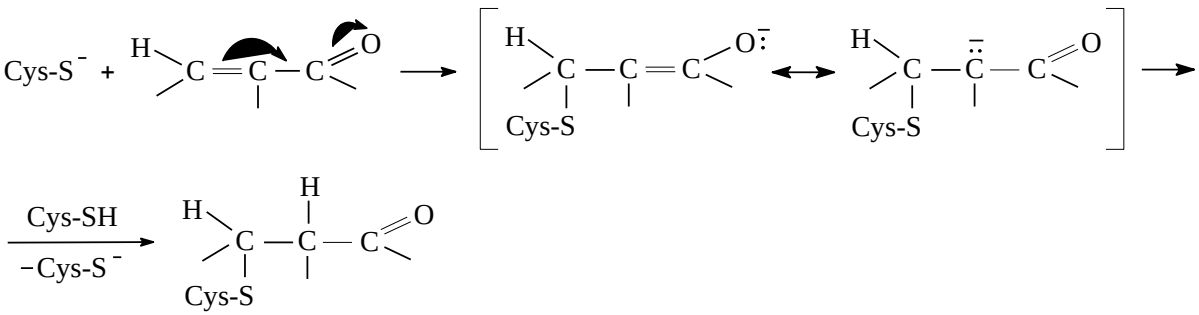
1988, 195(2), 151-213.
5. Thust, R., Mendel, J., Schwarz, H., Warzok, R., Nitrosated urea pesticide metabolites and other nitrosamides. Activity in clastogenicity and SCE assays, and aberration kinetics in Chinese hamster V79-E cells. Mutat. Res., 1980, 79(3), 239-248.
6. Buckley, N., A regioselective mechanism for mutagenesis and oncogenesis caused by alkylnitrosourea sequence-specific DNA alkylation. J. Am. Chem. Soc., 1987, 109(25), 7918-7920.
7. Spratt, T.E., Zydowsky, T.M., Floss, H.G., Stereochemistry of the in vitro and in vivo methylation of DNA by (R)- and (S)-N-[2H1,3H]methyl-N-nitrosourea and (R)- and (S)-N-nitroso-N-[2H1,3H]methyl-N-methylamine. Chem. Res. Toxicol., 1997, 10(12), 1412-1419.
8. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. Chem. Res. Toxicol., 2007, 20(1), 44-60.
9. Cain, J.D., A Theoretical Study of the Mechanism of the Alkylation of Guanine by N-Nitrso Compounds. PhD Thesis, University of North Carolina, Chapel Hill, USA, 1992.
10. Wiencke, J.K., Wiemels, J., Genotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Mutat. Res., 1995, 339(2), 91-119.
11. Kann, H.E. Jr, Kohn, K.W., Lyles, J.M., Inhibition of DNA repair by the 1,3-bis(2-chloroethyl)-l-nitrosourea breakdown product 2-chloroethyl isocyanate. Cancer Res., 1974, 34(2), 398-402.
12. Lawley, P.D., Thatcher, C.J., Methylation of deoxyribonucleic acid in cultered mammalian cells by N-methyl-N′-nitro-N-nitrosoguanidine. The influence of cellular thiol concentrations on the extent of methylation and the 6-oxygen atom of guanine as a site of methylation. Biochem. J., 1970, 116(4), 693-707.
13. Jansen, J.G., Mohn, G.R., Vrieling, H., van Teijlingen, C.M., Lohman, P.H., van Zeeland, A.A., Molecular analysis of hprt gene mutations in skin fibroblasts of rats exposed in vivo to N-methyl-N-nitrosourea or N-methyl-N-nitrosourea. Cancer Res., 1994, 54(9), 2478-2485.
14. Romert, L., Jenssen, D., Mechanism of N-acetylcysteine (NAC) and other thiols as both positive and negative modifiers of MNNG-induced mutagenicity in V79 Chinese hamster cells. Carcinogenesis, 1987, 8(10), 1531-1535.
15. Romert, L., Swedmark, S., Jenssen, D., Thiol-enhanced

	decomposition of MNNG, ENNG and nitrosocimetidine: relationship to mutagenicity in V79 Chinese hamster cells. Carcinogenesis, 1991, 12(5), 847-853.

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

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

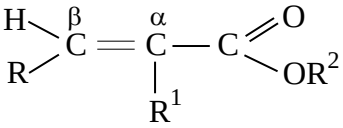
Scheme 1



The reactivity of polarized alkenes depends on the nature of the substituents R, R1, and R2. It is apparently that the electron-withdrawing groups on Cβ will activate the double bond although to a lesser extent than on Cα. Conversely, electron-donating groups such as methyl, ethyl, propyl, etc. will deactivate the double bond, more so when they are on Cα than when they are on Cβ. Moreover, the

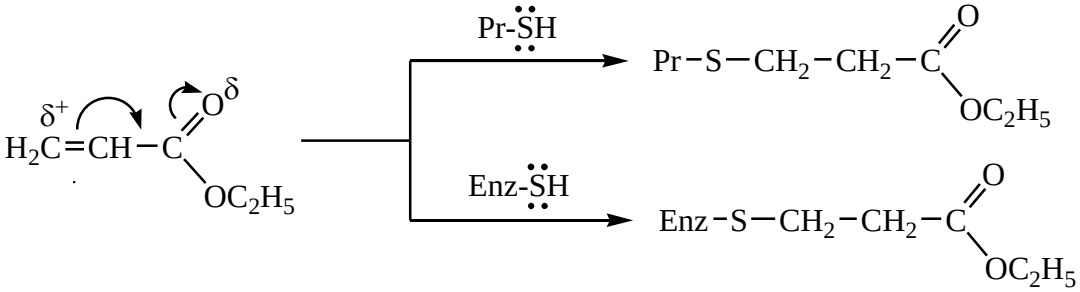
decrease in the reactivity of α,β-unsaturated carbonyls was found to be influenced by the combination of both steric and electronic factors [Schultz et al., 2005].	
Set of chemicals used for profile development	alpha,beta-Unsaturated Carbonyls and Related Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. T.W. Schultz, J.W. Yarbrough, R.S. Hunter, A.O. Aptula, Verification of the structural alerts for Michael acceptors. Chem. Res. Toxicol., 2007, 20(9), 1359–1363. 2. A.O. Aptula, G. Patlewicz, D.W. Roberts, T.W. Schultz, Nonenzymatic glutathione reactivity and in vitro toxicity: A non-animal approach to skin sensitization. Toxicol In Vitro, 2006, 20(2), 239–247. 3. S.J. Enoch, J.C. Madden, M.T.D. Cronin, Identification of mechanisms of toxic action for skin sensitisation using a SMARTS pattern based approach. SAR QSAR Environ. Res., 2008, 19(5-6), 555–578. 4. T.W. Schultz, K. Rogers, A.O. Aptula, (2009). Read-across to rank skin sensitization potential: Subcategories for the Michael acceptor domain. Contact Dermatitis, 2009, 60(1), 21–31. 5. R.M. LoPachin, D.S. Barber, T. Gavin, Genotoxicity and carcinogenicity of acrylamide: a critical review. Toxicol. Sci., 2008, 104(2), 235–249. 6. H. Tsuda, C.S. Shimizu, M.K. Taketomi, M.M. Hasegawa, A. Hamada, K.M. Kawata, N. Inui, Acrylamide: induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations. Mutagenesis, 1993, 8(1), 23–29. 7. B.I. Ghanayem, M.R. Elwell, S.R. Eldridge, Effects of the carcinogen, acrylonitrile, on forestomach cell proliferation and apoptosis in the rat: comparison with methacrylonitrile. Carcinogenesis, 1997, 18(4), 675–680. 8. D.W. Roberts, G. Patlewicz, A.O. Aptula, Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106

	chemicals tested in the mouse Local Lymph Node Assay. Chem. Res. Toxicol., 2007, 20(1), 44-60. 9. G.Y. Patlewicz, Z.M. Wright, D.A. Basketter, C.K. Pease, J.P. Lepoittevin, E. Gimenez Arnau, Structure-activity relationships for selected fragrance allergens. Contact Dermatitis, 2002, 47(4), 219-226. 10. O. Miyata, T. Shinada, I. Ninomiya, T. Naito, T. Date, K. Okamura, S. Inagaki, Stereospecific nucleophilic addition reactions to olefins. Addition of thiols to α,β-unsaturated carboxylic acid derivatives. J. Org. Chem., 1991, 56(23), 6556-6564. 11. S.R. Ahlfors, O. Sterner, C. Hansson, Reactivity of contact allergenic haptens to amino acid residues in a model carrier peptide, and characterization of formed peptide-hapten adduct. Skin Pharmacol. Appl. Skin Physiol., 2003, 16(1), 59-68. 12. T.W. Schultz, J.W. Yarbough, E.L. Johnson, Structure-activity relationships for reactivity of carbonyl-containing compounds with glutathione. SAR QSAR Environ. Res., 2005, 16(4), 313-322.

Individual profile/alert	
Name	alpha,beta-Unsaturated Carboxylic Acids and Esters
Type of profile	Structural alert
Description/applicability domain	 R = H atom, Csp3 (scy), Csp2 (scy), C(=O)OC R1 = H atom, Csp3, Csp2 (aryl) R2 = H atom, Csp3 (acy, scy), Csp2 (aryl, vinyl). Positive results for the in vitro chromosomal damage were seen mostly when ester hydrocarbon chain length (Csp3 acy) is between 1-10 carbon atoms [1]. Negative results were obtained in in vitro chromosome aberration test in Chinese hamster V79 cells for methacrylates, containing C12-C18 alkyl ester groups which may be branched or linear and may be even- or odd-numbered in chain length [2].
Mechanism	A_N2, Michael addition to *α,β*-unsaturated acids and esters

Michael addition, a nucleophilic addition on C_β atom of the double bond is suggested as the predominant reaction mechanism of *α,β*-unsaturated esters with intracellular nucleophiles, e.g., free sulfhydryl groups found in proteins, reduced glutathione, and in active sites of enzymes [9,15] The formation of corresponding adducts is presented in Scheme 1:

Scheme 1

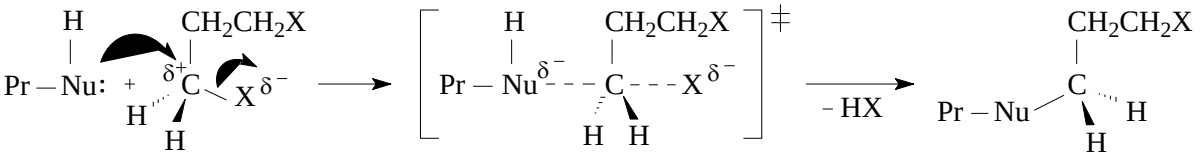


The difference in the reactivity of acrylates and methacrylates is probably determined by various factors. For example, hydrophilicity and lipophilicity (or hydrophobicity) were correlated with toxic potency, though these parameters are inversely related. Lower-molecular-weight substances were more toxic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

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

	Chem. Toxicol., **1992**, 30(6), 505-515. 8. Methacrylic Acid, SIDS Initial Assessment Profile, OECD SIDS, **2001**. 9. A.P. Freidig, H.J.M. Verhaar, J.L.M. Hermens, *Environ. Toxicol. Chem*., **1999**, 18(6), 1133-1139. 10. D.W. Potter, T.B. Tran, *Toxicol. Lett*., **1992**, 62(2-3), 275-285. 11. W. Geurtsen, G. Leyhausen, *J. Dent. Res*., **2001**, 80(12), 2046-2050. 12. F.P. Carney, C.A. Morris, B. Milthorpe, J.L. Flanagan, M.D. Willcox, *Eye Contact Lens*, **2009**, 36(6), 320-328. 13. B.I. Ghanayem, L.T. Burka, H.B. Matthews, *Fundam. Appl. Toxicol*., 1987, 9(3), 389-397. 14. J.M. Sanders, L.T. Burka, H.B. Matthews, *Drug Metab. Dispos*., **1988**, 16(3), 429-434. 15. T.J. McCarthy, E.P. Hayes, C.S. Scwartz, G. Witz, *Fundam. Appl. Toxicol*., **1994**, 22(4), 543-548. 16. K.L. Dearfield, C.S. Millis, K. Harrigton-Brock, C.L. Doerr, M.M. Moore, *Mutagenesis*, **1989**, 4(5), 381-393.

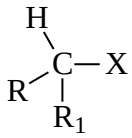
Individual profile/alert	
Name	alpha,omega-Dihaloalkanes
Type of profile	Structural alert
Description/applicability domain	$X^1—(CH_2)_n—X^2$ n = 3 - 6; $X^1 = X^2$ or $X^1 \neq X^2$ and X^1, X^2 = J, Br, Cl
Mechanism	S_N2, Nucleophilic substitution at sp^3 carbon atom
The disubstituted haloalkanes 1,3-dibromopropane and 1-bromo-3-chloropropane have been found to be positive in in vitro chromosomal aberration assays without metabolic activation [1-3]. According to many authors, the clastogenicity of these compounds is strongly dependent upon the carbon chain length (4 > 5 > 3 ~ 6) as well as the type of halogen involved [1-3]. The order of leaving group ability is I > Br > Cl, which is in accordance with the order in nucleophilic participation processes of different halogen atoms [4]. SN2 mechanism between α,ω-activated dihaloalkane and protein nucleophiles is shown in Scheme1. Scheme 1	



X = J, Br, Cl ; Nu–H = -NH_2, -SH

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

Individual profile/alert	
Name	alpha-Activated Haloalkanes
Type of profile	Structural alert

Description/applicability domain	 (X is Cl, Br, I) R_1 is H or any C-atom R is —C≡C, —C≡N, -C=C, -C=S, -C=O, -NO_2
Mechanism	S_N2, Alkylation by nucleophilic substitution at sp^3-Carbon atom

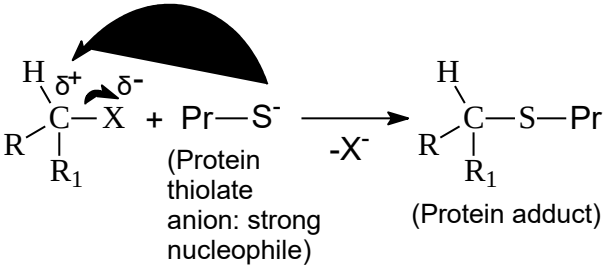
Alpha-Activated haloalkanes possess electron-withdrawing functional groups or sp2(sp)-carbon atoms directly bound to the alpha-C{sp3}-carbon atom. Chemicals from this sub-class can undergo nucleophilic aliphatic substitution reactions, in which the carbon-halogen bond is subject to heterolytic cleavage. Due to the presence of activated C-X bond with the carbon atom being sufficiently electrophilic center, halogens which are weak bases act as good leaving groups.

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

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

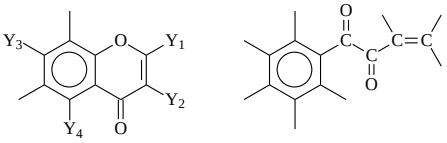
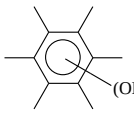
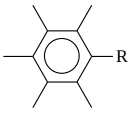
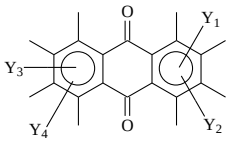
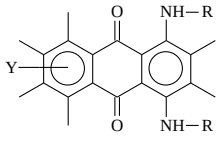
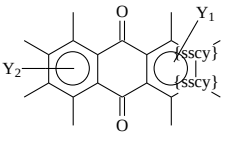
Other compounds with electron-withdrawing carbonyl-containing functionalities, adjacent to the halogen atom such as alachlor and butachlor can also undergo displacement reactions with strong nucleophiles and elicit in vitro genotoxic effects such as CA [10]. It has been shown that such compounds form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

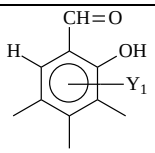
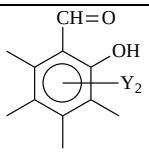
The proposed mechanistic scheme of the SN2-type reaction of alpha-activated haloalkane derivatives with the thiol functional groups of the cysteine fragments in histone/non-histone proteins is shown below:



Set of chemicals used for profile development	alpha-Activated Haloalkanes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Sofuni, T., Ed. (1998). Data Book of Chromosomal Aberration Test in vitro, Revised Edition. Life-Science Information Center, Tokyo, Japan. 4. Allyl Chloride, IARC Monographs on Evaluation of Carcinogenic Risks to Humans, 7 – 9 April 2014; https://monographs.iarc.fr/wp-content/uploads/2018/08/14-002.pdf. 5. Lin MF, Wu CL, Wang TC, Pesticide clastogenicity in CHO cells, Mutat. Res., 1987 Jul; 188(3):241 - 2 6. 3,4-Dichlorobut-1-ene, CAS No. 760-23-6, SIDS Initial Assessment Profile, OECD SIDS; https://hpvchemicals.oecd.org/ui/handler.axd?id=de9d1204-4d60-48e0-8e1f-41de0749ca1f. 7. K.S. Loveday, M.H. Lugo, M.A. Resnick, B.E. Anderson, E. Zeiger, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals. Environ. Mol. Mutagen., 1989, 13(1), 60-94. 8. Office of Environmental Chemicals Safety Environmental Health Bureau, Ministry of Health & Welfare in Japan, Toxicology Testing Reports of Environmental Chemicals, Chemicals Investigation Promoting Committee, Vol. 4, 1996, p. 529.

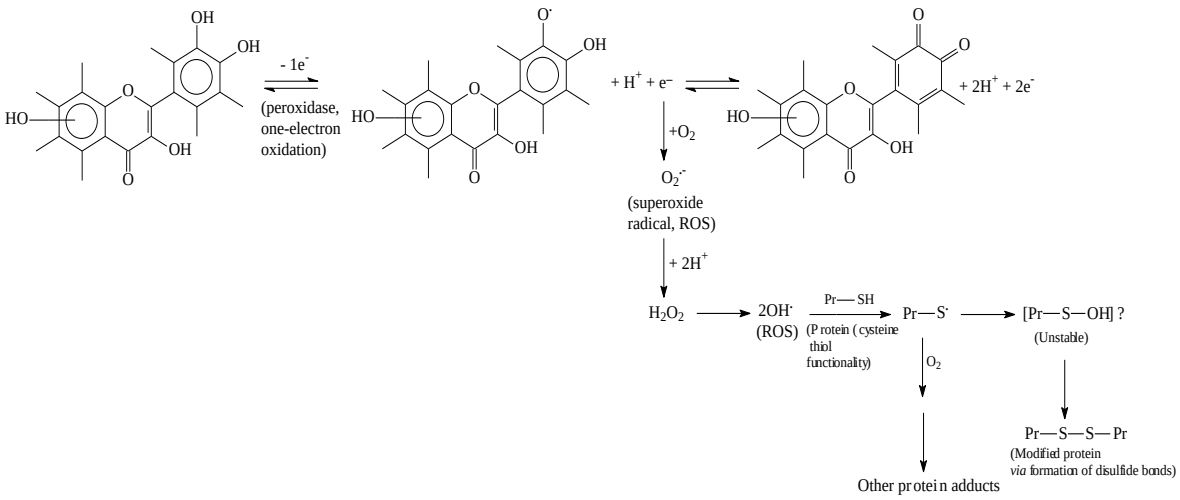
	9. E. Eder, T. Neudecker, D. Lutz, D. Henschler, Mutagenic potential of allyl and allylic compounds. Structure-activity relationship as determined by alkylating and direct in vitro mutagenic properties. Biochem. Pharmacol., 1980, 29(7), 993–998. 10. K. A. Lippa, S. Demel, I.H. Lau, A.L. Roberts, Kinetics and mechanism of the nucleophilic displacement reactions of chloroacetanilide herbicides: investigation of alpha-substituent effects. J. Agric. Food Chem., 2004, 52(10), 3010-3021. 11. D.M. Stamper, O.H. Tuovinen, Biodegradation of the herbicides alachlor, metolachlor, and propachlor. Crit. Rev. Microbiol., 1998, 24(1), 1 - 22. 12. G.R. Lambert, W.T. Padgett, M.H. George, K.T. Kitchin, S. Nesnow, Quantitative analysis of alachlor protein adducts by das chromatography-mass spectrometry. Anal. Biochem., 1999, 268(2), 289 - 296.

Individual profile/alert	
Name	Arenecarbonyl Compounds
Type of profile	Structural alert
Description/applicability domain	A. Flavonoid Compounds:  Y_1 can be H or  (n = 1 - 3) Y_2 is H or -OH or  (R is $-OCH_3$ (1) or -OH (1 - 3)) Y_3 is -OH; Y_4 is H or -OH B. Anthraquinone Derivatives:    (Y_1 is -OH (1 - 3); Y_2 is $-CH_2OH$ (1); or $-CH_3$ (1); or $-CH_2CH_3$ (1); or C{ar} (1) or -CH=O (1) or $-C(O)CH_3$ (1); Y_3 is H (all) or -OH (1 - 2); Y_4 is -C(O)OH (1 - 2) (if Y_3 is -OH) (R is H (both) or $-NHCH_2$ (both) or combinations); Y is -OH (1 - 2) or H (Y_1 is -OH (1 - 2); Y_2 is H combined with -OH (1 - 2); or H combined with $-OCH_3$ (1) C. Salicylaldehydes:

	  (Y_1 is H (all); or -OH (0 - 2); or Cl (0 - 2); or -NO_2 (0 - 2) (Y_2 is H (all); or -CH_3 (0 - 2) (No more than totally 4 substituents)
Mechanism	AN2, Schiff base formation AN2, Michael-type addition, quinoid structures Radical, ROS generation Non-covalent interactions, DNA/protein intercalation

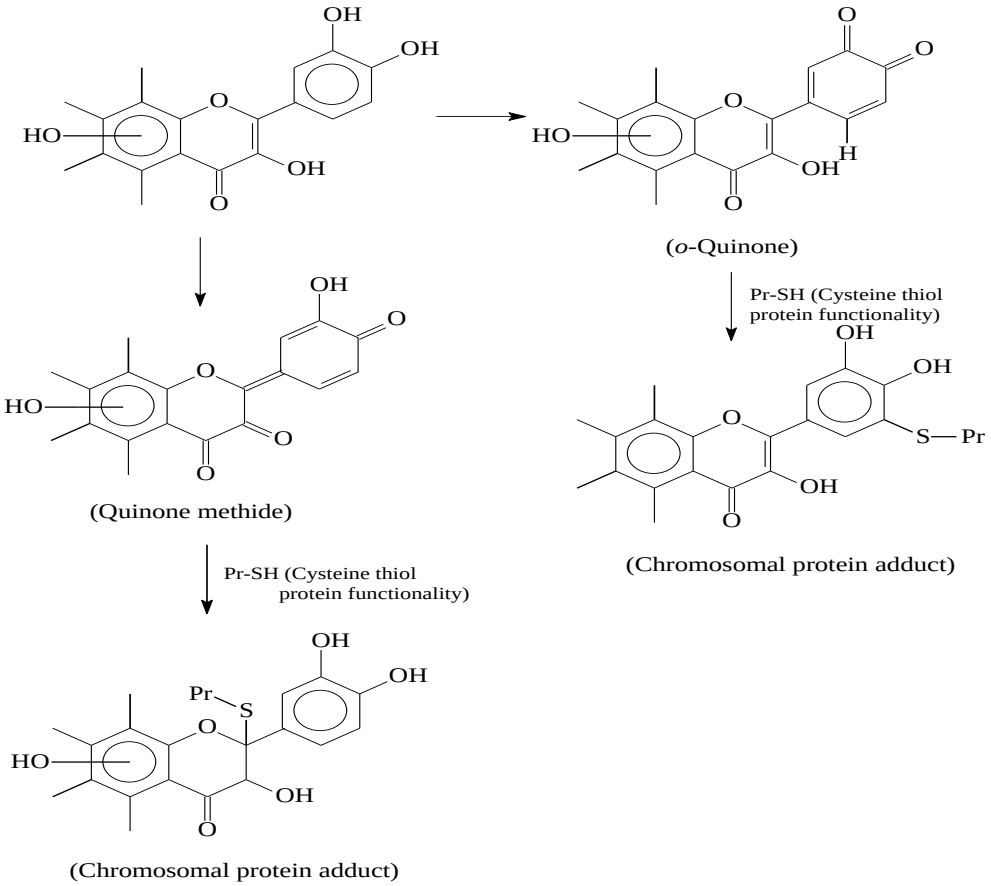
A. **Flavonoid compounds**

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



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

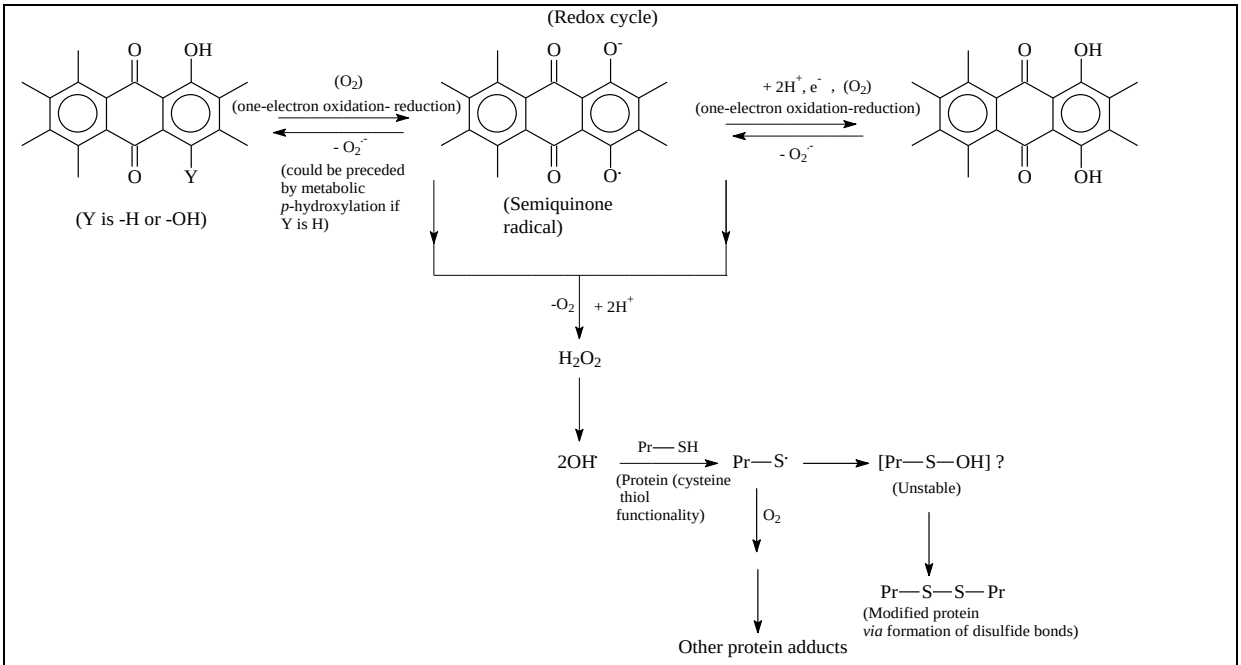
the following mechanistic scheme for protein adduct formation can be expertly proposed (Fig, 2):



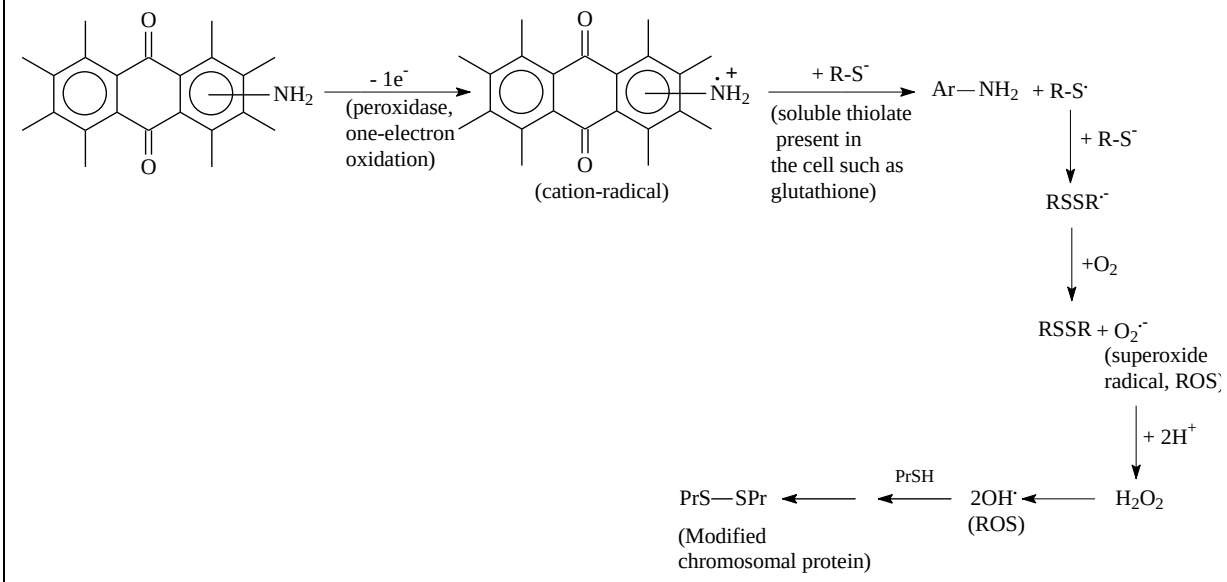
Intercalation – non-covalent interaction with DNA/protein complex. Flavonoids such as Quercetin can also bind to chromosomal DNA by non-covalent interactions such as intercalation [11]. This could be due to the combination of large planar molecules and electron-donating substituents such as –OH and –OCH3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

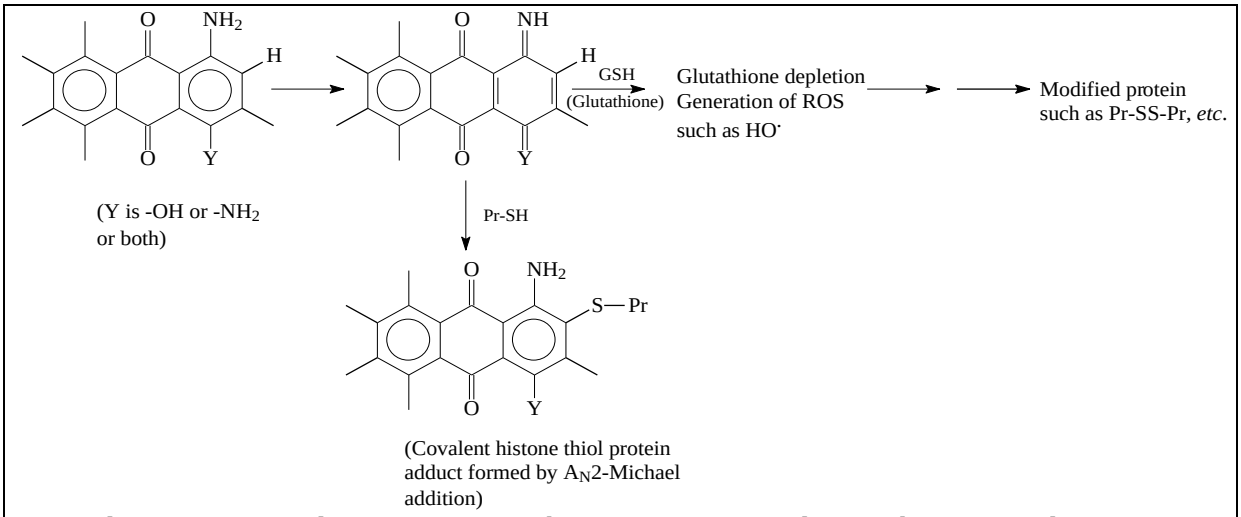
B. Anthraquinone Derivatives

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



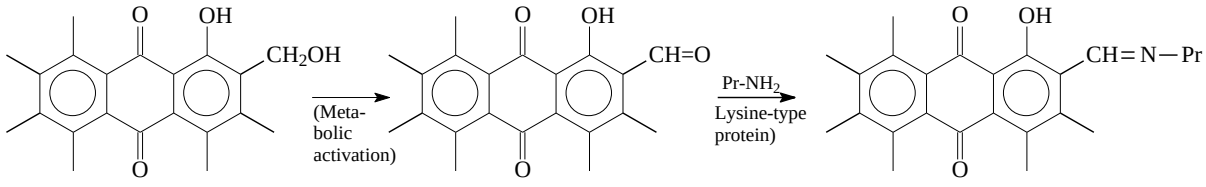
Similar mechanistic schemes associated with generation of ROS and protein modification can be proposed for aminoanthraquinones [24, 25, 27], (Figs. 4a, b):





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

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

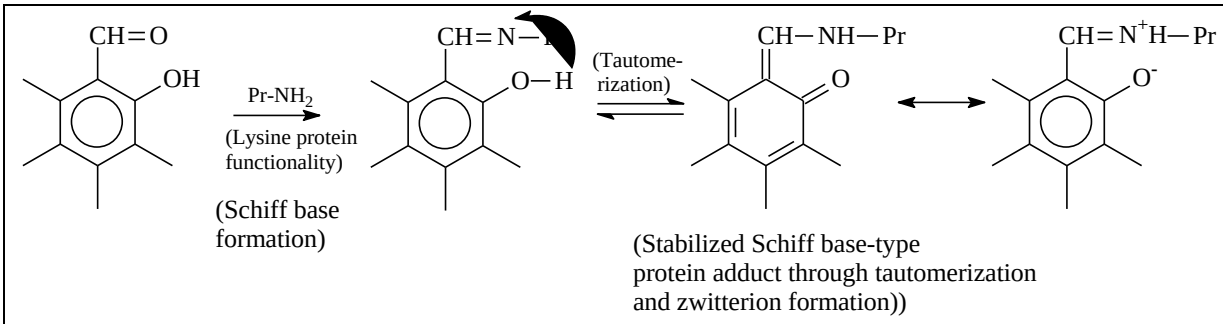


(Salicylic aldehyde-type anthraquinone derivative)

Protein adduct formed *via* A_N2 Schiff base formation mechanism

C. Salicylaldehydes

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



Set of chemicals used for profile development	Arenecarbonyl Compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Kovacic, P., Jacintho, J.D., Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. Curr. Med. Chem., 2001, 8(7), 773-796. 4. Do Céu Silva, M., Gaspar, J., Duarte Silva, I., Leão, D., Rueff, J., Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells. Mutagenesis, 2003, 18(6), 491-496. 5. Double, J. C., J. R. Brown, Evaluation of the Binding of Some Substituted Anthraquinones and Naphthacenequinones to DNA, Communications, J. Pharm. Pharmac. 1976, 28, 166 – 169. 6. Yordi, E. G., E. M. Perez, M. J. Matos, E.U. Villares, Structural Alerts for Predicting Clastogenic Activity of Pro-Oxidant Flavonoid Compounds: Quantitative Structure-Activity Relationship Study, J. Biomolecular Screening, 2012, 17(2), 216 – 224. 7. Enescu, M., Gardey, B., Mechanism of cysteine oxidation by a hydroxyl radical: a theoretical study. Chemphyschem., 2006, 7(4), 912-919. 8. Resende, Fl. A., W. Vileges, L. C. dos Santos, E. A. Varanda, Mutagenicity of Flavonoids Assayed by Bacterial Reverse Mutation (Ames) Test, Molecules, 2012, 17, 5255 – 5268. 9. Spencer, J. P. E., G. G. C. Kunhle, R. J. Williams, C. R. Evans, Intracellular Metabolism and Bioactivity of Quercetin and Its In Vivo Metabolites, Biochem. J. 2003, 372, 173 – 181. 10. Award, H. M., Studies on the pro-oxidant chemistry of flavonoids, Thesis, Wageningen University, 2002; https://core.ac.uk/download/pdf/29298240.pdf. Last visited:

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16. Decision on Testing Proposals Set Out in a Registration Pursuant to Article 40(3) of Regulation (EC), No. 1907/2006 for 2-Ethylanthraquinone, CAS No. 84-51-5, ECHA, 27 May 2015; https://echa.europa.eu/documents/10162/07e6629e-515f-137a-405b-4ea57f62d552. Last visited: 10.12.2019.
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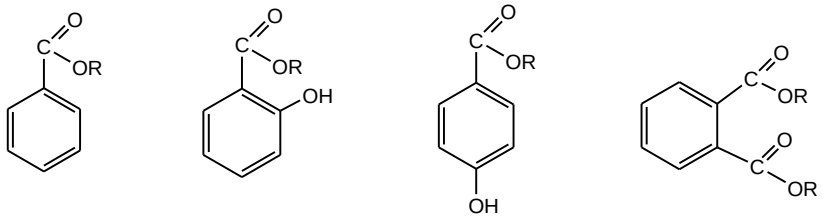
	25. Subrahmany, V. V., P. J. O., Brien, Peroxidase Catalysed Oxygen Activation by Arylamine Carcinogens and Phenol, Chem.-Biol. Interactions, 1985, 56, 185 – 199. 26. Makena, P. S., K. T. Chung. Evidence that 4-Aminobiphenyl, Benzidine and Benzidine Congeners Produce Genotoxicity Through Reactive Oxygen Species, Environ. Mol. Mutagenesis, 2007, 48, 404 – 413. 27. 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. 2010, 31(10), 50 – 58. 28. Salicylaldehyde; Exemption from the Requirements of a Tolerance, EPA, 40 CFR Part 180 Final Rule, Federal Register /Vol. 81, No. 61 /Wednesday, March 30, 2016 /Rules and Regulations. 29. Suto, M. J., J. M. Domagala, G. E. Roland, G. B. Mailloux, M. A. Cohen, Fluoroquinolones: Relationships between structural variations, mammalian cell cytotoxicity, and antimicrobial activity, J. Med. Chem. 1992, 35, 4745 – 4750. 30. Pelltari, E., E. Karhumaki, J. Langshaw, H. Perakyla, H. Elo, Antimicrobial Properties of Substituted Salicylaldehydes and Related Compounds, Z. Naturforsch. 2007, 62c, 487 – 497. 31. Patlewicz G., Basketter, D.A., Smith, C.K., Hotchkiss, S.A.M., Roberts, D.W: Skin-sensitisation structure-activity relationships for aldehydes, Contact Dermatitis, 2001, 44, 331-336. 32. Roberts D.W., Patlewicz, G., Mechanism based structure-activity relationships for skinsensitisation - the carbonyl group domain, SAR and QSAR in Enviromental Research, 2002, 13(1), 145-152. 33. Natsch, A., Gfeller, H., Haupt, T., Brunner, G. Chemical reactivity and skin sensitization potential for benzaldehydes: Can Schiff base formation explain everything? Chem. Res. Toxicol., 2012, 25 (10), 2203 – 2215. 34. M. Pr. K., Investigation on tautomeric equilibrium of Schiff base in mixed binary solvent, MSc in Chemistry Dissertation, National Institute of Technology, Rourkela, India, 2010; http://ethesis.nitrkl.ac.in/1598/2/Prakash_Malik.pdf. Last visited: 10.12.2019.

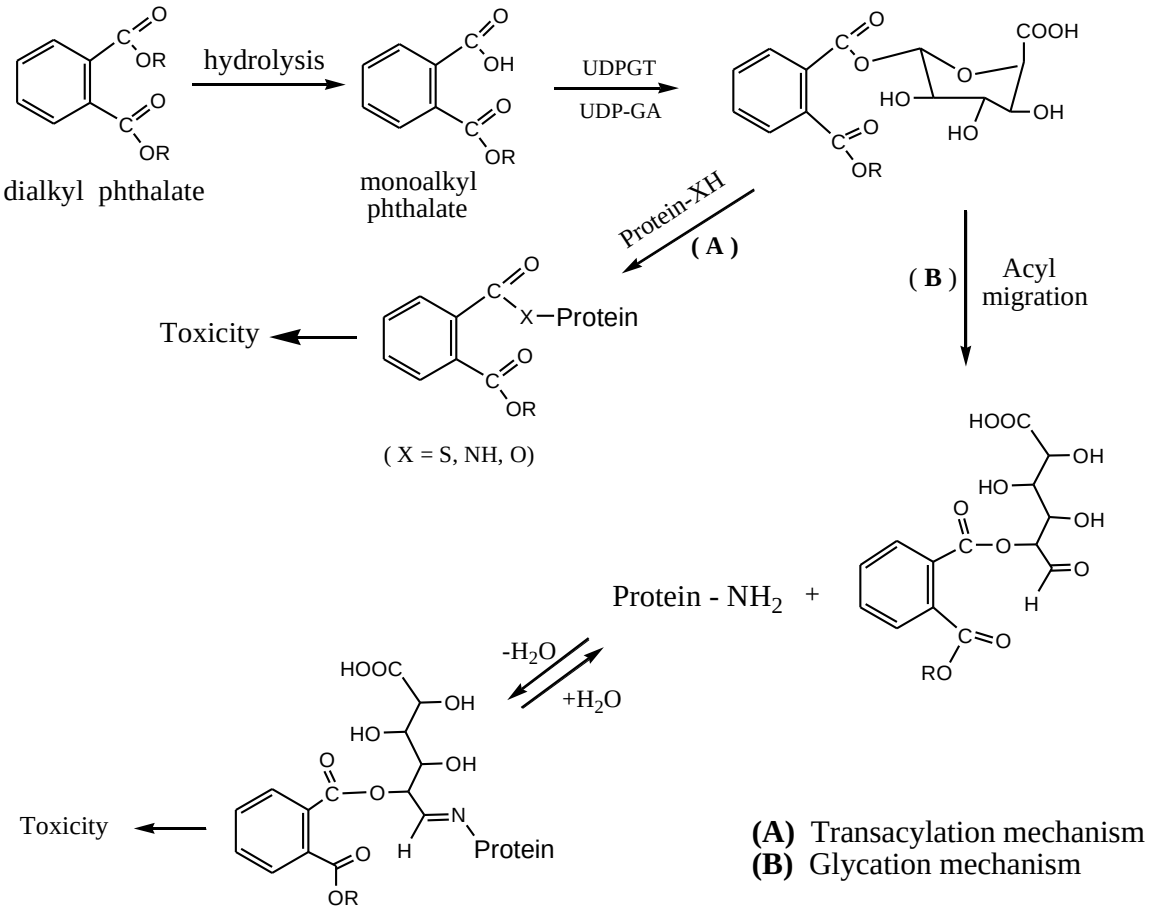
Individual profile/alert	
Name	Arenecarboxylic Acid Esters
Type of profile	Structural alert

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

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

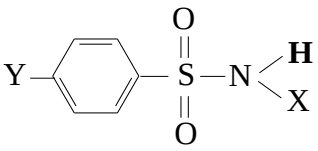
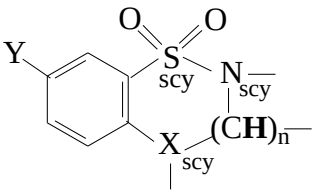
Bearing in mind these facts, it can be assumed that acyl glucuronides of hydrolyzed arenecarboxylic esters can form protein adducts by two mechanisms - transacylation mechanism and glycation mechanism (or Schiff's base mechanism) [6,15]):

Scheme 1



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

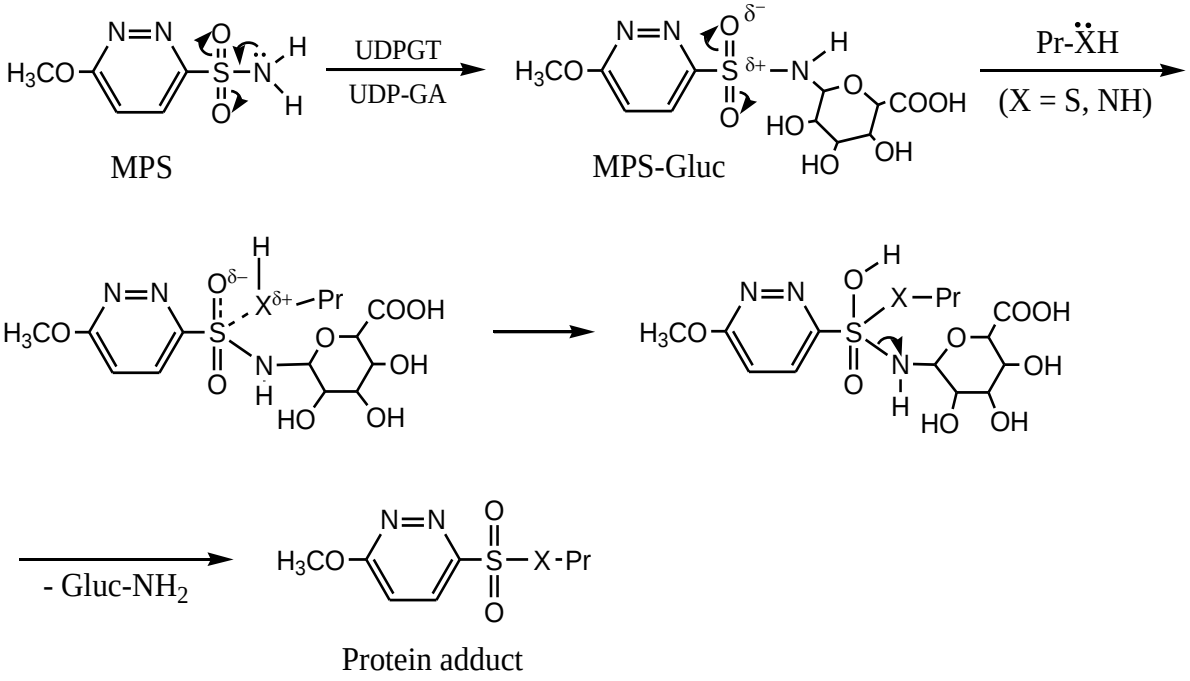
	8. Foster, P. M., Int. J. Androl. 2006, Vol. 29, pp. 140-147. 9. Schachter, D., and J. G. Manis, J. Clin. Invest. 1958, Vol. 37, pp. 800-807. 10. Morris, M. E., Drug Metab. Dispos. 1990, Vol. 18, pp. 809-811. 11. Foster, P. M., M. W. Cook, L. V. Thomas, D. G. Walters, and S. D. Gangolli, Drug Metab.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

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

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

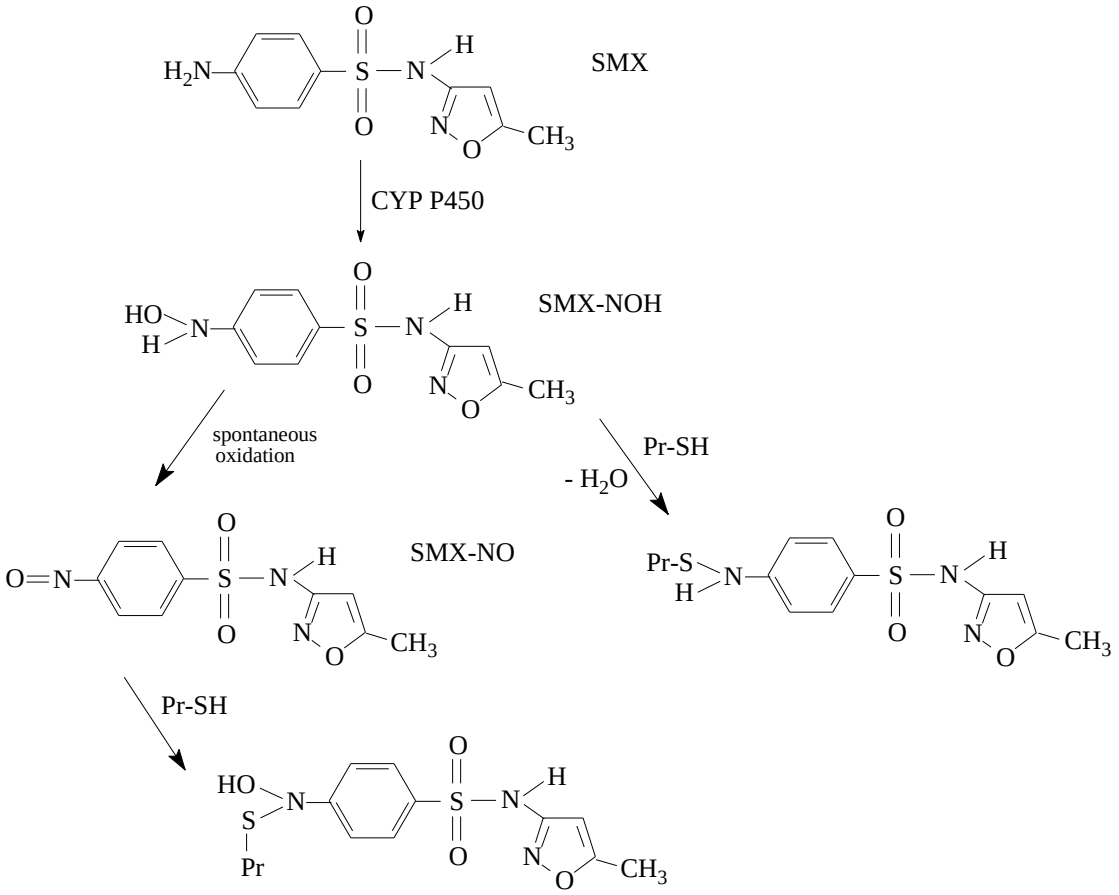
Chiu and Huskey [6] established that primary and secondary sulfonamides can undergo glucuronidation in vitro and in vivo in a large number of animal species and in humans. The bioactivation of sulfonamide non-antibiotics via N-glucuronidation results in the formation of more reactive intermediate [7]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

Scheme 1



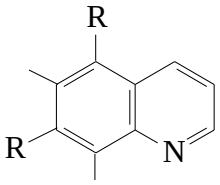
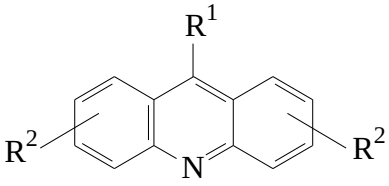
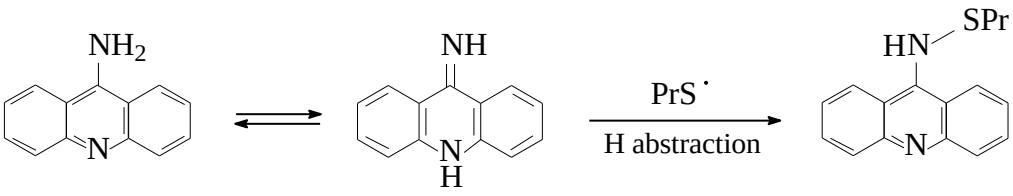
Sulfonamide antibiotics (antimicrobials) can be regarded as derivatives of the sulfanilamide having an aromatic amine group at the para-position towards SO2NH-group. The aromatic amine moiety is considered to be trigger for serious drug reactions which occur mainly in internal administration. For example,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

Scheme 2

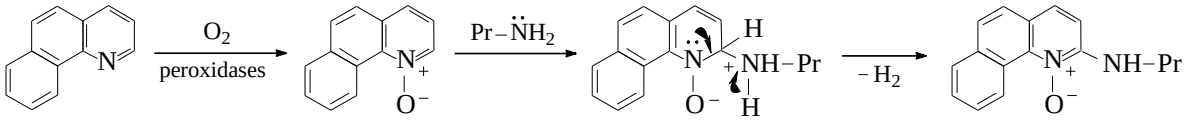


Set of chemicals used for profile development	Arenesulfonamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. S.R. Knowles, L.E. Shapiro, N.H. Shear, *Drug Saf.*, **2001**, 24(4), 239-247. 2. L.E. Shapiro, S.R. Knowles, E. Weber, M.G. Neuman, N.H. Shear, *Drug Saf.*, **2003,** 26(3), 187-195. 3. C.K. Svensson, E.W. Cowen, A.A. Gaspari, *Pharmacol. Rev.*, **2000**, 53(3), 357-379. 4. J. Clausen, *J. Pharmacol. Exp. Ther.*, **1966**, 153(1), 167-175. 5. M. Schafer-Korting, *Arzneimittelforschung*, **1985**, 35(12), 1828-1831. 6. S.-H. L. Chiu, S.-E. W. Huskey, *Drug Metab. Dispos.*, **1998**, 26(9), 838 – 847. 7. S. Zhou, E. Chan, W. Duan, M. Huang, Y.-Z. Chen, *Drug Metab. Rev.*, **2005**, 37(1), 41-213. 8. T. Manchandra, D.A. Hess, L. Dale, S.G. Ferguson, M.J. Rieder, *Mol. Pharmacol.*, **2002**, 62(5), 1011-1026.

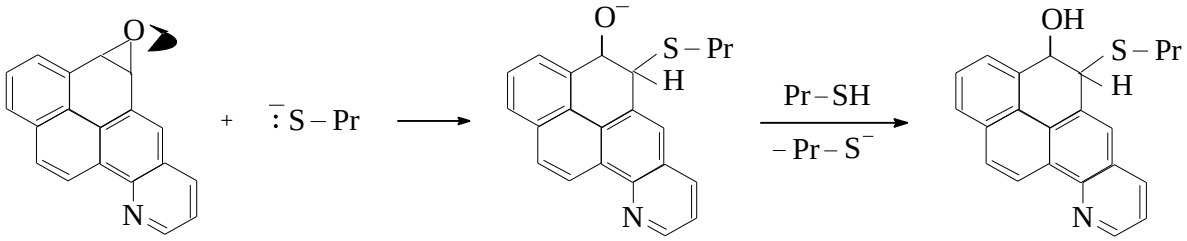
	9. P.M. Vyas, S. Roychowdhury, C.K. Svensson, *Drug Metab. Dispos*., **2006**, 34(1), 16-18. 10. P. Bhaiya, S. Roychowdhury, P.M. Vyas, M.A. Doll, D.W. Hein, C.K. Svensson, *Toxicol. Appl. Pharmacol*., **2006**, 215(2), 158-167. 11. D.A. Hess, M.E. Sisson, H. Suria, J. Wijsman, R. Puvanesasingham, J.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

Individual profile/alert	
Name	Benzoquinoline and Acridine derivatives
Type of profile	Structural alert
Description/applicability domain	 **R** = Csp^2(aryl) – fused rings  **R1** = –H, –Cl, –NH_2; **R2** = –H, –Cl, –NO_2, –OCH_3, –CF_3
Mechanism	AR, Radical-type addition to imino tautomer of aminoacridines SNAr, Nucleophilic substitution on activated Csp2-atoms in quinolines SN2, Mechanistic alert: Ring opening nucleophilic substitution involving arene oxide derivatives and proteins
9-Aminoacridine may exist in two tautomer forms, namely, amino and imino form. The latter has a reactive imino group, which is able to associate with protein thiol radicals via radical version of Michael addition, as is assumed by Aptula *et al*. [5]. The possible mechanism of protein binding of 9-aminoacridine is presented in Scheme 1.  	

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

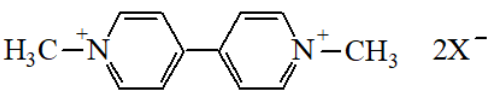
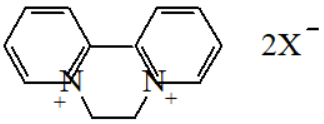


Pyrenoline 4,5-oxide has an epoxide ring in the molecule and is a direct acting mutagen as the bay region epoxides are the active metabolites of aza-PAHs *in vivo* [7,8]. Cleavage of the epoxide ring by various nucleophiles, such as amino and sulfhydryl groups, is one of the most frequently encountered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



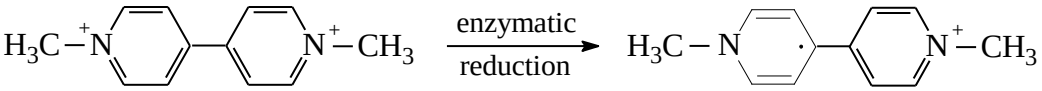
Set of chemicals used for profile development	Benzoquinoline and Acridine derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. E.A. Bleeker, S. Wiegman, P. De Voogt, M. Kraak, H.A. Leslie, E. De Haas, W. Admiraal, Toxicity of azaarenes. *Rev. Environ. Contam. Toxicol*., **2002**, 173, 39-83. 2. A. Matsuoka, K. Shudo, Y. Saito, T. Sofuni, M. Ishidate Jr, Clastogenic potential of heavy oil extracts and some azaarenes in Chinese hamster cells in culture. *Mutat. Res.*, **1982**, 102(3), 275-283. 3. M. Ishidate Jr, M.C. Harnois, T. Sofuni, A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 4. E.A.J. Bleeker, H.J. Van Der Geest, H.J.C. Klamer, P. De Voogt, E. Wind, M.H.S. Kraak, Toxic and genotoxic

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

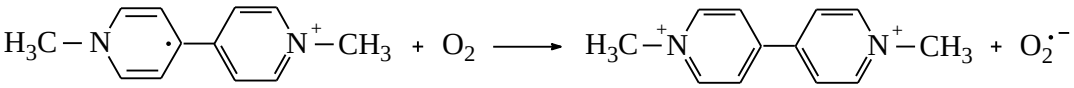
Individual profile/alert	
Name	Bipyridilium Herbicides
Type of profile	Structural alert
Description/applicability domain	 paraquat  diquat

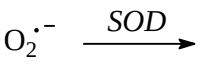
	where X = Cl^-, Br^-, I^-, $^-OSO_2OCH_3$, etc.
Mechanism	Radical mechanism, ROS generation and protein carbonylation A_N2, Nucleophilic addition to monopyridone moiety of paraquot or diquat

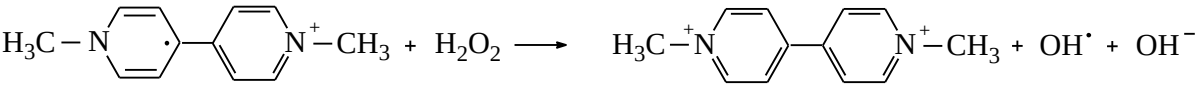
The herbicidal action and toxicity of PQ and DQ are thought to result from the generation of reactive oxygen species (ROS) through redox cycling [Bus et al., 1976, 1984; Winterbourn, 1981; Cohen and Doherty, 1987; Peng et al., 2004; Wang et al., 2007]. During redox cycling PQ and DQ undergo a single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



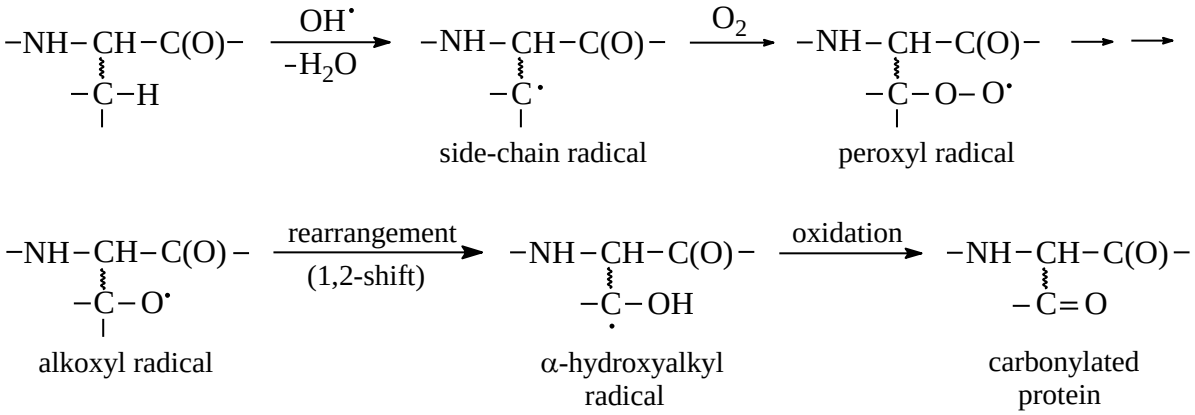
paraquat — paraquat radical



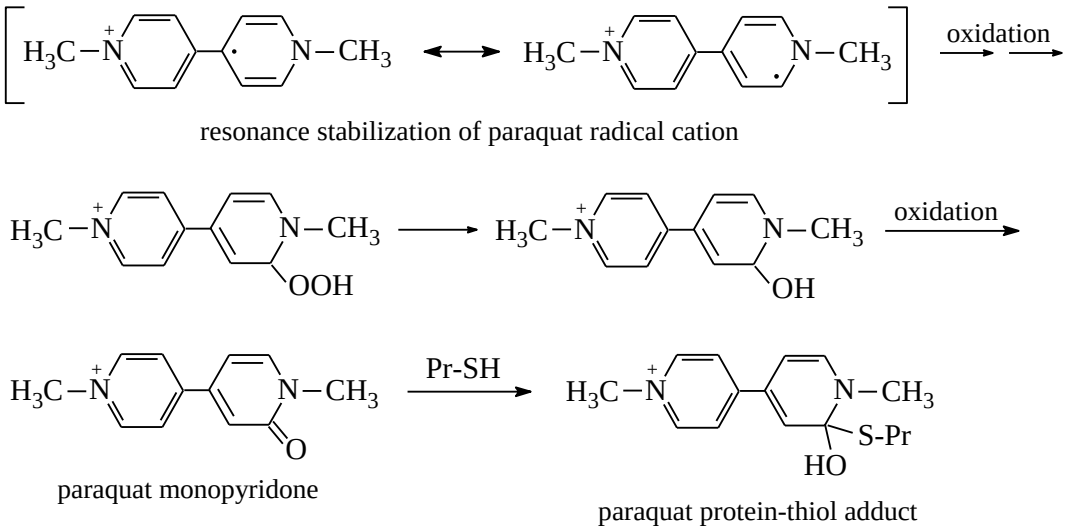




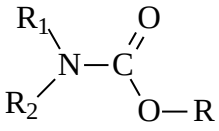
An excess of ROS (O2·⁻, H2O2, ·OH) causes oxidative damage to cellular macromolecules including proteins, nucleic acids and lipids. This results in many adverse effects such as the formation of mutagenic lesions, altered enzyme function, lipid peroxidation and inappropriate cell signaling [Black et al.,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



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

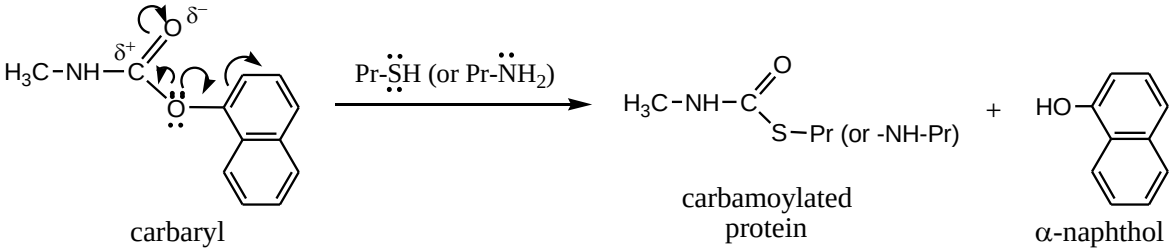


Set of chemicals used for profile development	Bipyridilium Herbicides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

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

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

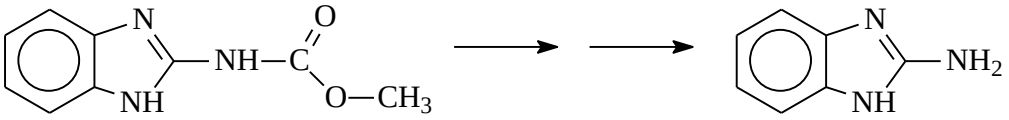
Covalent binding of carbamoyl moiety from carbaryl and other carbamates was also suggested [16]. The reaction takes place as a bimolecular nucleophilic substitution, which is enhanced if the ester moiety is aromatic. During carbamoylation of proteins (including chromosomal ones) by carbaryl and other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



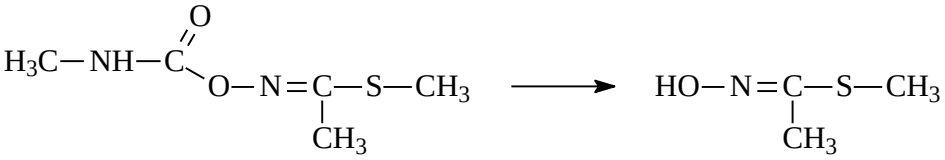
carbaryl — carbamoylated protein — α-naphthol

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

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

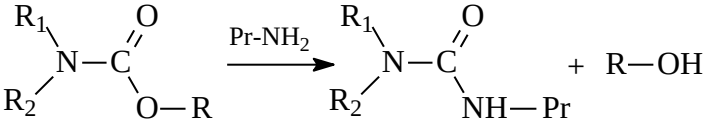


(Assumed potentially active metabolite: heterocyclic amine)



(Assumed potentially active metabolite: oxime)

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

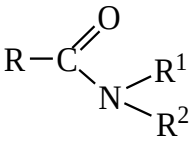
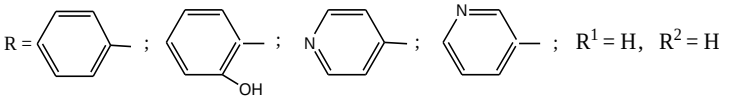
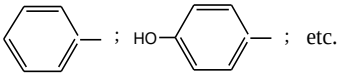


(Chromosomal protein adduct)

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

Set of chemicals used for profile development	Carbamates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Wei, L.Y., Chao, J.S., Hong, C.C., Assessment of the ability of propoxur, methomyl, and aldicarb, three carbamate insecticides, to induce micronuclei in vitro in cultured Chinese hamster ovary cells and in vivo in BALB/c mice, Environ. Mol. Mutagen., 1997, 29(4), 386-393.

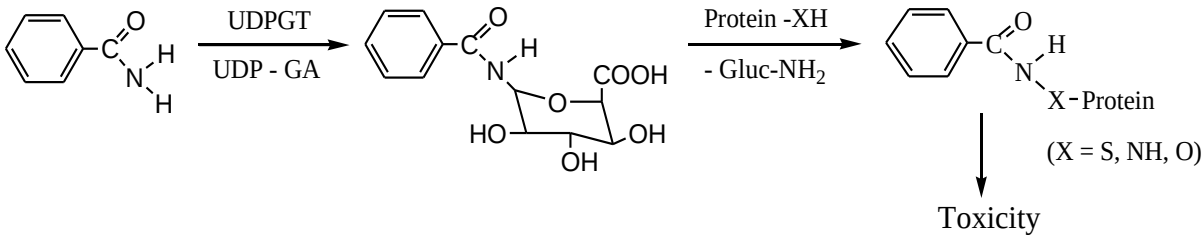
4. Carbaryl, EHC No. 153. World Health Organization, Geneva, 1994.
5. Carbamate pesticides: a general introduction, EHC No. 64, World Health Organization, Geneva, 1986.
6. Naravaneni, R., K. Jamil, Cytogenetic Biomarkers of Carbofuran Toxicity Utilizing Human Lymphocyte Cultures In Vitro, Drug Chem. Toxicol. 2005, 28(3), 359 – 372.
7. Soloneski, S., M. L. Larramendy, Genetic Toxicological Profile of Carbofuran and Pirimicarb Carbamic Insecticides, 2012; http://cdn.intechweb.org/pdfs/28277.pdf.
8. Sofuni, T. 1998. Data Book of Chromosomal Aberrations Test In Vitro, Revised Edition, Live-Science Information Center, Tokyo, Japan.
9. Murakami, M., Fukami, J.I., Uptake of benzo[a]pyrene, carbaryl, DDT and parathion in cultured human cells: re-evaluation, Bull. Environ. Contam. Toxicol., 1979, 21(1), 478 – 482.
10. Metcalf, R.L., Structure-activity relationships for insecticidal carbamates, Bull. World Health Organ., 1971, 44(1-3), 43 - 78.
11. Aldridge, W.N., Nature of reaction of organophosphorus compounds & carbamates with esterases, Bull. World Health Organ., 1971, 44(1-3), 25 - 30.
12. Pipy, B., Gaillard, D., Derache, R., Enzymatic activities of liver serine esterases during the reticuleondothelial system phagocytosis blockade by carbaryl, an anticholinesterasic insecticide, Toxicol. Appl. Pharmacol. 1982, 62(1), 11 - 18.
13. Murakami, M., Fukami, J.I., Incorporation of labeled pesticides and environmental chemicals into nuclear fraction of cultured human cells, Bull. Environ. Contam. Toxicol., 1980, 24(1), 27 - 30.
14. Murakami, M., Fukami, J.I., Carbaryl binds to proteins in human cells in culture but chlorinated organic chenicals do not, Bull. Environ. Contam. Toxicol., 1982, 28(4), 500 - 503.
15. Davies, J.H., Campbell, W.R., Kearns, Inhibition of fly head acetylcholinesterase by bis-(m-hydroxyphenyl)-trimethylammonium iodide) esters of polymethylenedicarbamic acids, C.W., Biochem. J., 1970, 117(2), 221 - 230.
16. Krug, H.F., Hamm, U., Berndt, J., Mechanism of inhibition of cyclo-oxygenase in human blood platelets by carbamate insecticides, Biochem. J., 1988, 250(1), 103 - 110.

Individual profile/alert	
Name	Carboxylic Acid Amides
Type of profile	Structural alert
Description/applicability domain	 where **Ist group:** R =  ; R^1 = H, R^2 = H **IInd group:** R = Alkyl (-CH_3, -C_2H_5, etc); R^1 = H; R^2 =  ; etc.
Mechanism	Acylation, Acylation involving an activated (glucuronidated) carboxamide group Acylation, Direct acylation involving a leaving group Michael addition, Quinone type compounds

Acylation involving an activated (glucuronidated) carboxamide group

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

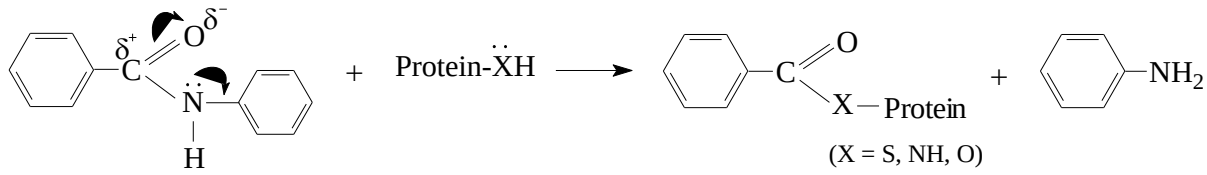
Scheme 1



Direct acylation involving a leaving group

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

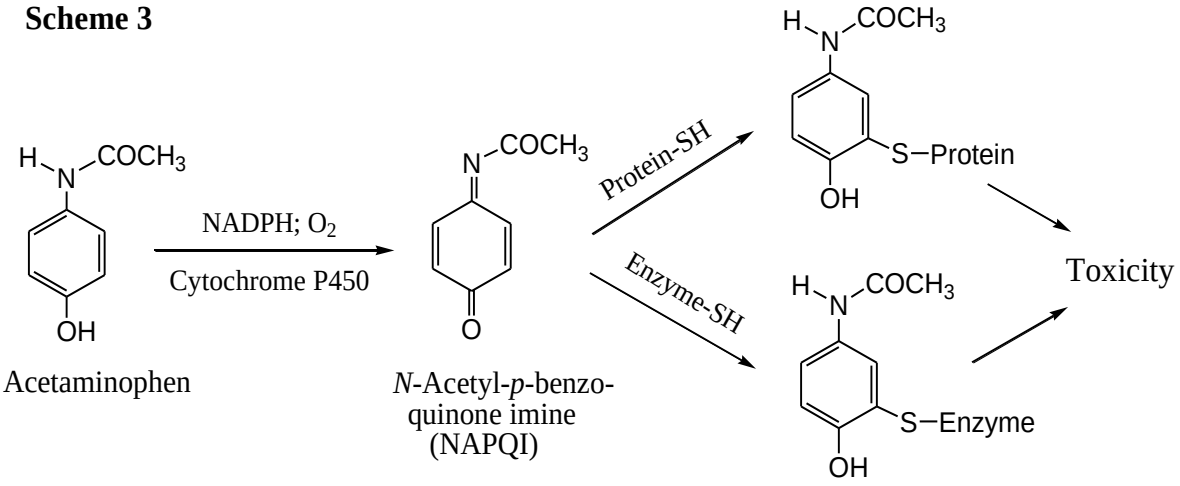
Scheme 2



Quinone type compounds

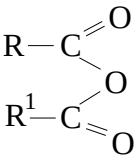
Acetaminophen is one of these secondary N-aryl amides which toxicity is related with its initial metabolism. It is metabolically activated by CYP450 to form a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [8-11]. In overdose, conjugation of this reactive metabolite with GSH leads to the GSH depletion and NAPQI (a soft electrophile) covalently binds to cysteine residues on proteins to form acetaminophen adducts [11]. Covalent binding of NAPQI to proteins is thought to be a critical step in the development of hepatotoxicity [8]. It was also reported that NAPQI is a topoisomerase II poison [10]. It induces DNA strand breaks, chromosomal aberrations, and sister chromatid exchanges in a variety of mammalian cells [7,19]. DNA cleavage in the presence of NAPQI is mediated by topoisomerase IIα. The binding of NAPQI to cellular proteins and to cellular enzymes is an excellent correlate of acetaminophen toxicity [9,11] (Scheme 3).

Scheme 3



Set of chemicals used for profile development	Carboxylic Acid Amides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. C.S. Song, N.A. Gelb, S.M. Wolff, *J. Clin. Invest*. 1972, **51**, 2959-2966. 2. M.E. Morris, G. Levy, *J. Pharm. Sci*. 1983, **72**, 612-617. 3. X. Xu, B.K. Tang, K.S. Pang, *J. Pharmacol. Exp. Ther*. 1990, **253**, 965-973. 4. R.G. Tirona, K.S. Pang, *Drug Metab. Dispos.* 1996, **24**, 821-833. 5. Final report of the safety assessment of niacinamide and niacin, *Int. J. Toxicol.* 2005, **24,** 1-31.

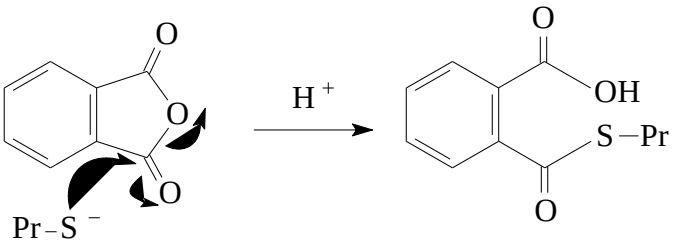
	6. A.G. Staines, M.W.H. Coughtrie, B. Burchell, *J. Pharmacol. Exp. Ther.* 2004, **311,** 1131-1137. 7. K. Bergmen, L. Müller, S.W. Teigen, *Mutat. Res.* 1996, **349**, 263-288. 8. K.L. Muldrew, L.P. James, L. Coop, S.S. McCullough, H.P. Hendrickson, J.A. Hinson, P.R. Mayeux, *Drug Metab. Dispos.* 2002, **30,** 446-451. 9. L. P. James, P. R. Mayeux, J. A. Hinson, *Drug Metab. Dispos.* 2003, **31,** 1499-1506. 10. R.P. Bender, R.H. Lindsey, Jr., D.A. Burden, N. Osheroff, *Biochemistry* 2004, **43**, 3731-3739. 11. A.B. Reid, R.C. Kurten, S.S. McCullough, R.W. Brock, J.A. Hinson, *J. Pharmacol. Exp. Ther.* 2005, **312**, 509-516. 12. D.J. Naisbitt, M. Britschgi, G. Wong, J. Farrell, J.P.H. Depta, D.W. Chadwick, W.J. Pichler, M. Pirmohamed, B.K. Park, *Mol. Pharmacol.* 2003, **63**, 732-741. 13. D. Zhang, W. Zhao, V.A. Roongta, J.G. Mitroka, L.J. Klunk, M. Zhu, *Drug Metab. Dispos.* 2004, **32,** 545-551. 14. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, *Chem. Res. Toxicol.* 2007, **20**, 1019–1030. 15. Toxic substances – Focus on Children; Developing a Canadian List of Substances of Concern to Children's Health, June 2004, p. A-64. 16. E.W. Scholes, D.A. Basketter, W.W. Lovell, A.E. Sarll, R.U. Pendlington, *Photodermatol. Photoimmunol. Photomed.* 1991, **8**, 249-254. 17. G.F. Gerberick, C.A. Ryan, E.C. Von Bargen, S.B. Stuard, G.M. Ridder, *J. Invest. Dermatol.* 1991, **97**, 210–218. 18. H. Spielmann, L. Müller, D. Averbeck, M. Balls, S. Brendler-Schwaab, J.V. Castell, R. Curren, O. de Silva, N.K. Gibbs, M. Liebsch, W.W. Lovell, H.F. Merk, J.F. Nash, N.J. Neumann, W.J. Pape, P. Ulrich, H.W. Vohr, *ATLA* 2000, **28**, 777-814. 19. E. Dybing, J.A. Holme, W.P. Gordon, E.J. Soderlund, D.C. Dahlin, S.D. Nelson, *Mutat. Res.* 1984, **138,** 21-32.

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

	R = R^1 = Csp^2 (scy), Csp^2 (aryl), Csp^2 (aryl fused)
Mechanism	SN2 Acylation, Ring opening acylation reaction

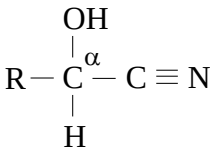
Maleic anhydride (MA), 2-acetoxybenzoic anhydride did induce chromosomal aberrations in cultured Chinese hamster lung (CHL) cells in the absence of exogenous metabolic activation and phthalic anhydride in cultured Chinese hamster ovary (CHO) cells in the absence of exogenous metabolic activation [2,3].

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



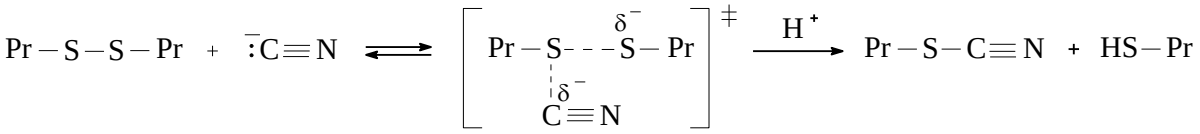
Set of chemicals used for profile development	Carboxylic Acid Anhydrides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.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

Individual profile/alert	
Name	Cyanohydrins
Type of profile	Structural alert

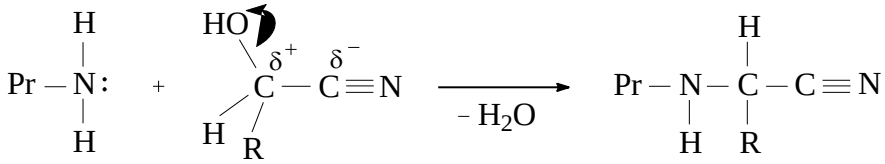
Description/applicability domain	 R = (Csp^3)n, where “n” represents up to 7 carbon atoms.
Mechanism	SN2, Thiocyanate formation via the nucleophilic-type substitution at the disulfide bond of proteins and enzymes SN2, yanoalkylation of proteins via the nucleophilic substitution at sp3-carbon atom of cyanohydrins

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

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



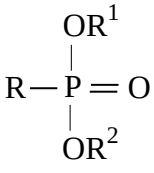
As the process of cyanide release is reversible and pH dependent, it would be expected that under physiological conditions (pH 7.2–7.4) there will be a certain amount of parent cyanohydrin. According to Horvath et al. [8], cyanohydrins may react with basic amino groups of proteins under physiological conditions, which leads to formation of the corresponding cyanoalkyl derivatives. This reaction proceeds as nucleophilic substitution of the hydroxyl group at the expence of increased electrophilicity of alpha-carbon atom, adjacent to the electron-withdrawing cyano group (Scheme 3).



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

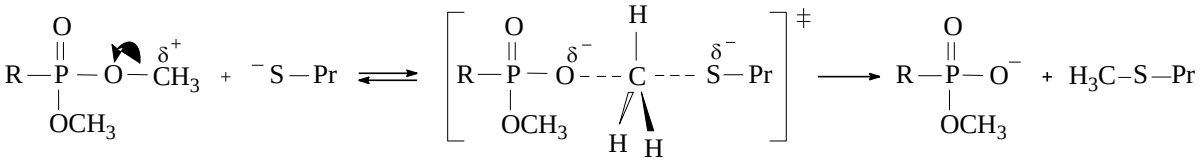
References	1. 2-Hydroxypropanenitrile: SIDS Initial Assessment Report for SIAM 2, July **1994**, pp. 3, 6, 11. 2. H. Kusakabe, K. Yamakage, S. Wakuri, K. Sasaki, Y. Nakagawa, M. Watanabe, M. Hayashi, T. Sofuni, H. Ono, N. Tanaka, Relevance of chemical structure and cytotoxicity to the induction of chromosome aberrations based on the testing results of 98 high production volume industrial chemicals. *Mutat. Res.*, **2002**, 517(1-2), 187-198. 3. T. Frisch, B.L. Møller, Possible evolution of alliarinoside biosynthesis from the glucosinolate pathway in *Alliaria petiolata*. *FEBS J.*, **2012**, 279(9), 1545-1562. 4. Acetone Cyanohydrin: *In* Acute Exposure Guideline Levels for Selected Airborne Chemicals, vol. 7, pp. 13-47, National Academies Press, Washington, D.C., **2009**. 5. P.V. Kaplita, R.P. Smith, Pathways for the bioactivation of aliphatic nitriles to free cyanide in mice. *Toxicol. Appl. Pharmacol.*, **1986**, 84(3), 533-540. 6. F.P. Simeonova, L. Fishbein, Hydrogen cyanide and cyanides: Human Health Aspects. Concise International Chemical Assessment Document, CICAD 61, WHO **2004**. 7. Cyanogenic glycosides in cassava and bamboo shoots, Technical Report Series No. 28. Food Standards Australia New Zealand, July **2004**. 8. V. Horváth, L. Trézl, T. Szarvas, J. Pipek, C. Vida, K. Bauer, Investigation of cyano-methylation reaction by cyanohydrin and its determination in tobacco-smoke. (Strecker-reactions). *Period. Polytech. Chem. Eng.*, **1992**, 36(3), 209-218.

Individual profile/alert	
Name	Dialkyl Alkylphosphonates
Type of profile	Structural alert

Description/applicability domain	 R, R^1, R^2 are Csp^3-atoms and R = R^1 = R^2 or R is different from R^1 and R^2
Mechanism	SN2, Protein and/ or DNA alkylation

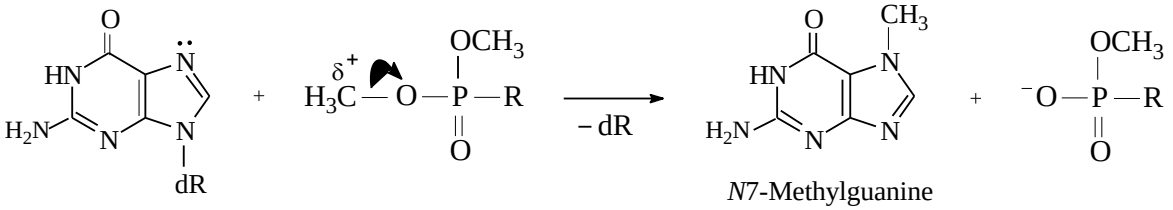
Protein alkylation via direct attack at the *O*-alkyl group

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



where R = Csp^3-atoms

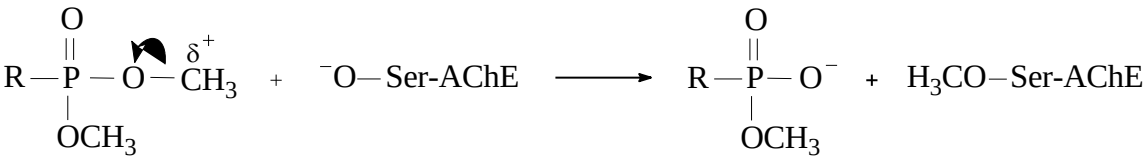
Trichlorfon and the other phosphonate esters with electron-withdrawing substituents possess higher electrophilicity on carbon atoms of the methoxy groups, compared to the non-substituted phosphonates. It was established that trichlorfon has a DNA-alkylating properties and may react with DNA *in vitro*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



*N*7-Methylguanine

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

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

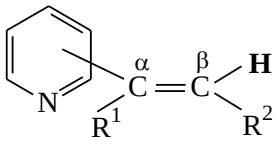


where R = Cl_3C-CH(OH)– or HOH_2C-NH-C(O)-$(CH_2)_2$–

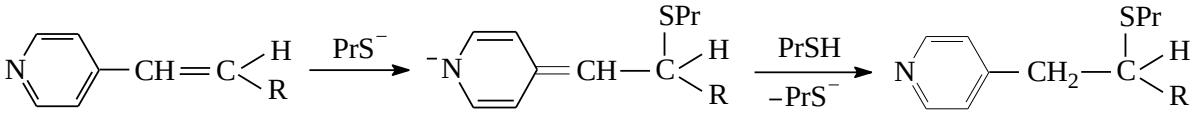
Set of chemicals used for profile development	Dialkyl Alkylphosphonates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. T.C. Wang, C.M. Lin, L.W. Lo, Genotoxicity of methoxyphosphinyl insecticide in mammalian cells. *Zool. Stud.*, **2003**, 42(3), 462-469. 2. T. Feng, Z.B. Li, X.Q. Guo, J.P. Guo, Effects of trichlorfon and sodium dodecyl sulphate on antioxidant defense system and acetylcholinesterase of *Tilapia nilotica* in vitro. *Pest. Biochem. Physiol*., **2008**, 92(3), 107-113. 3. G. Klopman, R. Contreras, H.S. Rosenkranz, M.D. Waters, Structure-genotoxic activity relationships of pesticides: comparison of the results from several short-term assays. *Mutat. Res*., **1985**, 147(6), 343-356. 4. NTP Toxicology and Carcinogenesis Studies of Dimethyl Methylphosphonate (CAS No. 756-79-6) in F344/N rats and B6C3F1 Mice (Gavage Studies). *Natl. Toxicol. Program Tech. Rep. Ser*., **1987**, NTP TR 323, pp. 1-172. 5. Toxicological Profile for Diisopropyl Methylphosphonate. U.S. Department of Health and Human Services, August **1998**. 6. S.M. Galloway, D.A. Deasy, C.L. Bean, A.R. Kraynak, M.J. Armstrong, M.O. Bradley, Effects of high osmotic strength on chromosome aberrations, sister chromatid exchanges and DNA strand breaks, and the relation to toxicity. *Mutat. Res*., **1987**, 189(1), 15-25. 7. M. Ishidate Jr, K. Yoshikawa, Chromosome aberration tests with Chinese hamster cells *in vitro* with and without metabolic activation. *Arch. Toxicol.*, **1980**, Suppl. 4, 41-44. 8. Trichlorfon: *In* IARC Monographs on the evaluation

	of the carcinogenic risk of chemicals to humans, Vol. 30, **1983**, pp. 207-231. 9. K. Baetcke, I.S. Rodgers, L. Tahan, G. Hard, R. McGaughy, *alpha*-2u-Globulin: Association with chemically-induced renal toxicity and neoplasia in the male rat. U.S. Environmental Protection Agency, February 1991. 10. J.A. Swenberg, α_{2u}-Globulin nephropathy: Review of the cellular and molecular mechanisms involved and their implications for human risk assessment. *Environ. Health Perspect*., **1993**, 101(Suppl. 6), 39-44. 11. K. Blumbach, A. Pähler, H.M. Deger, W. Dekant, Biotransformation and male rat-specific renal toxicity of diethyl ethyl- and dimethyl methylphosphonate. *Toxicol. Sci*., **2000**, 53(1), 24-32. 12. H.S. Rosenkranz, S. Rosenkranz, Reaction of DNA with phosphoric acid esters: gasoline additives and insecticides. *Experientia (Basel)*, **1972**, 28(4), 386-387. 13. W. Dedek, K. Lohs, G.W. Fischer, R. Schmidt, Alkylation of guanine in mice in vivo by organophosphorus insecticides. I. Trichlorphone and butonate. *Pest. Biochem. Physiol*., **1976**, 6(2), 101-110. 14. J.C. Sanchez-Hernandez, C.H. Walker, *In vitro* and *in vivo* cholinesterase inhibition in Lacertides by phosphonate- and phosphorothioate-type organophosphates. *Pestic. Biochem. Physiol*., **2000**, 67(1), 1-12. 15. T. Feng, Z.B. Li, X.Q. Guo, J.P. Guo, Effects of trichlorfon and sodium dodecyl sulphate on antioxidant defense system and acetylcholinesterase of *Tilapia nilotica* in vitro. *Pest. Biochem. Physiol*., **2008**, 92(3), 107-113. 16. D.M. Quinn, Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states. *Chem. Rev*., **1987**, 87(5), 955-979. 17. H. Dvir, I. Silman, M. Harel, T.L. Rosenberry, J.L. Sussman, Acetylcholinesterase: From 3D structure to function. *Chem.-Biol. Interact*., **2010**, 187(1-3), 10-22.

Individual profile/alert

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

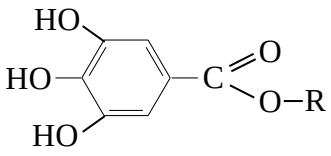
Vinyl pyridine isomers (ortho- and para-) are typical Michael acceptors due to the presence of a polarized double bond under the influence of electron-withdrawing effect of the nitrogen atom [7,8]. It has been suggested that di-substitution at the β-carbon atom of the alkene moiety sterically hinders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



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

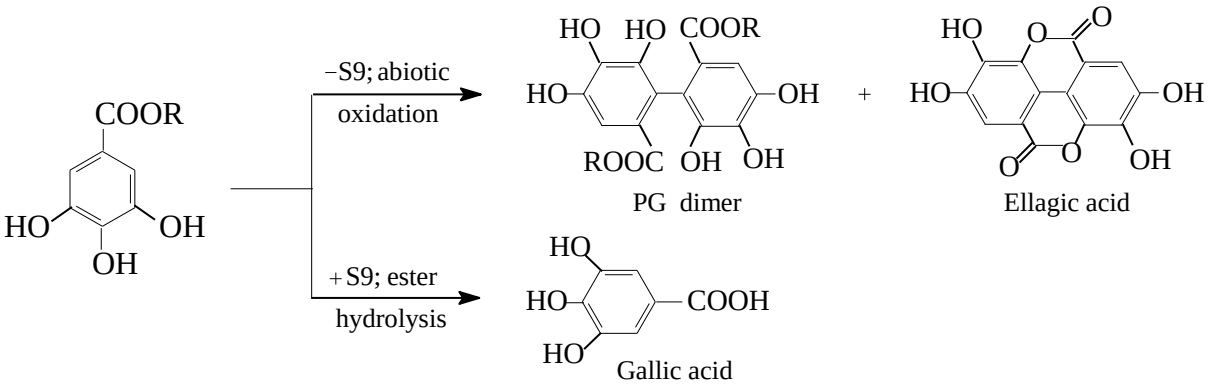
Set of chemicals used for profile development	Ethenyl Pyridines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References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

	8. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.T.D. Cronin, *Crit. Rev. Toxicol*., **2011**, 41(9), 783-802. 9. G.Y. Patlewicz, Z.M. Wright, D.A. Basketter, C.K. Pease, J.P. Lepoittevin, E.G. Arnau, *Contact Dermatitis*, **2002**, 47(4), 219–226. 10. T.W. Schultz, J.W. Yarbrough, R.S. Hunter, A.O. Aptula, *Chem. Res. Toxicol*., **2007**, 20(9), 1359-1363.

Individual profile/alert	
Name	Gallic Acid Esters
Type of profile	Structural alert
Description/applicability domain	 R can be H, Csp^3(alkyl, cycloalkyl), Csp^2(aryl), etc.
Mechanism	AN2, Michael-type addition to quinoid structures

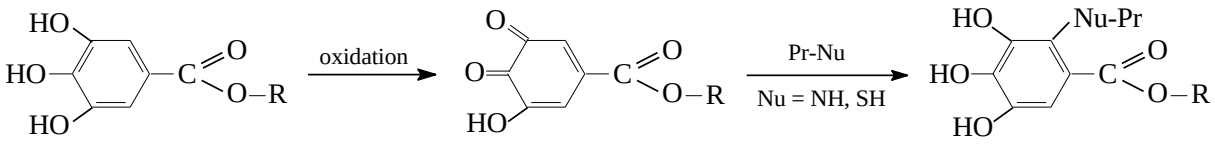
Gallate esters are used as synthetic antioxidants. Despite their presumed low toxicity, many authors were reported that some of linear alkyl gallates had many adverse effects. The cytotoxic effects of methyl, ethyl, propyl, butyl, octyl and dodecyl gallates have been studied by Nakagawa and Tayama [1,2].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

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



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

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



The reactivity of gallate esters toward proteins depends on the relative elongation of alkyl side-chains which determine their hydrophobicity. It was established that butyl gallate, octyl gallate and dodecyl gallate are more cytotoxic than propyl, ethyl, and methyl gallate [1].

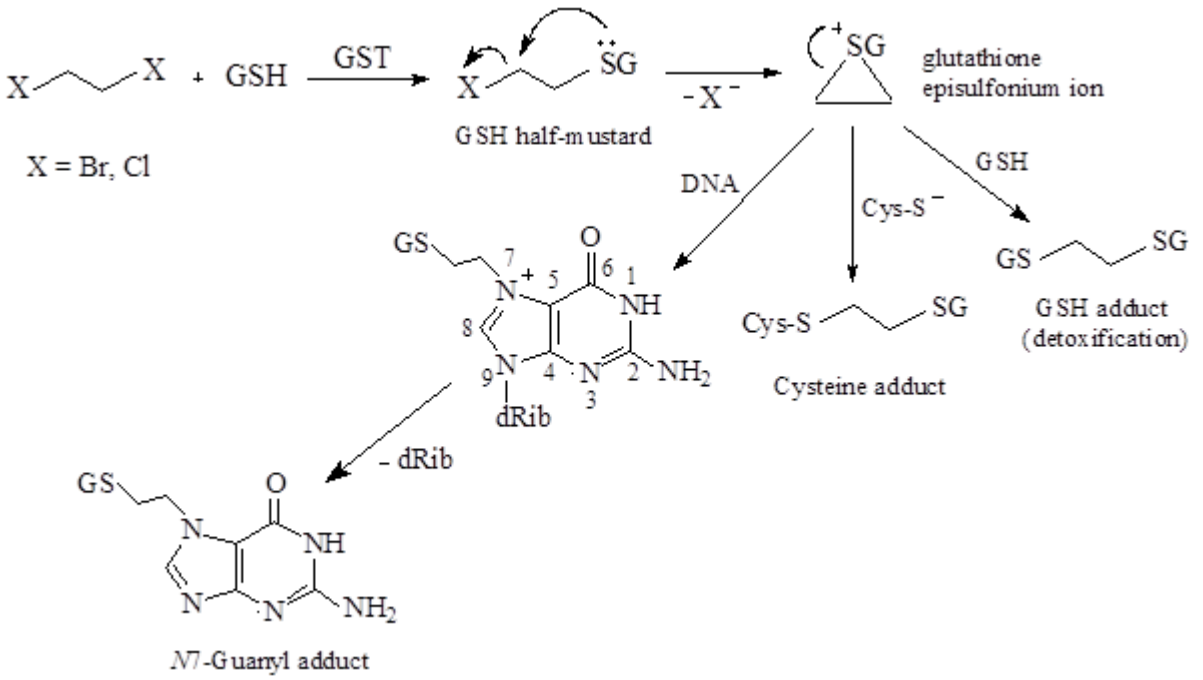
Set of chemicals used for profile development	Gallic Acid Esters
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Y. Nakagawa, S. Tayama, Cytotoxicity of propyl gallate and related compounds in rat hepatocytes. *Arch. Toxicol.*, **1995**, 69(3), 204-208. 2. S. Tayama, Y. Nakagawa, Cytogenic effects of propyl gallate in CHO-K1 cells. *Mutat. Res.*, **2001**, 498(1-2), 117-127. 3. M. Ishidate Jr, T. Sofuni, K. Yoshikawa, M. Hayashi, T. Nohmi, M. Sawada, A. Matsuoka, Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.*, **1984**, 22(8), 623-636.

	4. D.K. Gulati, K. Witt, B. Anderson, E. Zeiger, M.D. Shelby, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. III. Results with 27 chemicals. *Environ. Mol. Mutagen*., **1989**, 13(2), 133-193. 5. G. Patlewicz, D.W. Roberts, E. Uriarte, Skin sensitization: A comparison of reactivity schemes for the prediction skin sensitization potential. *Chem. Res. Toxicol*., **2008**, 21(2), 521-541.

Individual profile/alert	
Name	Halogenated Vicinal Hydrocarbons
Type of profile	Structural alert
Description/applicability domain	
Mechanism	SN2, Nucleophilic type substitution together with ring-opening of an episulfonium ion intermediate

- Non-alkylated vicinal haloalkanes

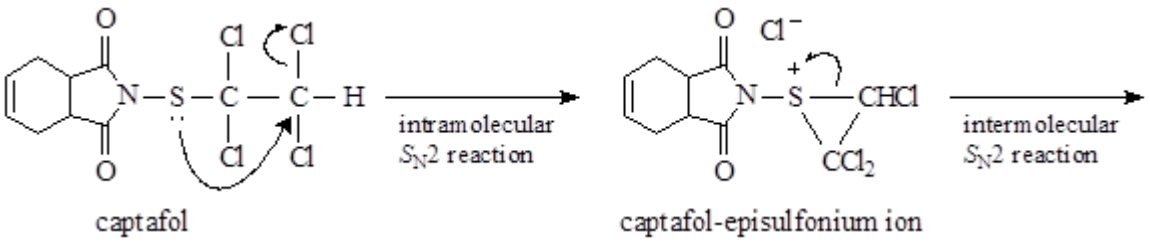
Vicinal dihaloalkanes such as 1,2-dichloroethane, 1,2-dibromoethane, and the mixed 1-bromo-2-chloroethane can be activated to electrophilic species by either oxidative metabolism or conjugation with glutathione [1-3]. Although GSH-conjugation is generally a route of detoxification, in this case it leads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

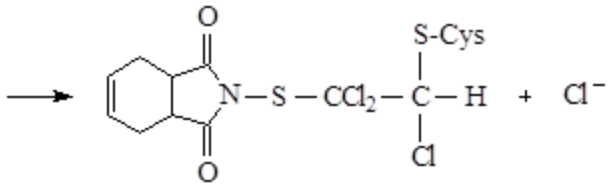


The other experimentally found DNA adducts of GSH-activated 1,2-dibromoethane are N2- and O6-guanyl derivatives. However, only with the N7-guanyl adduct depurination occurs, which could result in respective mutations [1]. For the series of 10 direct alkylating halogenated hydrocarbons a positive relationship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

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

The fungicide captafol differs structurally from vicinal dihaloalkanes having tetrachloroethylthio side chain moiety. This difference confers profound effect on its biological activity. Captafol can act directly as a clastogen without the need for GSH-dependent activation [11]. Captafol's tetrachloroethylthio side chain is able to form an intramolecular episulfonium ion due to the nucleophilicity of the sulfur atom. The opening of this intermediate involving cysteine thiolate residues of proteins is an intermolecular SN2 reaction. It is believed to proceed very fast since positively charged sulfur atom is a good leaving group and moreover, the strain in the three-membered ring is also released [12]. The mechanism of captafol's clastogenicity is shown in Scheme 2.



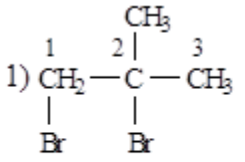


captafol-cysteine adduct

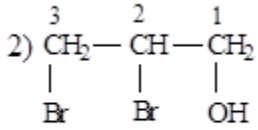
- Alkylated and C1-O-Arylated vicinal haloalkanes

  - Alkylated vicinal haloalkanes

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



1,2-dibromo-2-methylpropane
(non-mutagenic)

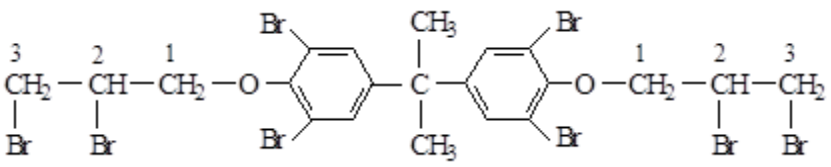


2,3-dibromopropan-1-ol
(mutagenic)

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

  - C1-O-Arylated vicinal haloalkanes

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

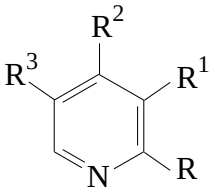
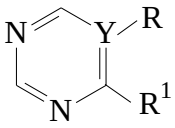


TBBPA bis(2,3-dibromopropyl) ether

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

Set of chemicals used for profile development	Halogenated Vicinal Hydrocarbons
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Guengerich, F.P., Activation of dihaloalkanes by thiol-dependent mechanisms. *J. Biochem. Mol. Biol.*, **2003**, 36(1), 20-27. 2. Tezuka, H., Ando, N., Suzuki, R., Terahata, M., Moriya, M., Shirasu, Y., Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. *Mutat. Res.*, **1980**, 78(2), 177-191. 3. Guengerich, F.P., Peterson, L.A., Cmarik, J.L., Koga, N., Inskeep, P.B., Activation of dihaloalkanes by glutathione conjugation and formation of DNA adducts. *Environ. Health Perspect.*, **1987**, 76, 15-18. 4. van Esch, G.J., Tris(2,3-dibromopropyl)phosphate and bis(2,3-dibromopropyl) phosphate. International Programme on Chemical Safety, Environmental Health Criteria 173. World Health Organization, Geneva, **1995**, pp. 18-21. 5. Nakamura, A., Noriyuki, T., Kojima, S., Kaniwa, M., Kawamura, T., The mutagenicity of halogenated alkanols and their phosphoric acid esters for *Salmonella typhimurium*. *Mutat. Res.*, **1979**, 66(4), 373-380. 6. Sofuni, T., Ishidate Jr., M., Induction of chromosomal aberrations in cultured Chinese hamster cells in a superoxide-generating system. *Mutat. Res.*, **1984**, 140(1), 27-31. 7. Gwinn, M.R., Johns, D.O., Bateson, T.F., Guyton, K.Z., A review of the genotoxicity of 1,2-dichloroethane. *Mutat. Res.*, **2011**, 727(1-2), 42-53. 8. Bolt, H.M., Laib, R.J., Peter, H., Ottenwälder, H., DNA adducts of halogenated hydrocarbons. *J. Cancer Res. Clin. Oncol.*, **1986**, 112(2), 92-96. 9. Inskeep, P.B., Guengerich, F.P., Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione-S-transferases and dibromoalkane strucrures. *Carcinogenesis*, **1984**, 5(6), 805-808.

10. van Bladeren, P.J., Breimer, D.D., Rotteveel-Smijs, G.M., de Knijff, P., Mohn, G.R., van Meeteren-Wälchli, B., Buijs, W., van der Gen, A., The relation between the structure of vicinal dihalogen compounds and their mutagenic activation via conjugation to glutathione. *Carcinogenesis*, **1981**, 2(6), 499-505.
11. Bernard, B.K., Gordon, E.B., An evaluation of the common mechanism approach to the food quality protection act: captan and four related fungicides, a practical example. *Int. J. Toxicol*., **2000**, 19(1), 43-61.
12. Kalsi, P.S., Kalsi, J.P., Bioorganic, Bioinorganic and Supramolecular Chemistry, New Age International, India, **2007**, p. 21.
13. Omichinski, J.G., Søderlund, E.J., Bausano, J.A., Dybing, E., Nelson, S.D., Synthesis and mutagenicity of selectively methylated analogs of tris(2,3-dibromopropyl)phosphate and 1,2-dibromo-3-chloropropane. *Mutagenesis*, **1987**, 2(4), 287-292.
14. Woo, Y.T., Lai, D., McLain, J.L., Manibusan, M.K., Dellarco, V., Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. *Environ. Health Perspect*., **2002**, 110 (Suppl 1), 75-87.
15. Perez-Garrido, A., Giron-Rodriguez, F., Morales Helguera, A., Borges, F., Combes, R.D., Topological structural alerts modifications of mammalian cell mutagenicity for halogenated derivatives. *SAR QSAR Environ. Res*., **2013**; doi: 10.1080/1062936X.2013.820791.
16. Flame Retardant Alternatives for Hexabromocyclododecane (HBCD). Final Report, EPA Publication 740R14001, June **2014**.
17. Knudsen, G.A., Jacobs, L.M., Kuester, R.K., Sipes, I.G., Absorption, distribution, metabolism and excretion of intravenously and orally administered tetrabromobisphenol A [2,3-dibromopropyl ether] in male Fischer-344 rats. *Toxicology*, **2007**, 237(1-3), 158-167.
18. McKee, R.H., Phillips, R.D., Traul, K.A., The genetic toxicity of 1,2-dibromo-3-chloropropane, 1,2-dibromo-3-chloro-2-methylpropane, and 1,2,3-tribromo-2-methylpropane. *Cell Biol. Toxicol.*, **1987**, 3(4), 391-406.
19. Låg, M., Omichinski, J.G., Dybing, E., Nelson, S.D., Søderlund, E.J., Mutagenic activity of halogenated propanes and propenes: effect of bromine and chlorine positioning. *Chem. Biol. Interact.*, **1994**, 93(1), 73-84.

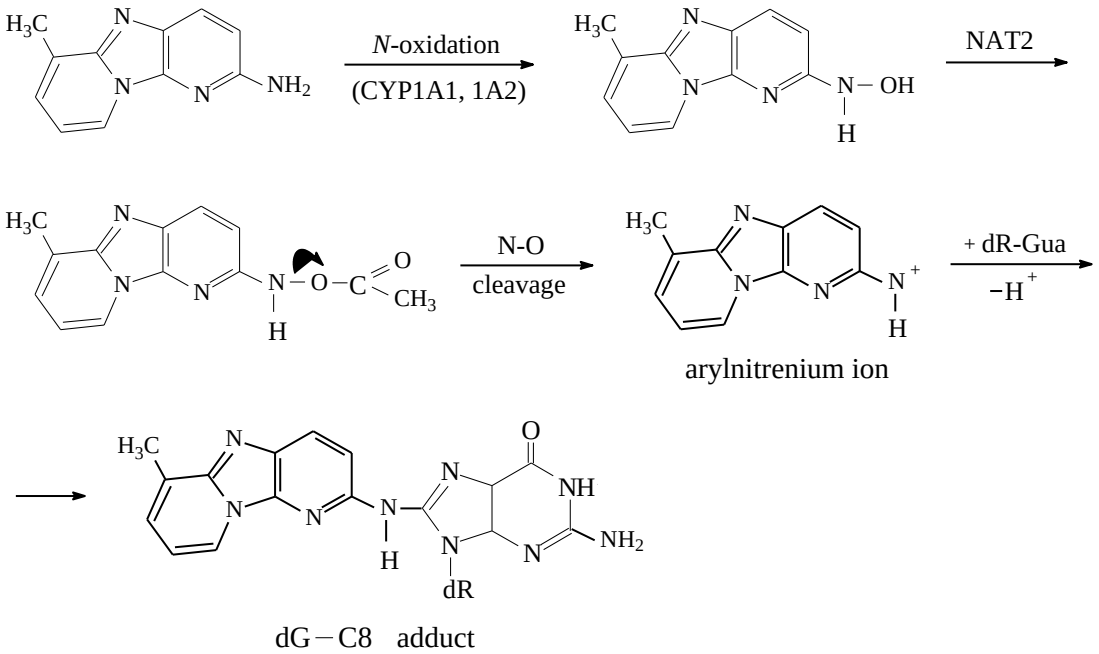
Individual profile/alert	
Name	Heterocyclic Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	 **R** and **R²** can be Csp^2(aryl) and Nsp^3(scy); **R¹** and **R³** can be Csp^2(aryl) or Nsp^2(scy) in five- or six-membered fused rings  Y = Nsp2(scy), if R1 ≠ H Y = Csp2(aryl), if R = R1 = Nsp2(scy)
Mechanism	**SE reaction (CYP450-activated heterocyclic amines)**, Direct attack of arylnitrenium cation to the C8 position of nucleoside base **SR reaction (peroxidase-activated heterocyclic amines)**, Direct attack of arylnitrenium radical to the C8 position of nucleoside base **Radical mechanism**, ROS generation and direct attack of hydroxyl radical to the C8 position of nucleoside base **AN2**, Nucleophilic addition to pyridonimine tautomer of aminopyridoindoles or aminopyridoimidazoles (hypothesized)

- SE reaction (CYP450-activated heterocyclic amines), Direct attack of arylnitrenium cation to the C8 position of nucleoside base

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

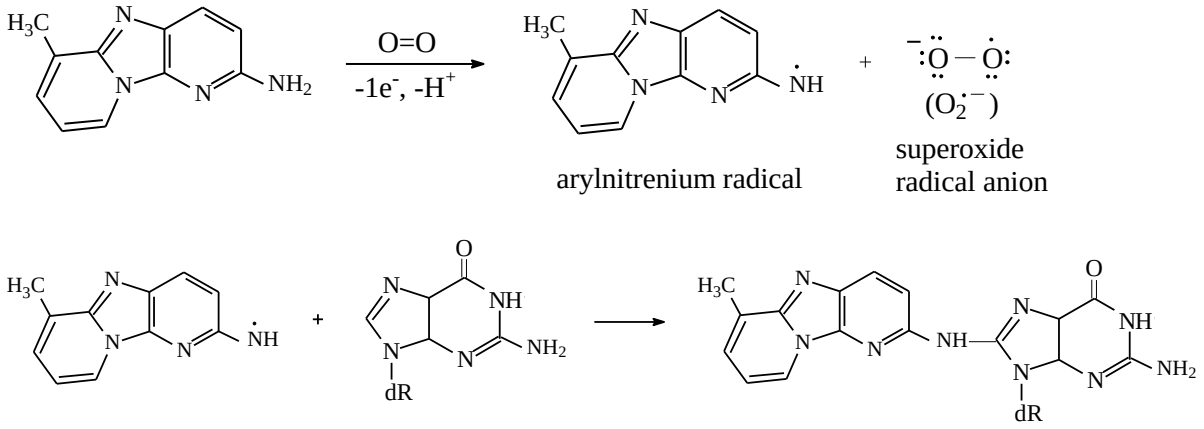
The major DNA adducts formed by activated HCAs in vivo and in vitro have been identified as N-(deoxyguanosyl-8-yl)-HCA (dG-C8) adducts and 5-(deoxyguanosyl-N2-yl)-HCA (dG-N2) adducts

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



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

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

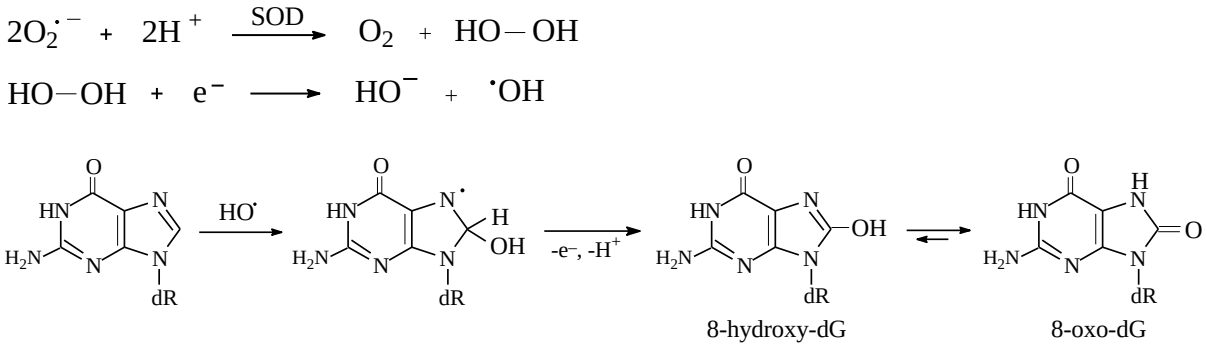


N-Hydroxy intermediates do not appear to be involved in the metabolism by peroxidases including prostaglandin H synthase [2]. Thus, the N-centered free radicals of HCAs are believed to play a crucial role in an extrahepatic pathway proving that this metabolic pathway proceeds via a one-electron mechanism.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

reduced by GSH, while, in turn, glutathione was oxidized to form a thiyl radical.

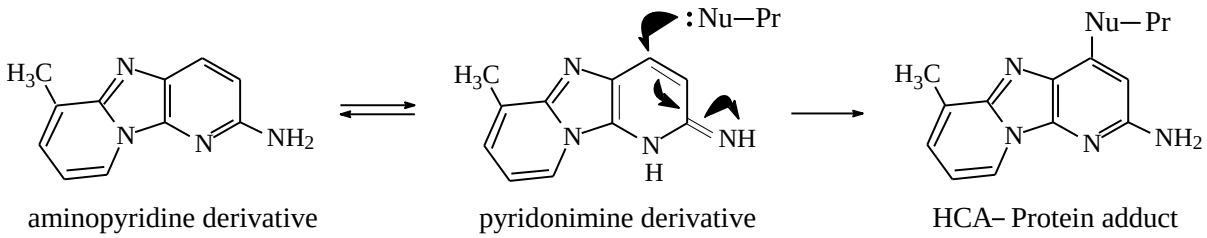
- Radical mechanism, ROS generation and direct attack of hydroxyl radical to the C8 position of nucleoside base

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



- AN2, Nucleophilic addition to pyridonimine tautomer of aminopyridoindoles or aminopyridoimidazoles (hypothesized)

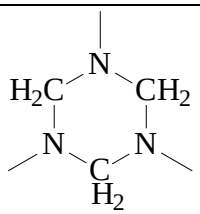
The reactivity of 2- and 4-aminopyridoindoles or pyridoimidazoles could also be explained by the possibility such compounds to form two tautomeric forms, the first one (aminopyridine form) being more stable. The second form, pyridonimine tautomer, may act as a Michael acceptor and can bind to protein nucleophiles according to Scheme 4.

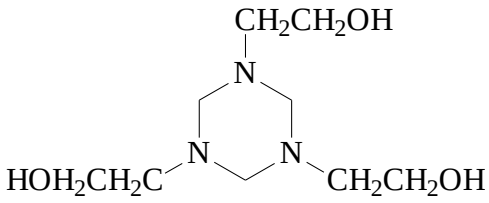


Set of chemicals used for profile development	Heterocyclic Aromatic Amines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the

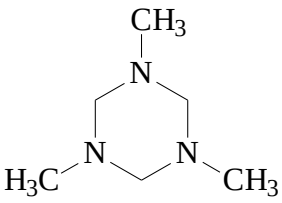
	references listed below.
References	1. H.A.J. Schut, E.G. Snyderwine, DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. *Carcinogenesis*, **1999**, 20(3), 353-368. 2. R.J. Turesky, L.L. Marchand, Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. *Chem. Res. Toxicol*., **2011**, 24(8), 1169-1214. 3. H. Frederiksen, Two food-borne heterocyclic amines: Metabolism and DNA adduct formation of amino-*α*-carbolines. *Mol. Nutr. Food Res*., **2005**, 49(3), 263-273. 4. Y.F. Sasaki, H. Yamada, K. Shimoi, N. Kinae, I. Tomita, H. Matsumura, T. Ohta, Y. Shirasu, Enhancing effects of heterocyclic amines and *beta*-carbolines on the induction of chromosome aberrations in cultured mammalian cells. *Mutat. Res.*, **1992**, 269(1), 79-95. 5. M. Ishidate, Jr, S. Odashima, Chromosome tests with 134 compounds on Chinese hamster cells *in vitro* - a screening for chemical carcinogens. *Mutat. Res.*, **1977**, 48(3-4), 337-353. 6. M. Ishidate, Jr, K.F. Miura, T. Sofuni, Chromosome aberration assays in genetic toxicology testing *in vitro*. *Mutat. Res.*, **1998**, 404(1-2), 167-172. 7. Y. Yanagawa, M. Sawada, T. Deguchi, F.J. Gonzalez, T. Kamataki, Stable expression of human CYP1A2 and *N*-acetyltransferases in Chinese hamster CHL cells: Mutagenic action of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline. *Cancer Res*., **1994**, 34(13), 3422-3427. 8. L.H. Thompson, R.W. Wu, J.S. Felton, Genetically modified Chinese hamster ovary (CHO) cells for studying the genotoxicity of heterocyclic amines from cooked foods. *Toxicol. Lett*., **1995**, 82-83, 883-889. 9. R.J. Turesky, S.C. Rossi, D.H. Welti, J.O. Lay Jr, F.F. Kadlubar, Characterization of DNA adducts formed *in vitro* by reaction of *N*-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline and *N*-hydroxy-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline at the C8 and N^2 atoms of guanine. *Chem Res Toxicol*., **1992**, 5(4), 479-490. 10. Z.Z. Yang, S.F Qi, D.X. Zhao, L.D. Gong, Insight into mechanism of formation of C8 adducts in

	carcinogenic reactions of arylnitrenium ions with purine nucleosides. *J. Phys. Chem.*, **2009**, 113(1), 254-259. 11. H.J. Moonen, J.J. Briedé, J.M. van Maanen, J.C. Kleinjans, T.M. de Kok, Generation of free radicals and induction of DNA adducts by activation of heterocyclic aromatic amines via different metabolic pathways *in vitro*. *Mol. Carcinog*., **2002**, 35(4), 196-203. 12. C.J. Burrows, J.G. Muller, Oxidative nucleobase modification leading to strand scission. *Chem. Rev*., **1998**, 98(3), 1109-1151. 13. J.E. Klaunig, L.M. Kamendulis, B.A. Hocevar, Oxidative stress and oxidative damage in carcinogenesis. *Toxicol. Pathol*., **2010**, 38(1), 96-109. 14. M. Dizdaroglu, Oxidatively induced DNA damage: Mechanisms, repair and disease. *Cancer lett*., **2012**, 327(1-2), 26-47. 15. P. Møller, H. Wallin, U. Vogel, H. Aytrup, L. Risom, M.T. Hald, B. Daneshvar, L.O. Dragsted, H.E. Poulsen, S. Loft, Mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline in colon and liver of Big Blue rats: role of DNA adducts, strand breaks, DNA repair and oxidative stress. *Carcinogenesis*, **2002**, 23(8), 1379-1385.

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



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

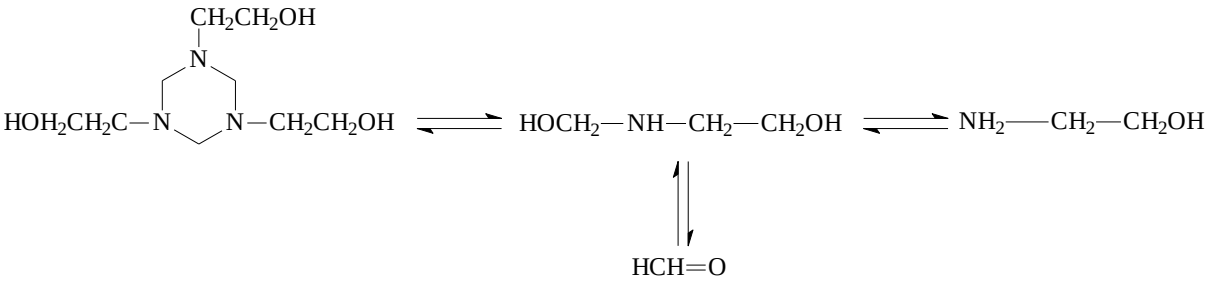


Hexahydro-1,3,5-Trimethyl-1,3,5-Triazine (CAS No. 108-74-7)

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

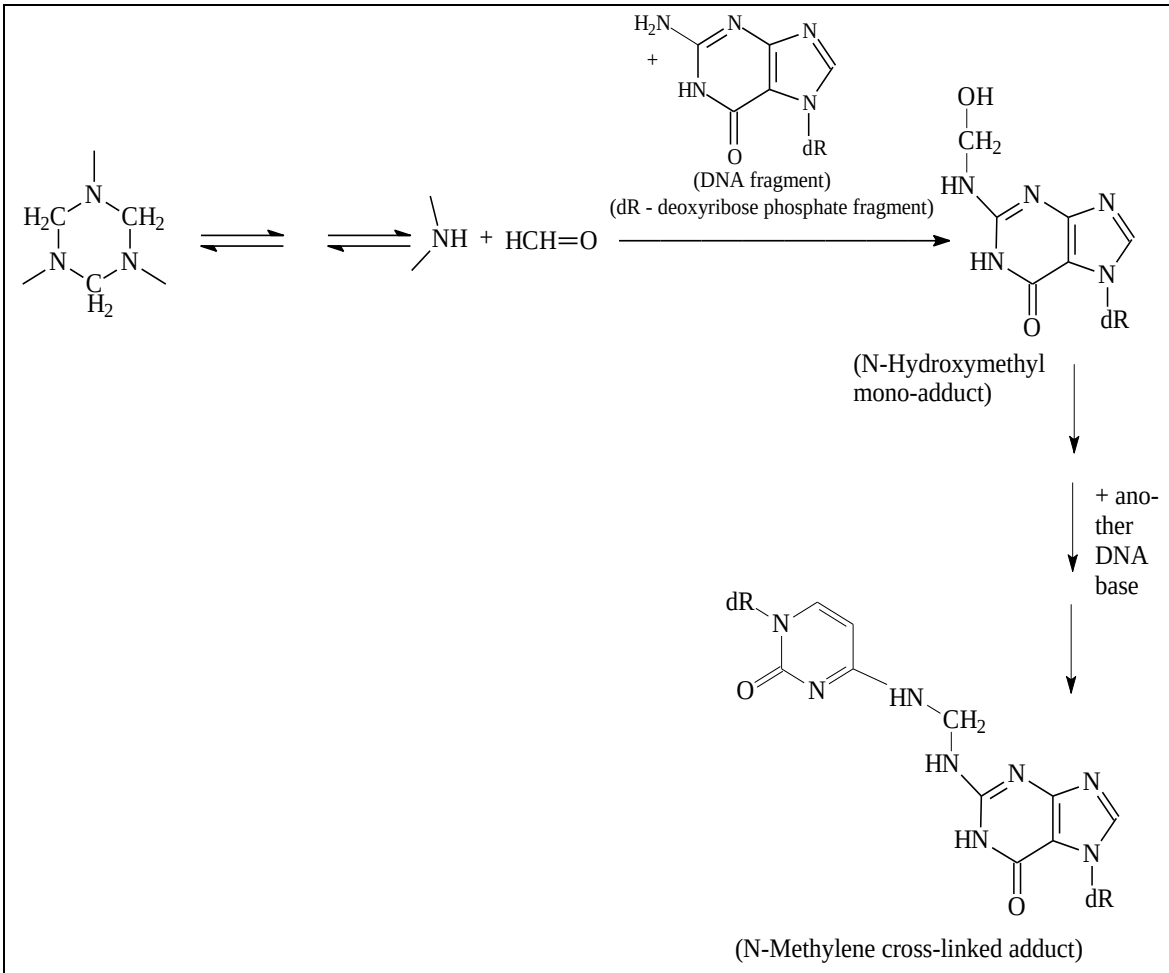
Positive results for another structurally similar chemical, hexahydro-1,3,5-trimethyl-1,3,5-triazine, have also been reported [3].

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



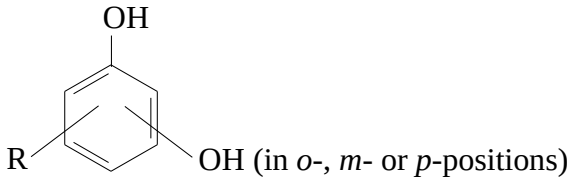
The released formaldehyde, which is genotoxic substance induces DNA-DNA and DNA-protein cross-links as the primary DNA lesions. This is related to the cytotoxicity and clastogenicity (chromosomal aberrations) [6]. Formaldehyde induces mainly N-hydroxymethyl mono-adducts on guanine, adenine and cytosine,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

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



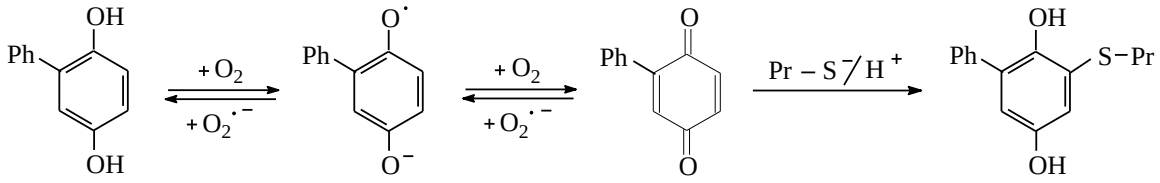
Set of chemicals used for profile development	Hexahydrotriazine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. *Reregistration Eligibility Decision (RED) for Crotan (HHT)*, US EPA, OPP, June 27, 2008. 2. Speit, G., *Genotoxicity of Formaldehyde In Vitro and In Vivo*, CEFIC 2012 (Presentation); https://www.scribd.com/document/91977532/Genotoxicity-of-Formaldehyde-in-Vitro-and-in-Vivo-by-Gunter-Speit. 3. *Hexahydro-1,3,5-Trimethyl-1,3,5-Triazine*, Exp Key Genetic Toxicity In Vitro.002; https://echa.europa.eu/registration-dossier/-/registered-dossier/13293/7/7/2/?documentUUID=8822835a-905f-46dc-9489-09c35a57989b 4. Bakke, J. M., J. Buhaug, J. Riha, *Hydrolysis of 1,3,5-Tris(2-Hydroxyethyl)Hexahydro-s-Triazine and Its Reaction with H_2S*, Ind. Eng. Chem. Res. **40** (2001), 6051 – 6054.

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

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

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

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



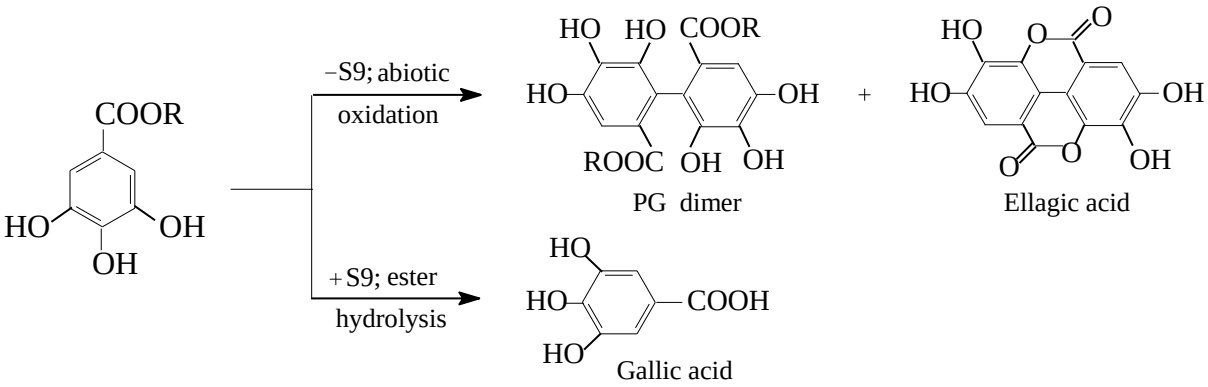
phenylhydroquinone — phenylsemiquinone anion-radical — phenylbenzoquinone — protein-adduct formation

According to Zhao et al. [8], phenylbenzoquinone can also covalently bind to nucleophilic sites on DNA in vitro. The proposed mechanism involves nucleophilic attack of the exocyclic amine nitrogen

of deoxyquanosine in position 2 (N2) on the electrophilic quinone carbon. Different types of DNA adducts were characterized by spectral analysis [8]

Gallate esters are used as synthetic antioxidants. Despite their presumed low toxicity, many authors were reported that some of linear alkyl gallates had many adverse effects. Propyl gallate was positive in in vitro chromosomal aberration tests [10,11], and was both positive and negative in the sister-chromatid test [11]. The effects of propyl gallate on carcinogenesis and mutagenesis have been reported to be both enhancing and suppressing [12].

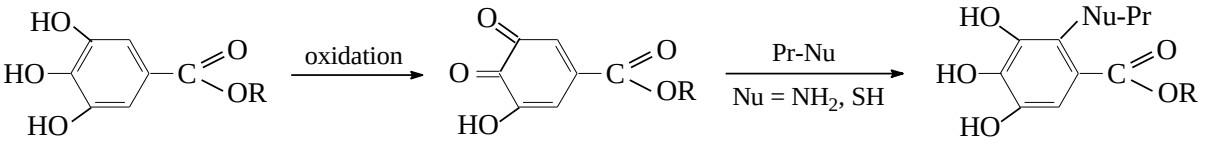
The adverse effects of gallates may be related to their antioxidizing potential. For example, when propyl gallate (PG) is autoxidized in the test media of CHO cells in the absence of metabolic activation, it is converted via a PG radical into PG dimer and finally into ellagic acid [13]. The authors established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



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

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

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

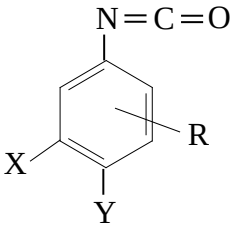


The reactivity of gallate esters toward proteins depends on the relative elongation of alkyl side-chains which determine their hydrophobicity. It was established that butyl gallate, octyl gallate and dodecyl gallate are more cytotoxic than propyl, ethyl, and methyl gallate [13].

Set of chemicals used for profile	Hydroxylated Phenols

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

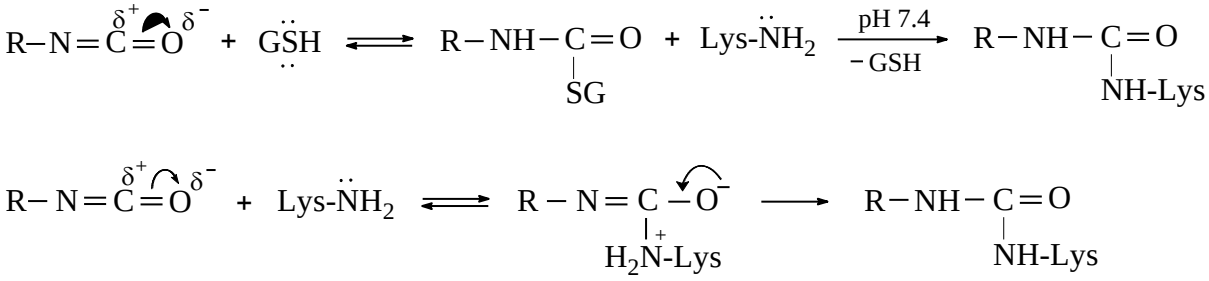
	exchange tests in Chinese hamster ovary cells *in vitro*. III. Results with 27 chemicals. *Environ. Mol. Mutagen.*, **1989**, 13(2), 133-193. 12. Tayama, S., Nakagawa, Y., Cytogenic effects of propyl gallate in CHO-K1 cells. *Mutat. Res.*, **2001**, 498(1-2), 117-127. 13. Nakagawa, Y., Tayama, S., Cytotoxicity of propyl gallate and related compounds in rat hepatocytes. *Arch. Toxicol.*, **1995**, 69(3), 204-208. 14. Patlewicz, G., Roberts, D.W., Uriarte, E., Skin sensitization: A comparison of reactivity schemes for the prediction skin sensitization potential. *Chem. Res. Toxicol.*, **2008**, 21(2), 521-541.

Individual profile/alert	
Name	Isocyanates and Diisocyanates
Type of profile	Structural alert
Description/applicability domain	Isocyanates are regarded as chemicals containing one, two or more isocyanate (-N=C=O) functional groups. They can be classified as aliphatic (acyclic or alicyclic) isocyanates and the aromatic isocyanates. The main structures of aliphatic and aromatic iso- and diisocyanates can be presented as follows: $(Csp^3)n-N=C=O$ where n = 1–4 at Csp^3 (acyclic) and n = 5–7 at Csp^3 (alicyclic). Arene iso- and diisocyanates structure for the compounds with positive effect in the chromosomal aberration test without metabolic activation is given below:  R = H, Csp3(acy); X = H, Csp2(aryl), N = C = O group; Y = H, Csp2(aryl).
Mechanism	Acylation, Acyl transfer via nucleophilic addition reaction
The electrophilic isocyanate moiety of iso- and diisocyanates is capable of undergoing nucleophilic addition with a variety of active hydrogen species including amines, alcohols, phenols and thiols [2]. The isocyanates can also react with water to form amines. In addition, the isocyanates may react	

reversibly with sulfhydryl groups of GSH, and, in the presence of appropriate nucleophiles, GSH-isocyanate adducts could react further to yield transcarbamoylating products [8]. These reversible thiocarbamates might shelter iso- and diisocyanates from hydrolysis thereby allowing further penetration into the body and increasing toxicity and allergenicity under physiological conditions [10]. The preferred reaction of isocyanates with GSH and their subsequent transfer to nucleophilic sites of peptides and proteins is favored under physiological conditions, i.e. at pH ~ 7.2-7.4. It is well known that carbamoylated organic isocyanates are stable at acidic pH conditions but not at physiological pH [9].

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

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



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

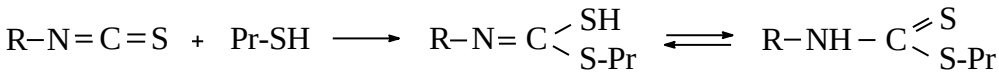
Set of chemicals used for profile development	Isocyanates and Diisocyanates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. A. Pronk, L. Preller, M. Raulf-Heimsoth, I.C.L. Lonkers, J.-W.

Lammers, I.M. Wouters, G. Doekes, A.V. Wisnewski, D. Heederik, Respiratory symptoms, sensitization, and exposure-response relationships in spray painters exposed to isocyanates. *Am. J. Respir. Crit. Care Med*., **2007**, 176(11), 1090-1097.

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

Individual profile/alert	
Name	Isothiocyanates
Type of profile	Structural alert
Description/applicability domain	R—N=C=S where R can be Csp3 (alkyl, cycloalkyl), Csp2 (aryl), excluding vinyl group.
Mechanism	Acylation, Acyl transfer via nucleophilic addition reaction

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

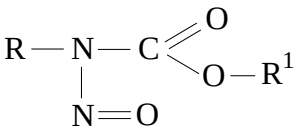


protein thiocarbamoylation

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

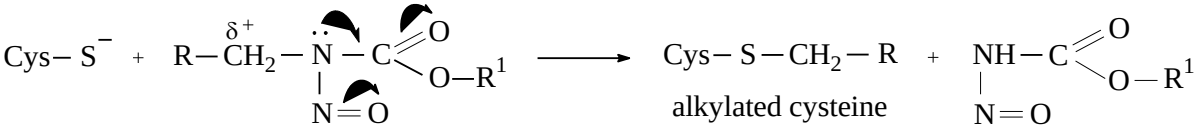
Set of chemicals used for profile development	Isothiocyanates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. F. Kassie, B. Pool-Zobel, W. Parzefall, S. Knasmuller, Genotoxic effects of benzyl isothiocyanate, a natural chemopreventive agent. *Mutagenesis*, **1999**, 14(6), 595-603. 2. B.E. Cavell, S.S. Syed Alwi, A. Donlevy, G. Packham, Anti-angiogenic effects of dietary isothiocyanates: Mechanisms of action and implications for human health. *Biochem. Pharmacol*., **2011**, 81(3), 327-336. 3. Y. Peng, C. Bao-An, L. De-Long, Anticancer mechanisms and researchers of isothiocyanates. *Chin. J. Nat. Med*., **2008**, 6(5), 325-332. 4. C. Fimognary, E. Turrini, L. Ferruzzi, M. Lenzi, P. Hrelia, Natural isothiocyanates: Genotoxic potential *versus*

	chemoprevention. *Mutat. Res*., **2012**, 750(2), 107-131. 5. F. Kassie, S. Knasmuller, Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC). *Chem. Biol. Interact*., **2000**, 127(2), 173-180. 6. F. Kassie, B. Laky, E. Nobis, M. Kundi, S. Knasmuller, Genotoxic effects of methyl isothiocyanate. *Mutat. Res*., **2001**, 490(10, 1-9. 7. L. Mi, Z. Xiao, T.D. Veenstra, F.L. Chung, Proteomic identification of binding targets of isothiocyanates: A perspective on techniques. *J. Proteomics*, **2011**, 74(7), 1036-1044. 8. Y. Zhang, E.C. Callaway, High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate. *Biochem. J*., **2002**, 364(Pt 1), 301-307. 9. K.K. Brown, M.B. Hampton, Biological targets of isothiocyanates. *Biochim. Biophys. Acta*, **2011**, 1810(9), 888-894.

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

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

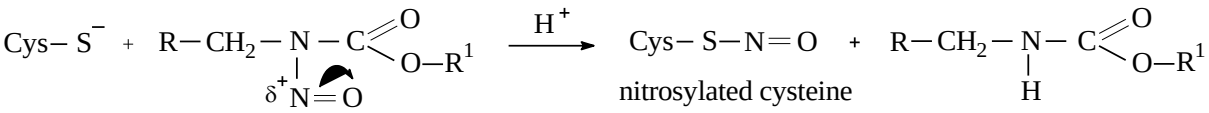
N-Nitrosocarbamates were found to react with cysteine and glutathione at room temperature and at neutral pH [7]. There appear to be different possibilities for the reaction of protein thiolate ion with N-alkyl-N-nitrosocarbamates [1,8]. Such a possibility is associated with direct attack of the cysteine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



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

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

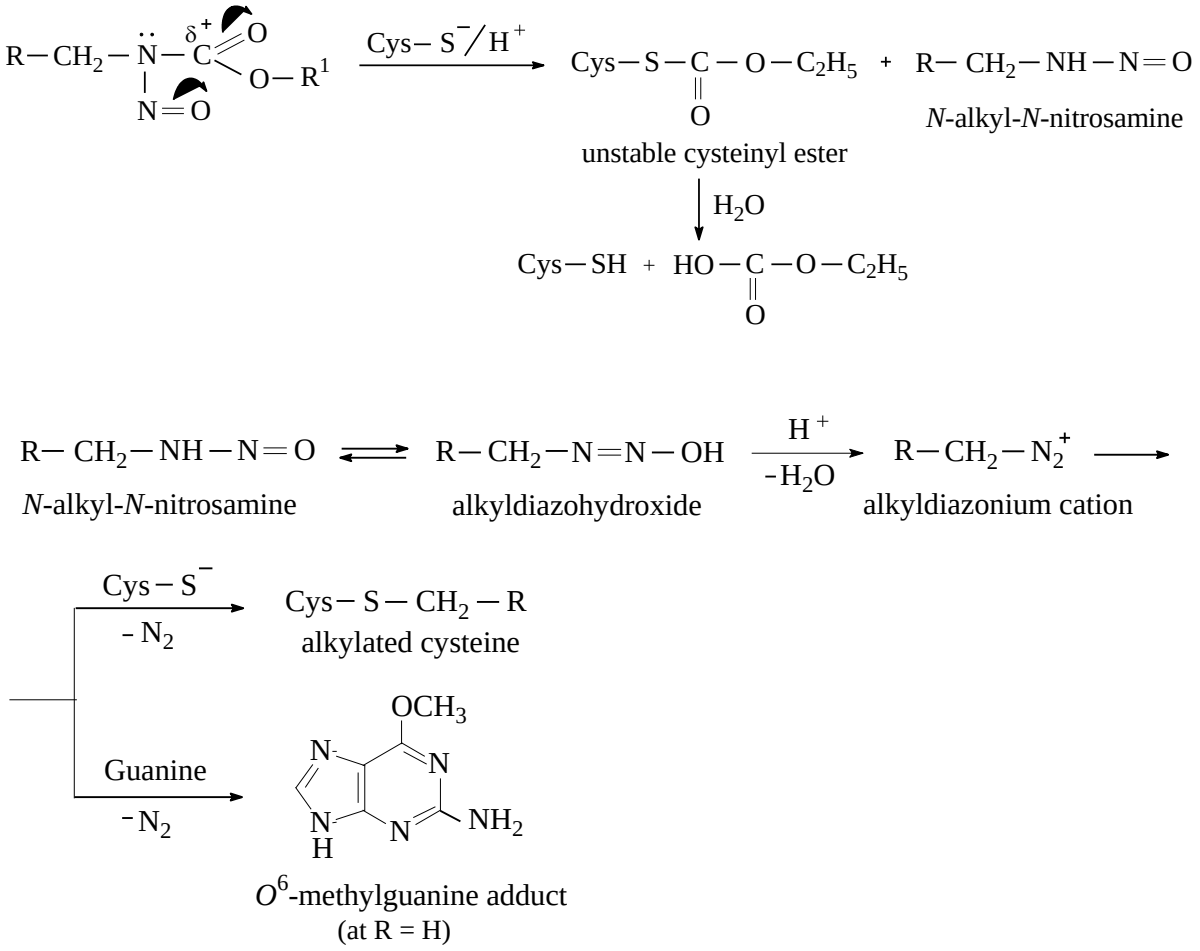
Another pathway involves nucleophilic attack of cysteine thiolate ion at the nitrogen of the nitroso group, leading to nitrosylated protein [1,8]. Thus, N-nitrosocarbamates can be used as N=O donors for the formation of S-nitrosothiols [1] (Scheme 2):



- SN1 and SN2, DNA and protein alkylation via direct attack at carbonyl carbon atom and the formation of alkyldiazonium ion

N-Alkyl-N-Nitrosocarbamates are potent direct acting mutagens and carcinogens and can be considered as SN1 and SN2 type alkylating agent [4,5]. Results from chromosome aberration and hprt gene mutation indicated the O6-methylguanine adduct (O6-MeG) is the major mutagenic base derivative formed in DNA on exposure of cells to DNA methylating agents. It is generally accepted that alkylation-induced cell killing is largely attributable to apoptosis and the O6-MeG acts as a trigger of this toxic response [5].

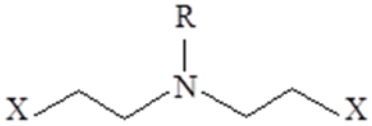
According to Schoental and Rive [7], in the presence of ionizable free thiol groups, N-alkyl-N-nitrosocarbamates decompose rapidly at neutral pH even in the dark, with evolution of nitrogen gas. The products formed with cysteine comprised the alkyl ester of substituted cysteine and N-alkyl-N-nitrosamine. The alkyl ester of cysteine was rather unstable and hydrolysed on standing at room temperature, even at neutral pH [7]. N-Alkyl-N-nitrosamine undergoes tautomerization to the corresponding alkyldiazohydroxide, which is able to form an alkyldiazonium ion as an ultimate electrophilic alkylating agent [8]. The corresponding reaction tranformations are shown in Scheme 3.



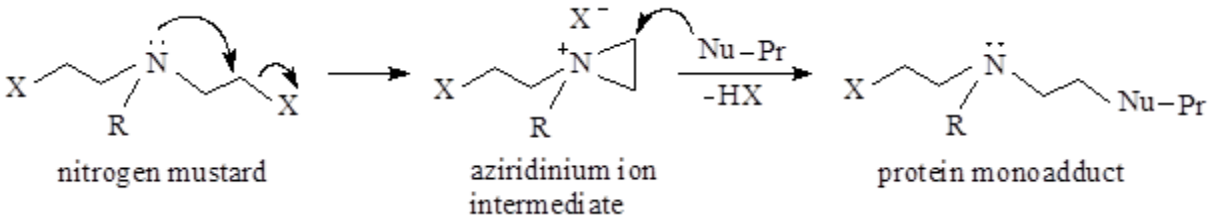
N-Alkyl-N-nitrosocarbamates as methylating agents are potent carcinogens that are mutagenic and cytotoxic towards bacteria and mammalian cells [2,5,9]. Their effects can be ascribed to an ability to modify DNA covalently.

Set of chemicals used for profile development	N-Alkyl-N-nitrosocarbamates
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Cai, T., Janczuk, A.J., Nitric oxide donors: chemical activities and biological applications. *Chem. Rev.*, **2002**, 102(4), 1091-1134. 2. Wang, T.C., Chiou, C.M., Chang, Y.L., Genetic toxicity of *N*-methylcarbamate insecticides and their *N*-nitroso derivatives. *Mutagenesis*, **1998**, 13(4), 405-408. 3. Lin, C.M., Wei, L.Y., Wang, T.C., The delayed genotoxic effect of *N*-nitroso *N*-propoxur insecticide

	in mammalian cells. *Food Chem. Toxicol.*, **2007**, 45(6), 928-934. 4. Wang, T.C., Chiou, J.M., Chang, Y.L., Hu, M.C., Genotoxicity of propoxur and its *N*-nitroso derivative in mammalian cells. *Carcinogenesis*, **1998**, 19(4), 623-629. 5. Yoon, J.Y., Oh, S.H., Yoo, S.M., Lee, S.J., Lee, H.S., Choi, S.J., Moon, C.K., Lee, B.H., N-Nitrosocarbofuran, but not carbofuran, induces apoptosis and cell cycle arrest in CHL cells. *Toxicology*, **2001**, 169(2), 153-161. 6. Eya, B.K., Talcott, R.E., Effect of *N*-alkyl chain length on the mutagenicity of *N*-nitrosated 1-naphthyl *N*-alkylcarbamates. *Environ. Mutagen.*, **1980**, 2(3), 395-404. 7. Schoental, R., Rive, D.J., Interaction of N-alkyl-N-nitrosourethanes with thiols. *Biochem. J.*, **1965**, 97(2), 466-474. 8. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol.*, **2007**, 20(1), 44-60. 9. Bignami, M., O'Driscoll, M., Aquilina, G., Karran, P., Unmusking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.*, **2000**, 462(2-3), 71-82.

Individual profile/alert	
Name	Nitrogen mustards
Type of profile	Structural alert
Description/applicability domain	 X = Cl, Br, I; R = any atom/group
Mechanism	SN2, Nucleophilic ring opening on aziridinium ion intermediate of N-mustards
N-mustards are typical alkylating agents. In aqueous conditions they spontaneously form reactive aziridinium ion that can covalently bind to nucleophilic sites within proteins and other biomolecules. The initial formation of a reactive aziridinium intermediate is followed by covalent binding to protein-	

nucleophilic sites, such as cysteine thiols, lysine amines and histidine groups. A ring opening SN2-mechanism is shown in Scheme 1 [3,4].

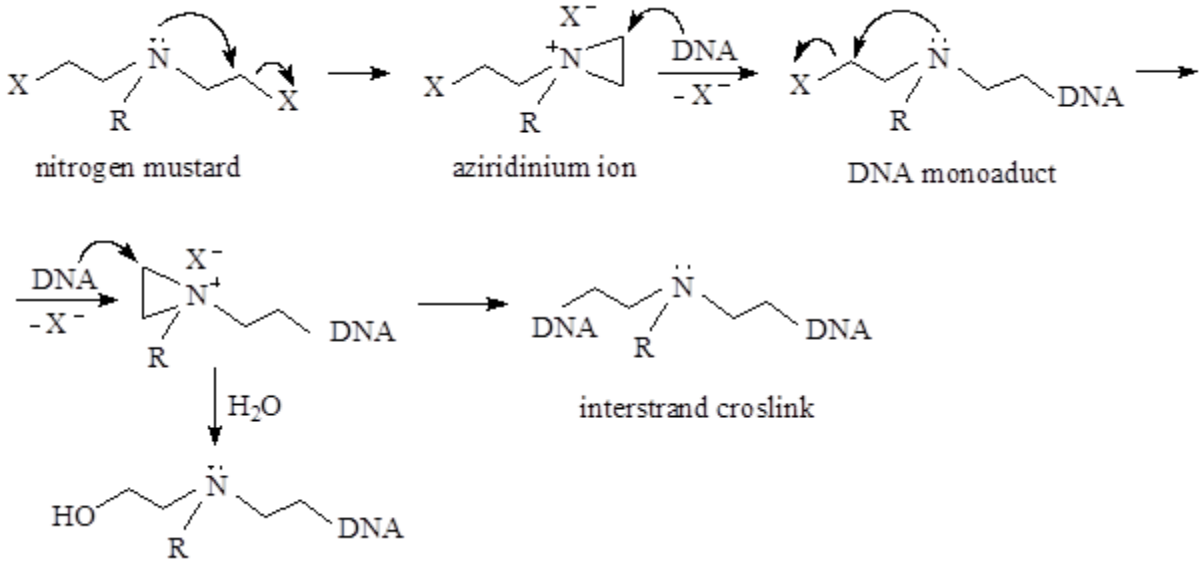


Nu = -SH (Cysteine residue), -NH_2 (Lysine residue), -NH (Histidine residue)

In the same manner N-mustard–protein monoaduct is able to bind another molecule of protein-nucleophile.

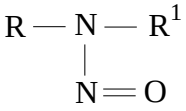
Nitrogen mustards are mutagenic in cultured mammalian cells. The aziridinium group can alkylate DNA mainly by attacking the N-7 nucleophilic center on the guanine base. The interstrand crosslinks can arise from the covalent binding of the alkylating agent to both strands of the double helix and it is considered to be the most toxic lesion. Monoalkylated adducts and intrastrand crosslinked products may be formed when the alkylating agent is smaller than the width of the minor groove of the DNA strand, while the interstrand product may be formed when the alkylating agent is longer than the width of the minor groove of the DNA strand.

Thus, the type of product that is formed depends on the nucleophiles present in the system as well as on the structure of the alkylating agent and DNA. The mechanism of DNA alkylation by N-mustards is shown in Scheme 2 [3].



Set of chemicals used for profile development	Nitrogen mustards

Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. NAC/AEGL Committee. Acute exposure guideline levels for nitrogen mustards, **2007**, Interim1:11, p. 5. 2. R. Benigni, C. Bossa, Structure alerts for carcinogenicity, and the *Salmonella* assay system: A novel insight through the chemical relational databases technology. *Mutat. Res.*, **2008**, 659(3), 248-261. 3. V.R. Thompson, A.P. DeCaprio, Covalent adduction of nitrogen mustards to model protein nucleophiles. *Chem. Res. Toxicol.*, **2013**, 26(8), 1263-1271. 4. D. Florea-Wang, Reactions of chlorambucil and its main metabolite, phenylacetic acid mustard, with 2'-deoxyribonucleosides and calf thymus DNA. PhD Thesis, University of Turku, **2009**, 401A, pp. 14, 18.

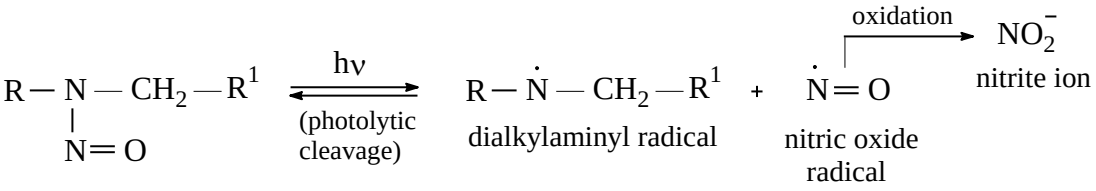
Individual profile/alert	
Name	N-Nitrosoamine derivatives
Type of profile	Structural alert
Description/applicability domain	 where: R = (Csp3)n acy at n ≥ 1; (Csp3)nacy–O– at n ≥ 1, preferably linear or branched short-chain alkyl groups with up to five carbons; (Csp3)acy–NH–; >Nacy–C(=O)–, etc. R1 = (Csp3)nacy at n ≥ 1, linear or branched short-chain alkyl groups with up to five carbons; (Csp3)nacy–O– at n ≥ 1; Csp2(aryl), such as phenyl, 1,3,5-triazinyl, etc.
Mechanism	SN1 and SN2 , DNA and protein alkylation via the formation of alkyldiazonium ion
The activation of N-nitrosoamine derivatives in the in vivo and in vitro systems is most frequently attributed to the cytochrome P450-dependent mixed function oxidases [2,7-9]. It proceeds via the	

formation of short-lived alpha-hydroxynitrosamines, which decompose into diazohydroxides and aldehydes [2]. However, the activation of nitrosamines into electrophilic or mutagenic species in cells, lacking CYP-450 activity (e.g., CHL and CHO cells), requires the action of either hydroxyl radicals or ultraviolet light [3,6,10-12].

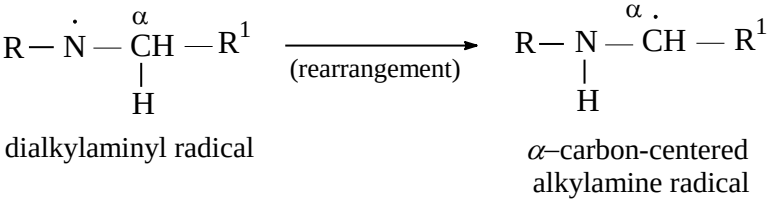
For example, the irradiation of nitrosamines with near-UVA light results in homolytic N–N(O) bond cleavage, leading to the initial formation of nitric oxide radical (˙N=O) and extremely short-lived dialkylaminyl radical (R(R1CH2)N˙) (Scheme 1a). Subsequently, carbon-centered radicals are generated by rearrangement of the initially formed aminyl radical via homolytic cleavage of the α–C-H bond or by its disproportionation involving parent N-nitrosalkylamine [7,10] (Scheme 1b). Moreover, during the photolysis of nitrosamines, superoxide anions may be formed through reduction of oxygen by alkylaminyl radicals and may thus contribute to the production of α–carbon-centered free radicals [11].

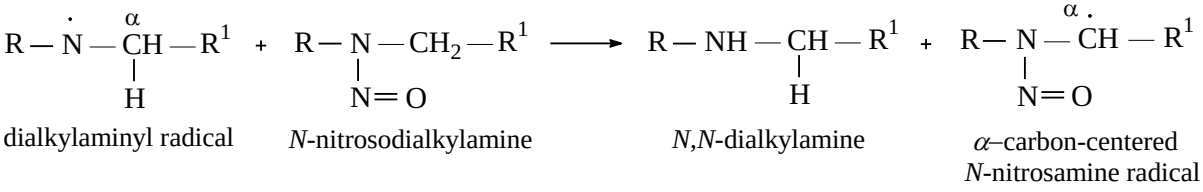
Scheme 1

a) homolytic cleavage of N–N(O) bond under the influence of ultraviolet light, sunlight, etc. and formation of a dialkylaminyl radical (the so called spontaneous denitrosation):



b) transformation of the short-lived dialkylaminyl radical to the corresponding alpha–carbon-centered alkylamine radical and alpha–carbon-centered N-nitrosamine radical:



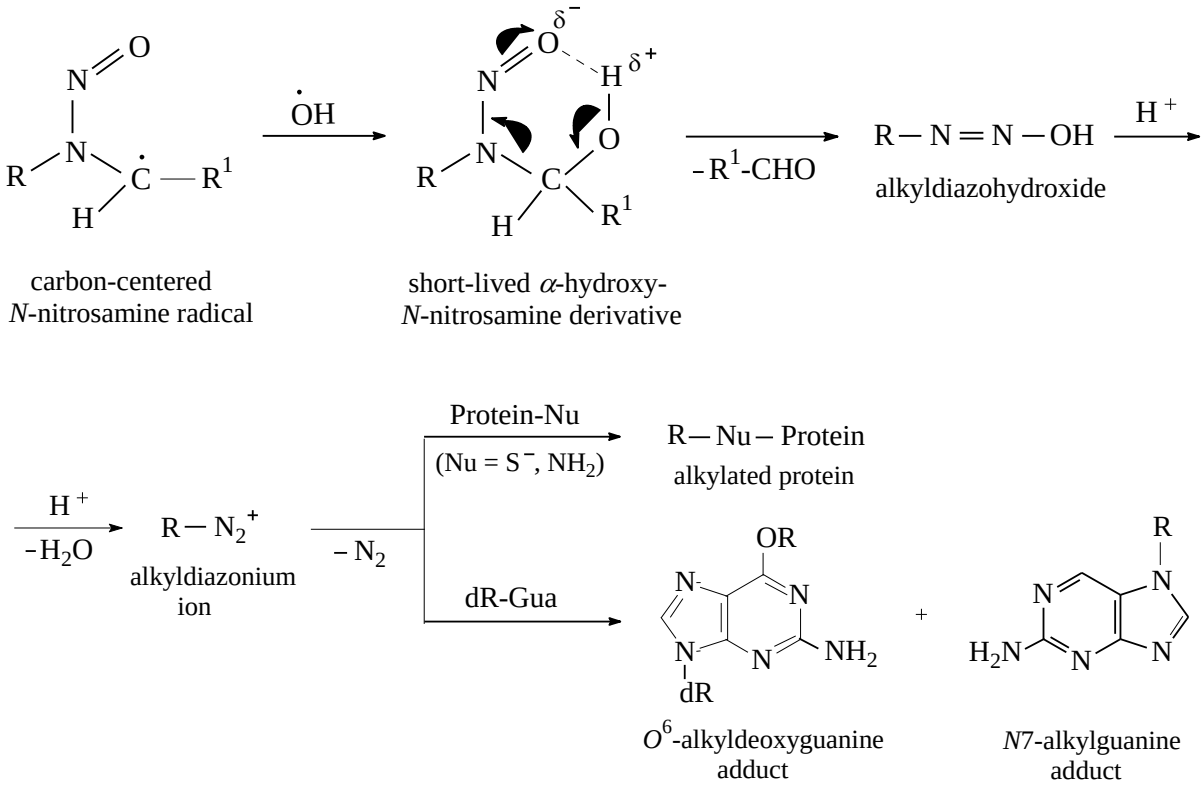


alpha–Carbon-centered N-nitrosamine radicals may undergo further transformations leading to the formation of the active alkylating species.

The rate-limiting step in the metabolism of N-nitrosamines involves the cleavage of alpha–C-H bond, followed by the interaction of alpha–carbon-centered radicals with reactive oxygen species such as ˙OH, H2O2, etc. (in the absence of CYP-450 activation). The hydroxylation usually occurs at longer

alkyl chains or at benzyl moiety. The resulting short-lived α–hydroxylated derivatives are able to decompose into alkyldiazohydroxydes and aldehydes [1,10,11]. This decomposition may be achieved by a concerted pathway or by two-step mechanism [13]. In the case of concerted mechanism the reaction is able to be enhanced by the formation of an intramolecular hydrogen bond, as shown in Scheme 2.

The active alkylating species is believed to be the alkyldiazonium ion, formed after dissociation of the alkyldiazohydroxyde [5,7,13]. The most likely mechanism is associated with direct attack of alkyldiazonium ion on the nucleophilic sites of DNA and protein molecules [1]. The results indicate that the more nucleophilic sites on the DNA (i.e., N7-position of guanine) react with primary diazonium ions via an SN2 mechanism while the less nucleophilic sites (i.e., O6-position of guanine) react via an SN1 mechanism with the secondary carbenium ions produced from decomposition and subsequent rearrangement of the primary diazonium ion [14] (Scheme 2).



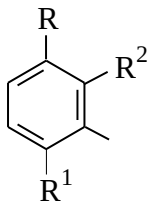
According to Arimoto-Kobayashi et al. [6], alkylation of DNA with UVA activated N-nitrosodimethylamine leads to the formation of N7-methylguanine adduct that is 40–70 times more than the respective O6-methyldeoxyguanine adduct.

Overall, N-nitrosodialkylamines can cause chromosomal aberrations on irradiation with near-UV light. Bearing in mind that the irradiation used by many authors is much weaker than that of the sunlight [3,6], positive clastogenic effects could be expected for N-nitrosodialkylamines, which are not exposed to activation by CYP-450 enzymes.

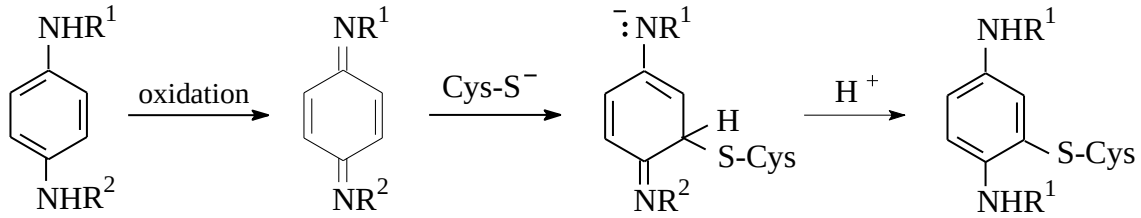
Set of chemicals used for profile	N-Nitrosoamine derivatives

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. Guttenplan, J.B., *N*-Nitrosamines: bacterial mutagenesis and in vitro metabolism. *Mutat. Res*., **1987**, 186(2), 81-134. 2. Mochizuki, M., Anjo, T., Okada, M., Isolation and characterization of *N*-alkyl-*N*-(hydroxymethyl)nitrosamines from *N*-alkyl-*N*-(hydroperoxymethyl)nitrosamines by deoxygenation. *Tetrahedron Lett*., **1980**, 21(38), 3693-3696. 3. Yamashita, Y., Sumi, N., Arimoto, S., Hayatsu H., Synergistic action of N-nitrosodialkyl-amines and near-UV in the induction of chromosome aberrations in Chinese hamster lung fibroblasts in vitro. *Mutat. Res*., **1995**, 348(4), 163-168. 4. Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Cai, T., Janczuk, A.J., Nitric oxide donors: chemical activities and biological applications. *Chem. Rev*., **2002**, 102(4), 1091-1134. 5. Liu, Y.X., Guttenplan, J.B., Mutational specificities of *N*-nitrosoamines in a host-mediated assay: comparison with direct-acting *N*-nitroso compounds in vitro and an approach to deducing the nature of ultimate mutagens in vivo. *Mol. Carcinog*., **1992**, 6(4), 232-237. 6. Arimoto-Kobayashi, S., Kaji, K., Sweetman, G.M., Hayatsu, H., Mutation and formation of methyl- and hydroxylguanine adducts in DNA caused by *N*-nitrosodimethylamine and *N*-nitrosodiethylamine with UVA irradiation. *Carcinogenesis*, **1997**, 18(12), 2429-2433. 7. Hebels, D.G., Briedé, J.J., Khampang, R., Kleinjans, J.C., de Kok, T.M., Radical mechanisms in nitrosamine- and nitrosamide-induced whole-genome gene expression modulations in Caco-2 cells. *Toxicol. Sci*., **2010**, 116(1), 194-205. 8. Singer, B., Kuśmierek, J.T., Chemical mutagenesis. *Annu. Rev. Biochem*., **1982**, 51, 655-693. 9. Yoo, J.S., Yang, C.S., Enzyme specificity in the metabolic activation of *N*-nitrosodimethylamine to a mutagen for Chinese hamster V79 cells. *Cancer Res*., **1985**, 45(11 Pt 1), 5569-5574. 10. Grover, T.A., Ramseyer, J.A., Piette, L.H., Photolysis of nitrosamines and nitrosamides at neutral pH: A spin-trap

study. *Free Radic. Biol. Med*., **1987**, 3(1), 27-32.
11. Bartsch, H., Hietanen, E., Malaveille, C., Carcinogenic nitrosamines: free radical aspects of their action. *Free Radic. Biol. Med*., **1989**, 7(6), 637-644.
12. Fujiwara, M., Honda, Y., Inoue, H., Hayatsu, H., Arimoto, S., Mutations and oxidative DNA damage in phage M13mp2 exposed to *N*-nitrosomorpholine plus near-ultraviolet light. *Carcinogenesis*, **1996**, 17(2), 213-218.
13. Andreozzi, P., Klopman, G., Hopfinger, A.J., Theoretical study of N-nitrosamines and their presumed proximate carcinogens. *Cancer Biochem. Biophys*., **1980**, 4(4), 209-220.
14. Spratt, T.E., Zydowsky, T.M., Floss, H.G., Stereochemistry of the *in vitro* and *in vivo* methylation of DNA by (*R*)- and (*S*)-*N*-[$^{2}H_{1}$,^{3}H]methyl-*N*-nitrosourea and (*R*)- and (*S*)-*N*-nitroso-*N*-[$^{2}H^{1}$,^{3}H]methyl-*N*-methylamine. *Chem. Res. Toxicol*., **1997**, 10(12), 1412-1419.

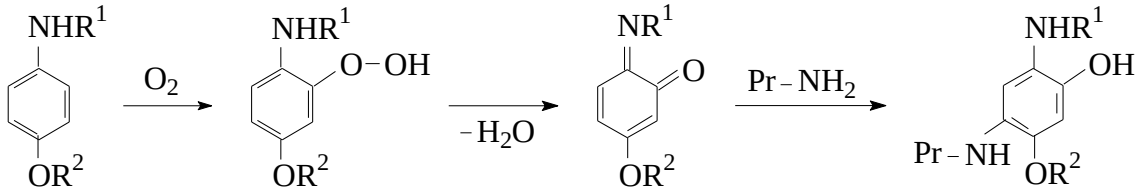
Individual profile/alert	
Name	N-Substituted Aromatic Amines
Type of profile	Structural alert
Description/applicability domain	 Where: R = NH2, NH-Csp3 (acy), NH-Csp2 (aryl) – fused or non-fused, O-Csp3 (acy); R1 = H, NH2, NH-Csp2(aryl); R2 = H, Csp2 (scy) – fused.
Mechanism	AN2, Michael addition to the quinoid type structures such as quinone-diimines, quinone-imines, etc.
N-Substituted para-phenylenediamines, alkoxyanilines and N-alkylated anilines are susceptible toward oxidation in the presence of air oxygen or peroxidases in the cellular systems [5,6]. For example, N,N'-diphenylamines can be oxidized to the corresponding electrophilic intermediates quinone-diimines. Then the quinone-diimines can bind to proteins mainly by the attack of protein-	

associated thiolate anion at a ring carbon atom (Scheme 1) [7].



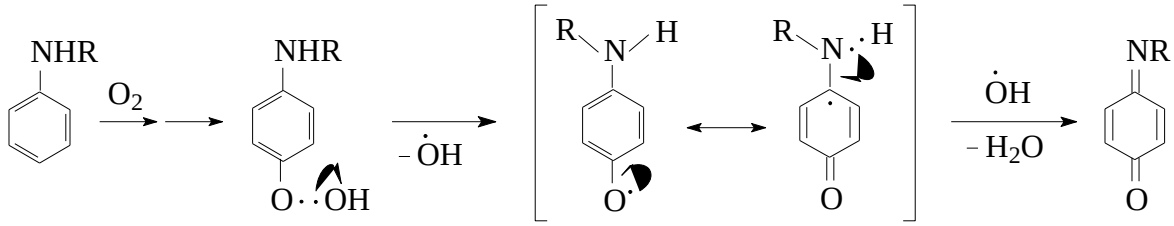
R^1 = H, Csp^3 (*iso*-propyl, 1,3-dimethylbutyl), Csp^2 (phenyl, 2-naphthyl); R^2 = H, Csp^2 (phenyl, 2-naphthyl).

On the other hand, the clastogenicity of para-alkoxyanilines could be explained by the possibility to undergo perhydroxylation which is analogous to that of meta-phenylenediamines [7]. The perhydroxylation is able to occur mainly in ortho-position toward amino group due to the strong stabilizing effect of nitrogen atom in the formation of free radicals. The ortho-quinone imine formed undergoes Michael-type addition reaction (Scheme 2).

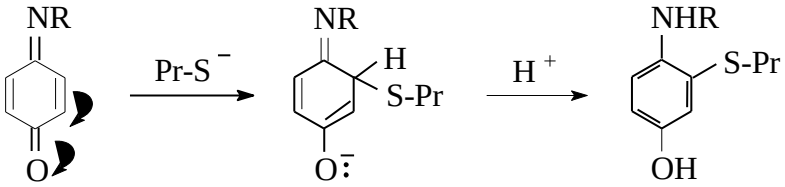


R1 = C6H5, p-C6H4OCH3; R2 = –CH3, –C2H5, –iso-C3H7, etc.

N-Alkylanilines such as N-methyl- and N-ethylaniline are susceptible to oxidation by a variety of reagents, including the oxygen in air [7-9]. The oxidation to the corresponding benzoquinone imine occurs in the ring preferably in para-position relative to the amino group. The possible mechanism is analogous to that of the oxidation of ring-alkylated anilines [8] and is associated with the preliminary formation of a phenyl hydroperoxide as shown in Scheme 3.



N-Alkylbenzoquinone imine thus obtained is able to undergo Michael-type addition reaction involving protein nucleophiles such as Pr-S⁻ or Pr-NH2 (Scheme 4).

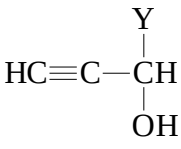


R = Csp3 (acy) - CH3, C2H5, etc.

N-Ethylaniline induced chromosomal aberrations (25.0%) after 6-h treatment without S9 mix at the highest concentration of 9.1 mM. According to Morita et al. [10], the chromosomal aberrations observed might be due to high toxicity. However, there is no supporting evidence to reduce the level of concern and the minimal concern for its clastogenicity still exists.

Set of chemicals used for profile development	N-Substituted Aromatic Amines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. T. Sofuni, A. Matsuoka, M. Sawada, M. Ishidate Jr, E. Zeiger, M.D. Shelby, A comparison of chromosome aberration induction by 25 compounds tested by two Chinese hamster cell (CHL and CHO) systems in culture. Mutat. Res., 1990, 241(2), 175-213. 2. S.M. Galloway, M.J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A.D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B.H. Margolin, M.A. Resnick, B. Anderson, E. Zeiger, Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ. Mol. Mutagen., 1987, 10 (Suppl. 10), 1-175. 3. N-Isopropyl-N'-phenyl-1,4-benzenediamine, CAS No. 101-72-4. Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257-8523, Japan. 4. N-Ethylaniline, CAS No. 103-69-5. Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257, Japan. 5. M. Uchimiya, A.T. Stone, Reversible redox chemistry of quinones: Impact on biogeochemical cycles. Chemosphere, 2009, 77(4), 451-458. 6. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, Mechanistic applicability domain classification of a Local Lymph Node Assay dataset for skin sensitization. Chem. Res. Toxicol.,

	2007, 20(7), 1019-1030. 7. A.O. Aptula, S.J Enoch, D.W. Roberts, Chemical mechanisms for skin sensitization by aromatic compounds with hydroxyl and amino groups. Chem. Res. Toxicol., 2009, 22(9), 1541-1547. 8. D.W. Roberts, G. Patlewicz, S.D. Dimitrov, L.K. Low, A.O. Aptula, P.S. Kern, G.D. Dimitrova, M.I.H. Comber, R.D. Phillips, J. Niemelä, C. Madsen, E.B. Wedebye, P.T. Bailey, O.G. Mekenyan, TIMES-SS – A mechanistic evaluation of an external validation study using reaction chemistry principles. Chem. Res. Toxicol., 2007, 20(9), 1321-1330. 9. A. Brunmark, E. Cadenas, Redox and addition chemistry of quinoid compounds and its biological implications, Free Radic. Biol. Med., 1989, 7(4), 435-477. 10. T. Morita, M. Honma, K. Morikawa, Effect of reducing the top concentration used in the in vitro chromosomal aberration test in CHL cells on the evaluation of industrial chemical genotoxicity. Mutat. Res., 2012, 741(1-2), 32-56.

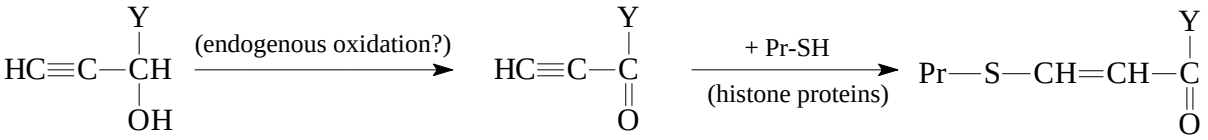
Individual profile/alert	
Name	Propargyl Alcohol Derivatives
Type of profile	Structural alert
Description/applicability domain	 (Y is -H or -$(CH_2)_nH$ (n = 1 - 3)
Mechanism	AN2, Nucleoophilic addition to alpha, beta - unsaturated carbonyl compounds

Propargyl alcohol was found to be clastogenic in vitro by inducing chromosomal aberrations in CHO cells with and without metabolic activation, while this chemical was not bacterial mutagen [1].

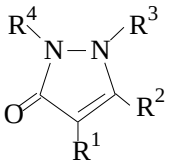
According to an evaluation report, in a chromosomal aberration (CA) test using CHO cells, cells collected 16 h following treatment with propargyl alcohol showed a small but statistically significant increase in chromosomal aberrations in the absence of metabolic activation. Although only the response at the highest dose was significantly higher than the control, the trend was positive. In the presence of exogenous metabolic activation, a larger, dose-related increase was induced. However, in

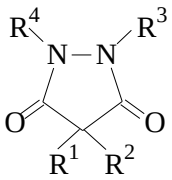
cells after 10 h of treatment, there was no increase in CAs, either with or without metabolic activation [2].

One possible (and oversimplified) mechanistic scheme for in vitro bioactivation of propargyl alcohol and other short-chain derivatives, which could elicit clastogenicity by nucleophilic interaction with histone proteins, can be expressed as follows:



Set of chemicals used for profile development	Propargyl Alcohol Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Blakey, D. H., K.aus, R. bell, J. Bayley, G. R. Douglas, E. R. Nestmann, *Mutagenic Activity of 3 Industrial Chemicals in a Battery of In Vitro and In Vivo Tests*, Mutat. Res. **320** (1994), 273 – 283. 2. *Robust Summaries and Test Plan for Propargyl Alcohol (CAS No. 107-19-7)*, Final Revised Submission, High Production Volume Chemical Challenge Program, July 22, 2009; http://www.epa.gov/HPV/pubs/summaries/propargy/c14222rt2.pdf.

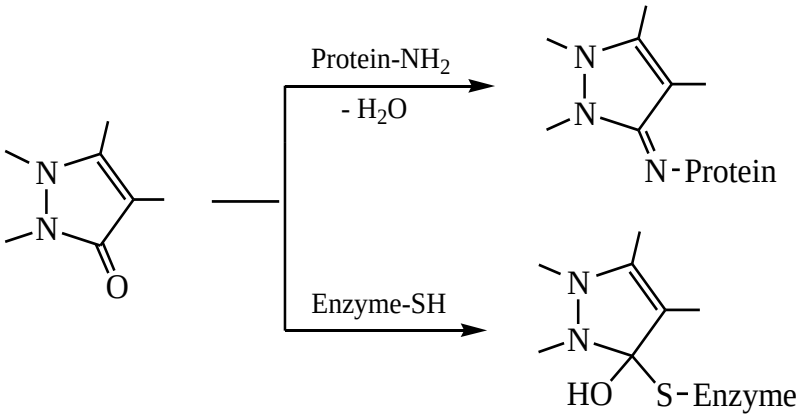
Individual profile/alert	
Name	Pyrazolone and Pyrazolidine-3,5-dione Derivatives
Type of profile	Structural alert
Description/applicability domain	**Pyrazolone derivatives**  R^1 = H, C_1-C_4 alkyl, allyl, propargyl, benzyl, dialkylamino, etc. R^2 = C_1-C_4 alkyl, benzyl, aryl, etc. R^3 = H or alkyl R^4 = C_4-alkyl, cycloalkyl, aryl, etc.

	Pyrazolidine-3,5-dione derivatives  R^1 = H, C_1-C_4 alkyl, cycloalkyl, allyl, benzyl, aryl, etc. R^2 = C_1-C_4 alkyl, allyl, benzyl, aryl, etc. R^3 = H, C_1-C_4 alkyl, acyl, carbamoyl, aryl, etc. R^4 = alkyl, cycloalkyl, aryl, etc.
Mechanism	AN2, Michael addition to activated double bonds in heterocyclic ring systems AN2, Shiff base formation with carbonyl compounds

- Shiff base formation with carbonyl compounds

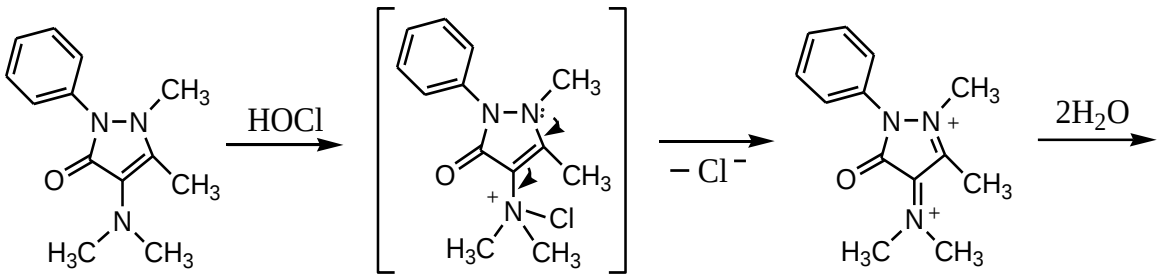
One of the reasons for the adverse effects of pyrazolone and pyrazolidine-3,5-dione derivatives should be the possibility to interact with different proteins. For example, antipyrine binds irreversibly to hepatic protein in vivo and in metabolizing liver microsomes [6], dipyrone and its metabolites bind to plasma protein [7], phenylbutazone and its hydroxylated metabolites bind covalently to plasma and human serum albumin [8,9]. All of these drugs and their metabolites contain carbonyl groups and it may be assumed that they react with the active sites of proteins or enzymes acting as Schiff base formers (Scheme 1):

Scheme 1

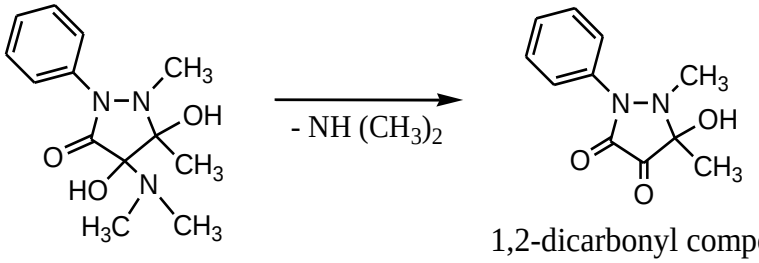


On the other hand, as a result of the bioactivation of aminopyrine derivatives, 1,2-dicarbonyl fragment can be form in the molecule under the influence of different oxidizing systems (myeloperoxidase, hydrogen peroxide, chloroperoxidase, hypochlorus acid, etc.), as it is shown in Scheme 2 [3,10].

Scheme 2



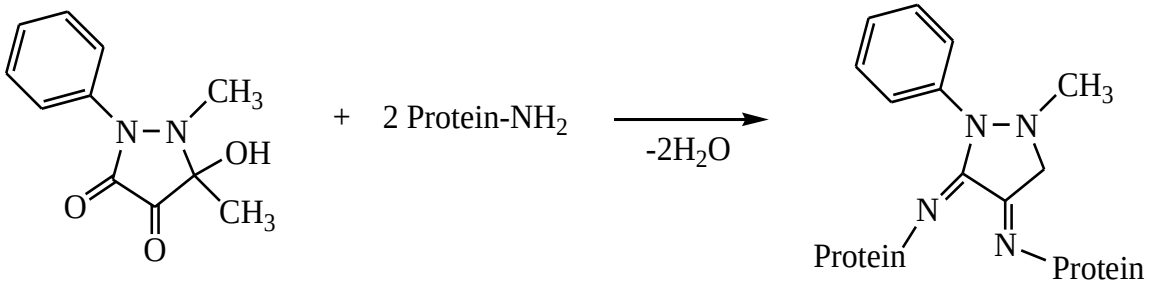
Aminopyrine *N*-chloroaminopyrine Reactive intermediate



1,2-dicarbonyl compound

The carbon atoms in 1,2-dicarbonyl compounds possess high electrophilicity because of the π,π-delocalization between two carbonyl groups. The following reaction scheme may be proposed as a result of the interaction with protein amines (Scheme 3).

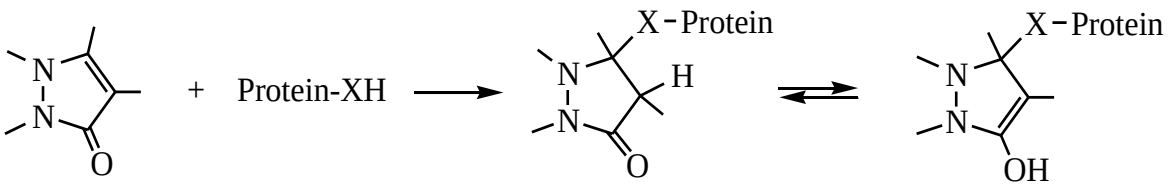
Scheme 3



- Michael addition to activated double bonds in heterocyclic ring systems

Pyrazolone derivatives may also undergo the Michael type addition reaction represented in Scheme 4.

Scheme 4

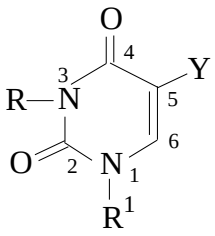
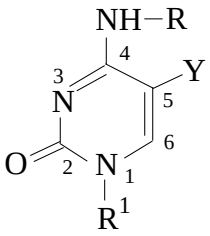
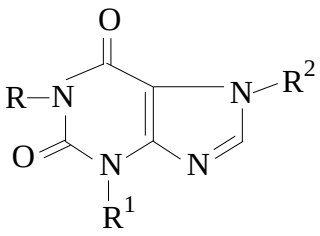
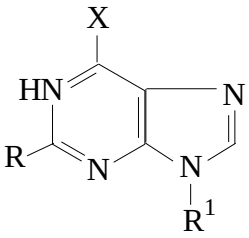


(X = S, NH, O)

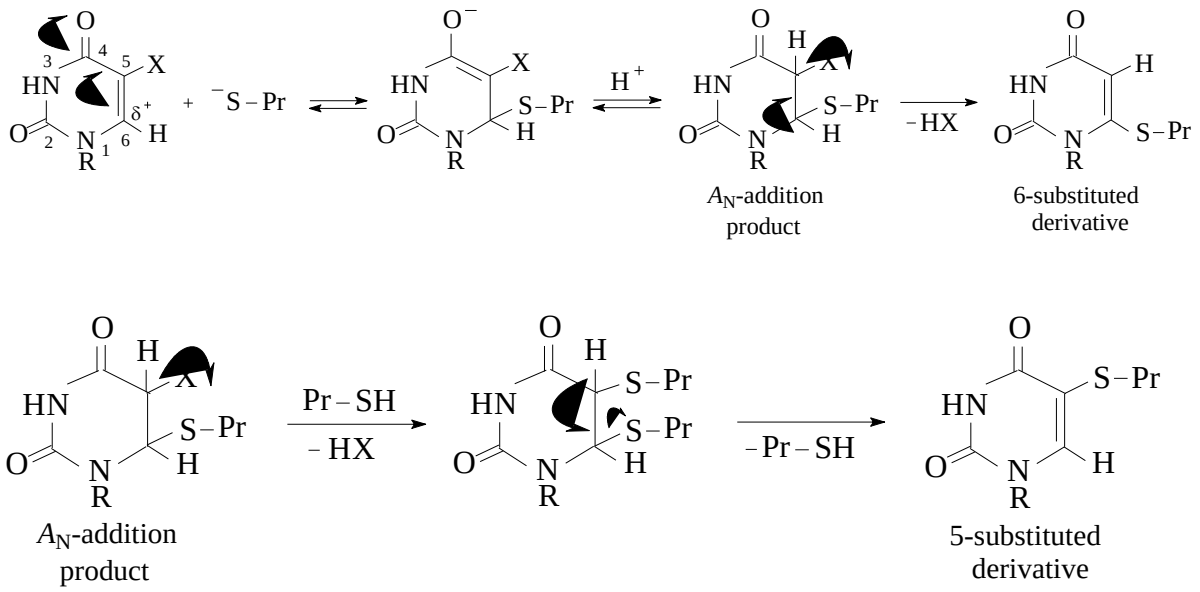
Thus, the pyrazolone derivatives can bind covalently to proteins and enzymes both by Schiff base

formation and/or Michael type addition reactions.	
Set of chemicals used for profile development	Pyrazolone and Pyrazolidine-3,5-dione 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. A.K. Giri, A. Mukhopadhyay, *Mutat. Res.*, **1998**, 420, 15-25. 2. R.N. Brogden, *Drugs*, **1986**, 32, 60-70. 3. A.S. Kalgutkar, D.K. Dalvie, J.P. O'Donnell, T.J. Taylor, D.C. Sahakian, *Current Drug Metab*., **2002**, 3, 379-424. 4. Y. Bentur, O. Cohen, *J. Toxicol. Clin. Toxicol*., **2004**, 42, 261-265. 5. S. Mao, S. Yang, D. Bi, *Biol. Pharm. Bull*., **2006**, 29, 1355-1359. 6. S. Tarabelli-Poplawski, H. Uehleke, *Naunyn-Schmiedeberg's Arch. Pharmacol*., **1977**, 297, 105-110. 7. Zylber-Katz, L. Granit, M. Levy, *Eur. J. Clin. Pharmacol*., **1985**, 29, 67-71. 8. W. Dieterle, J.W. Faigle, F. Fruh, H. Mory, W. Theoblad, K.O. Alt, W.J. Richter, *Arzneimittelforschung*, **1976**, 26, 572-577. 9. F. Chignell, *Mol. Pharmacol*., **1969**, 5, 244-252. 10. J.P. Uetrecht, H.M. Ma, E. MacKnight, R. McClelland, *Chem. Res. Toxicol*., **1995**, 8, 226-233.

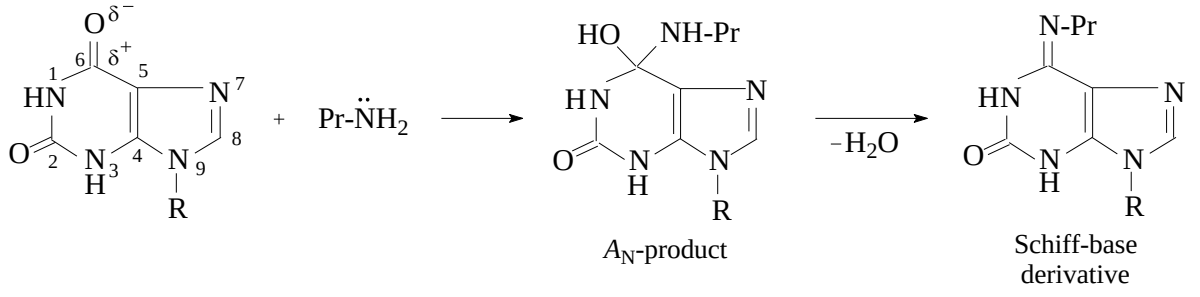
Individual profile/alert	
Name	Pyrimidines and Purines
Type of profile	Structural alert
Description/applicability domain	Pyrimidine derivatives, that are able to cause chromosomal aberrations in in vitro assays are presented with the following general structures:

	Structure 1  Structure 2  Structure 1: R = H or Csp3(scy), such as tetrahydrofuran-2-yl residue; R1 = H or Csp3(scy) such as tetrahydrofuran-2-yl residue, deoxyribose or ribose moieties, which are bound with nitrogen atom in position 2 of the five-membered heterocyclic ring; Y = F or Cl atoms. Structure 2: R = H or C(=O)-Csp3(acy) R1 = ribose or deoxyribose moieties; Y = H or Csp3(acy) short chains (CH3, C2H5). Purines, that possess clastogenic activity, may be presented with the following structures: Structure 1  Structure 2  Structure 1: R = R1 = CH3; R2 = H, CH3. Structure 2: X = OH, SH; R = H, NH2; R1 = H or ribose moiety, bound with nitrogen atom in position 2 of the five-membered heterocyclic ring.
Mechanism	AN2, Michael-type addition reaction; Schiff base formation
Genotoxicity of pyrimidines and purines were studied by the reverse mutation assay in bacteria and the chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells. The chemicals such as fluorouracil, tegafur, caffeine, theophylline, 4-amino-1-pentofuranosyl-2(1H)-pyrimidinone, enocitabine, 6-mercaptopurine, disodium 5'-guanylate were found to induce chromosomal aberration in in vitro assay in CHL cells without metabolic activation [1,2].	

Good leaving groups such as fluorine and chlorine atoms, attached at position 5 of pyrimidine ring may undergo elimination or, in the presence of the excess of nucleophile, subsequent substitution. As a result, a mixture of 6- and 5-substituted derivatives was obtained, as shown in Scheme 1 [3,4].

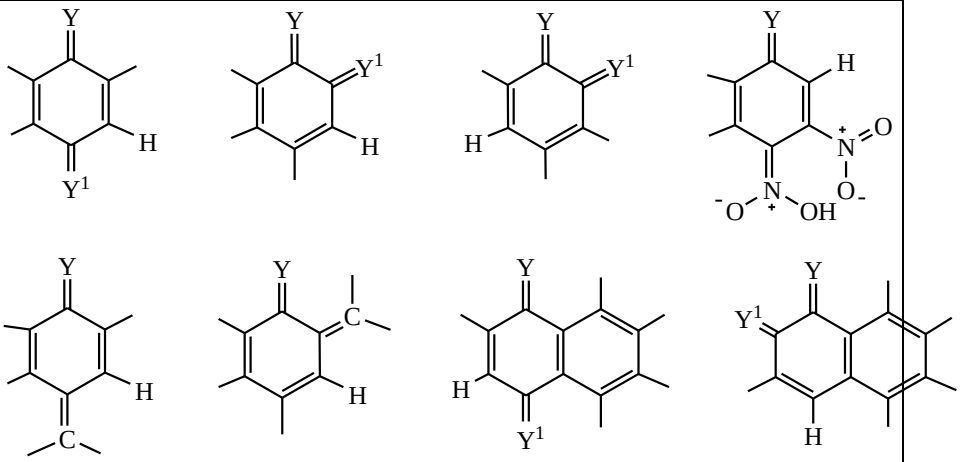


The carbon-oxygen double bonds at C-2 and/or C-6 positions in the purine derivatives and at C-2 and/or C-4 positions in the pyrimidines can also undergo nucleophilic addition reactions [4,5]. The initially formed product could be stable under appropriate reaction medium or may spontaneously undergo an elimination reaction, especially dehydration, leading to the Schiff base formation (Scheme 2).



Set of chemicals used for profile development	Pyrimidines and Purines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Yajima, N., K. Kondo, K. Morita, Reverse mutation tests in *Salmonella typhimurium* and chromosomal aberration tests in mammalian cells in culture on fluorinated pyrimidine derivatives. *Mutat Res.*, **1981**, 88(3), 241-254. 2. Ishidate, M. Jr., M.C. Harnois, T. Sofuni, A comparative analysis of data on the clastogenicity of 951 chemical substances tested in

	mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 3. Boncel, S., A. Gondela, K. Walczak, Uracil as a target for nucleophilic and electrophilic reagents. *Curr. Org. Synth.*, **2008**, 5(4), 365-396. 4. Hermanson, G.T., Bioconjugate Techniques, 2nd ed., Elsevier Inc., **2008**, pp. 55-59. 5. Shabarova, Z.A., A.A. Bogdanov, Advanced Organic Chemistry of Nucleic Acids, 2nd ed., John Wiley & Sons, **2008**, pp. 44-47, 393-394.

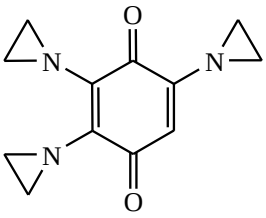
Individual profile/alert	
Name	Quinoid compounds
Type of profile	Structural alert
Description/applicability domain	 **Classification:** ➢ *ortho*- and *para*-Quinones, quinoneimines and quinonedimines: Y and Y^1 = O, N ➢ 1,4- and 1,2-Naphthoquinones, naphthoquinoneimines and naphthoquinonedimines: Y and Y^1 = O, N ➢ *ortho*- and *para*-Quinone methides and quinoneimine methides: Y = O, N and Y^1 = Csp^2 {acy, scy} ➢ *para*-Quinoid oximes: Y = O, N and Y^1 = N-OH ➢ *ortho*- and *para*-Nitroquinones and nitroquinoneimines: Y = O, N
Mechanism	AN2, Michael-type addition, quinoid structures Radical mechanism , ROS generation
<u>Direct formation of covalent adducts with chromosomal proteins</u> Quinones as the most representative sub-class of quinoid compounds constitute an important class of	

naturally occurring chemicals found in plants, fungi and bacteria [7]. Quinones are Michael acceptors, and modifications of cellular processes could occur through alkylation of crucial cellular proteins and/or DNA [3].

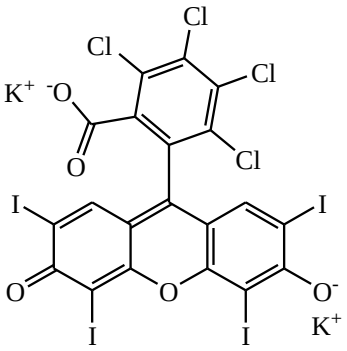
As "soft" electrophiles, quinones are particularly susceptible to conjugation via Michael addition reactions with cellular nucleophiles such as glutathione as well as cysteine and lysine residues in proteins [3,8]. These reactions can lead to loss or disruption of protein function [8] and induction of structural and numerical chromosomal aberration in mammalian cells [9].

It was suggested that chromosomal aberrations in cells might affect chromosomal translocations by inhibition of topoisomerase II [10]. For instance, para-benzoquinone at micromolar concentrations causes concentration-dependent inhibition of topoisomerase II activity, probably by interaction with the essential thiol groups of topoisomerase II [10,11].

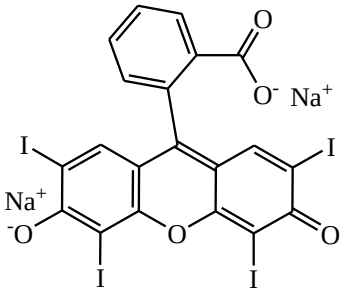
Some compounds with quinone or quinone methide fragment in their molecular structures such as as Trenimon, Rose Bengal and Erythrosine were reported to cause in vitro chromosomal aberrations in mammalian cells when tested without metabolic activation [12]:



Trenimon



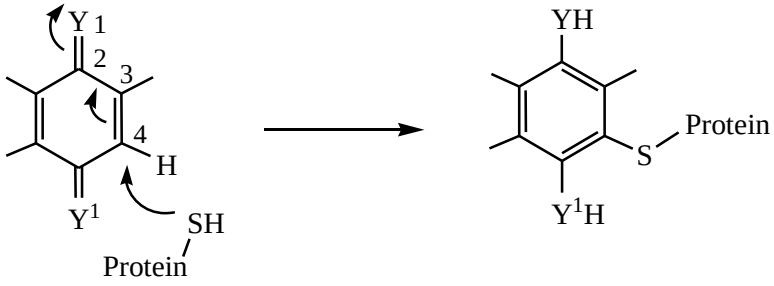
Bengal rose



Erythrosine

The proposed reaction schemes for formation of protein adducts between quinone-type compounds and cysteine and lysine residues in proteins are shown below:

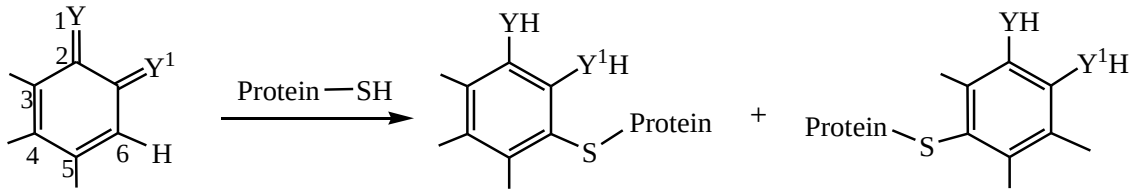
- 1,4-Michael addition reaction and formation of covalent bonded protein adduct with *para*-quinone type compounds



para-Quinones

Protein adduct

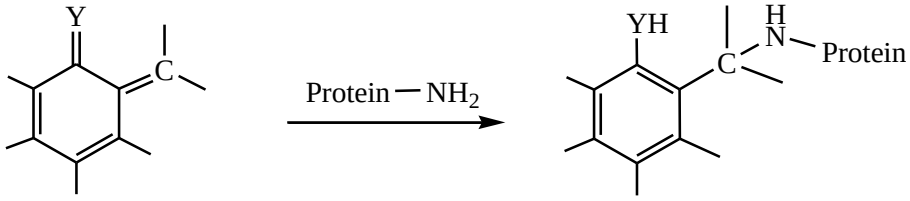
- 1,4- and 1,6-Michael addition reactions and formation of covalent bonded protein adducts with *ortho*-quinone type compounds



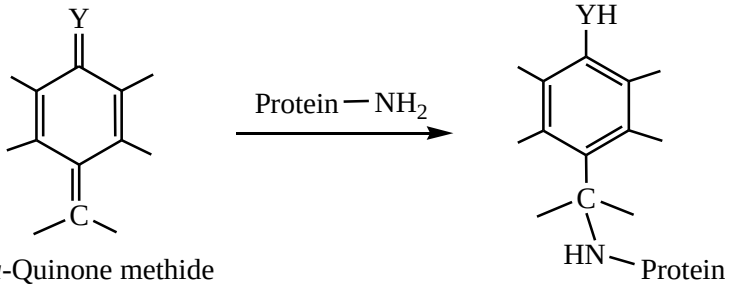
ortho-Quinones Major protein adduct Minor protein adduct

These reactions can lead to loss or disruption of protein function [8] and induction of structural and numerical chromosomal aberration in mammalian cells [9].

Moreover, *ortho*- and *para*- quinone methides interacting as Michael acceptors can form benzylic-type adducts when binding thiol and amino groups in proteins to their exocyclic methylene fragments, according to the following schemes [13,14]:



ortho-Quinone methide Protein adduct



para-Quinone methide

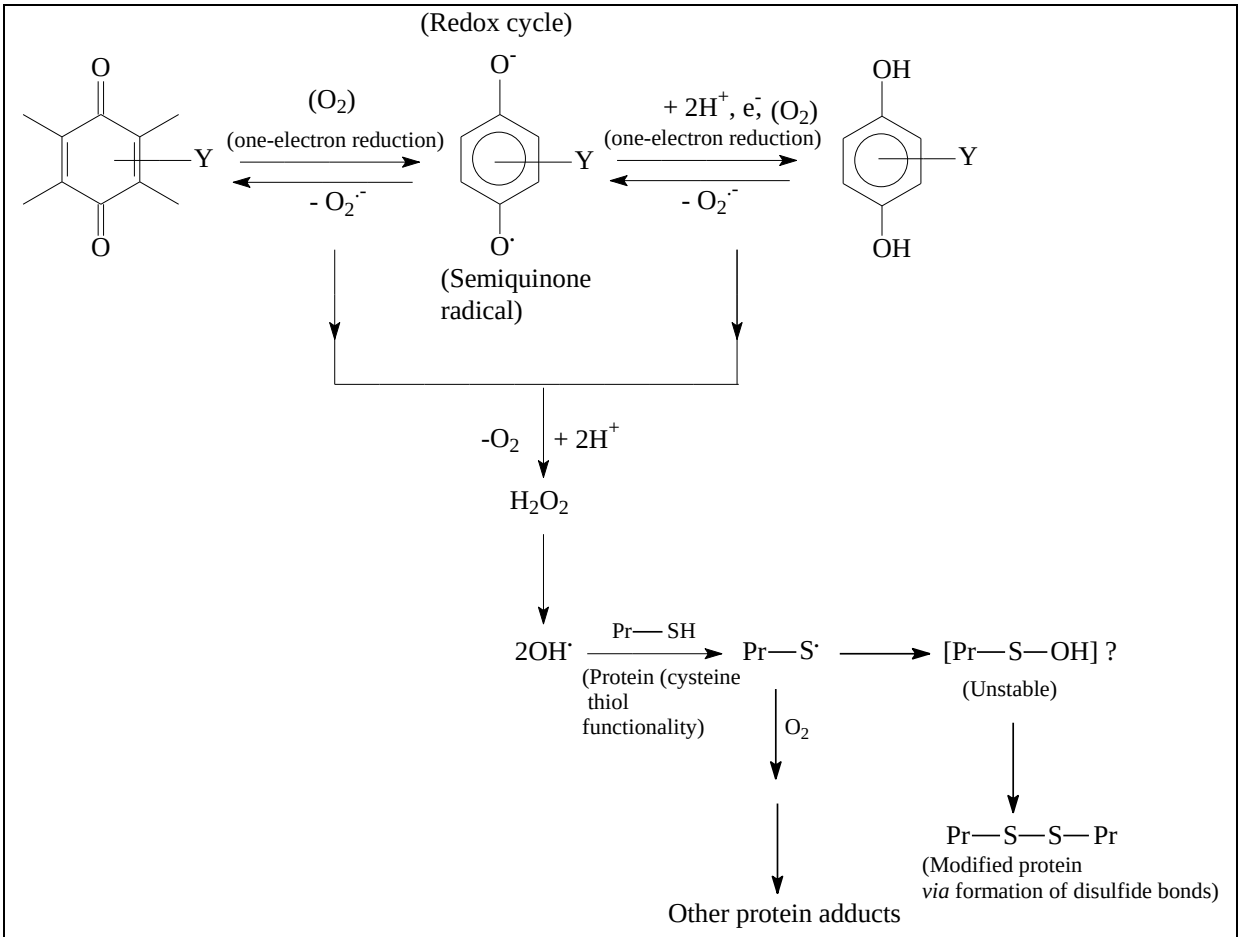
Protein adduct

The conversion of thiol groups to S-benzyl derivatives may alter the redox status of cells, but the susceptibility of these species to nucleophilic displacement reactions suggests that the relatively stable N-benzyl adducts may be of more toxicological importance [13].

Oxidative stress

Chemical interactions between thiol-containing compounds and reactive oxygen species (ROS) play a central role in the oxidation-reduction balance in the living cell. Experiments in aqueous solutions have indicated that the various thiol compounds (cysteine, cysteamine, glutathione, captopril, N-acetylcysteine, etc.) are very efficiently oxidized by hydroxyl radical (HO.) generated as ROS [15].

Not only cysteine residues in proteins are affected. Scheme I below provides an example of suggested mechanistic pathway of generation of ROS such as hydroxyl radical from quinones, with the subsequent modification of the cysteine thiol functionalities in proteins [3-5]:



Set of chemicals used for profile development	Quinoid compounds
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis*, **1987**, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. *Chem. Res. Toxicol*., **2017**, 30(1), 376-387. 3. Bolton, J.L., Dunlap, T., Formation and biological targets of quinones: cytotoxic versus cytoprotective effects. *Chem. Res. Toxicol.*, **2017**, 30(1), 13-37. 4. Yu, D., Berlin, J.A., Penning, T.M., Field, J., Reactive oxygen species generated by PAH *o*-quinones cause change-in-function mutations in *p*53. *Chem. Res. Toxicol*., **2002**, 15(6), 832-842. 5. Kovacic, P., Jacintho, J.D., Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. *Curr. Med. Chem*., **2001**, 8(7), 773-796. 6. do Céu Silva, M., Gaspar, J., Duarte Silva, I., Leão, D., Rueff, J., Mechanisms of induction of chromosomal aberrations by

hydroquinone in V79 cells. *Mutagenesis*, **2003**, 18(6), 491-496.
7. Monks, T.J., Jones, D.C., The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. *Curr. Drug Metab.*, **2002**, 3(4), 425-438.
8. Penning, T.M., Genotoxicity of *ortho*-quinones: reactive oxygen species *versus* covalent modification. *Toxicol. Res.*, **2017**, 6(6), 740-754.
9. Turchi, G., Glatt, H.R., Seidel, A., Puliti A, Sbrana I. Structure-activity relationship in the induction of chromosomal aberrations and spindle disturbances in Chinese hamster epithelial liver cells by regioisomeric phenanthrene quinones. *Cell Biol. Toxicol.*, **1997**, 13(3), 155-165.
10. Hutt, A.M., Kalf, G.F., Inhibition of human DNA topoisomerase II by hydroquinone and *p*-benzoquinone, reactive metabolites of benzene. *Environ. Health Perspect.*, **1996**, 104 Suppl. 6, 1265-1269.
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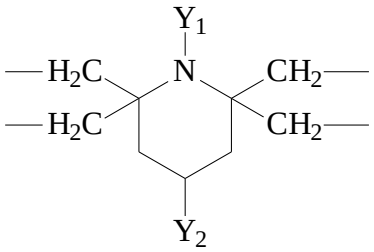
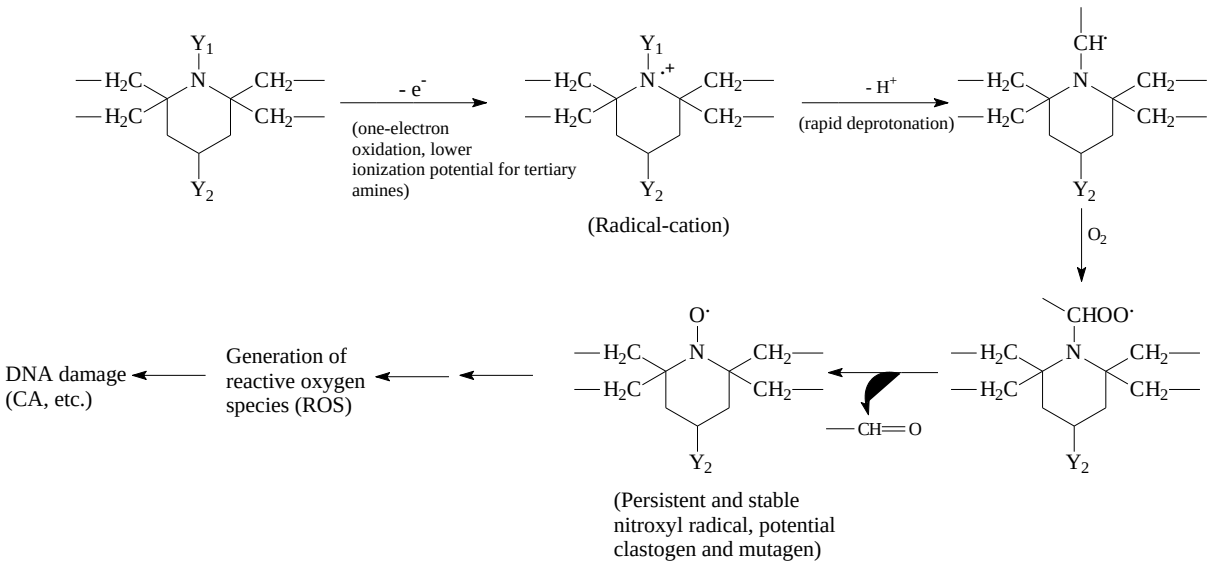
Individual profile/alert	
Name	Sterically Hindered Piperidine Derivatives
Type of profile	Structural alert

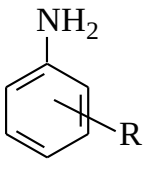
Description/applicability domain	 (Y_1 is -H or short-chain radicals such as $-CH_3$, $-CH_2CH_3$ or $-CH_2CH_2OH$ Y_2 is -H, -OH or -O-C{sp3})
Mechanism	Radical mechanism, ROS generation

Tertiary sterically hindered amines such as 2,2,6,6-tetramethyl-substituted piperidines are easily oxidized by electron transfer to cation-radicals. These cation-radicals have been directly observed in non-polar medium by optical spectroscopy. In the presence of oxygen, the amine-derived radicals are oxidized to nitroxyl radicals. The probable mechanistic scheme of generation of active radical intermediate(s) for the sterically hindered piperidine derivatives, which is also associated with generation of reactive oxygen species (ROS) and in vitro chromosomal aberrations can be outlined as follows [6]:



It is known that 2,2,6,6-Tetramethylpiperidine-1-oxyl (Tempol) is stable nitroxyl-type free radical. Tempol is mutagenic in the in vitro mouse lymphoma assay (MLA) and induces micronuclei in TK6 cells. Oxidative stress may account for part of genotoxicity induced by Tempol in both cell lines and resulting in large genetic alterations, including chromosomal breakage [7]. Stable, membrane-permeating nitroxyl radicals possess antioxidant activity in various experimental systems, however, in parallel, they are considered as possible harmful oxidants. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is simultaneously anti-genotoxic agent but, also, cytotoxic and clastogenic chemical, depending on the concentration applied. Nitroxyl-type radicals are mutagenic in Salmonella typhimurium at concentrations > 50 mM and can aggravate the mutagenic effects of H2O2 in Salmonella typhimurium strain TA104, which is sensitive to oxidative DNA damage. Thus the mutagenic action of nitroxides is assumed to be due to their pro-oxidant properties, and to be mediated by compounds formed upon oxidation of glutathione by nitroxides. The oxidative shift in

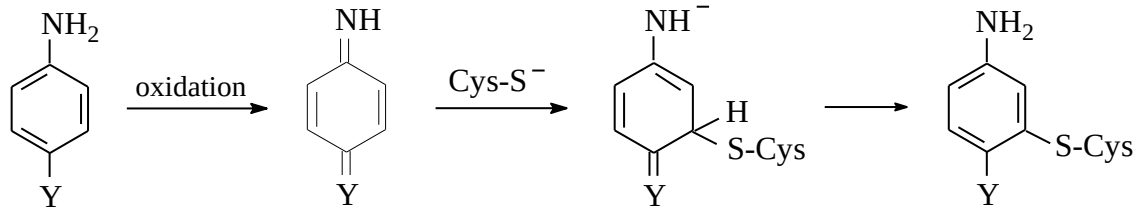
the GSH/GSSG ratio induced by nitroxides, which affects the redox homeostasis in the cell may be also a factor favoring mutagenesis [8].	
Set of chemicals used for profile development	Sterically Hindered Piperidine Derivatives
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Allen, N. S., J. F. McKellar, D. Wislon, Photo-Stabilisation of Commercial Polypropylene by Piperidine Compounds: The Role of Stable Free Radicals, Polym. Degrad. Stability 1(3) (1979), 205 – 215. 2. 4-Hydroxy-2,2,6,6-Tetramethylpiperidine-1-Ethanol, Exp Key Genetic Toxicity in vitro.003, ECHA CHEM 3. VP Sanduvor PR-31, Full Public Report, National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (September 1997); 4. *2,2',6,6'-Tetramethylpiperidin-4-ol CAS No. 2403-88-5*, SIDS Initial Assessment Report for SIAM 14, OECD SIDS, (26 – 28th March 2002); 5. Brede, O., D. Beckert, C. Windolph, H. A. Gottinger, *One-Electron Oxidation of Sterically Hindrede Amines to Nitroxyl Radicals: Intermediate Amine Radical Cations, Aminyl, α-Aminoalkyl, and Aminylperoxyl Radicals*, J. Phys. Chem. A **102** (1998), 1457 – 1464. 6. Guo, X., R. A. Mittelstaedt, L. Guo, J. G. Shaddock, R. H. Heflich, A. H. Bigger, M. M. Moore, N. Mei, *Nitroxide TEMPO: A Genotoxic and Oxidative Stress Inducer in Cultured Cells*, Toxicol. In Vitro **27** (2013), 1496 – 1502. 7. Lewinska, A., M. Wnuk, E. Slota, Gr. Bartosz, *The Nitroxide Antioxidant Tempol Affects metal-Induced Cyto- and genotoxicity in Human Lymphocytes In Vitro*, Mutat. Res. **649** (2008), 7 – 14.

Individual profile/alert	
Name	Substituted Anilines
Type of profile	Structural alert
Description/applicability domain	 (R can be located in *o*-, *m*- or *p*-positions)

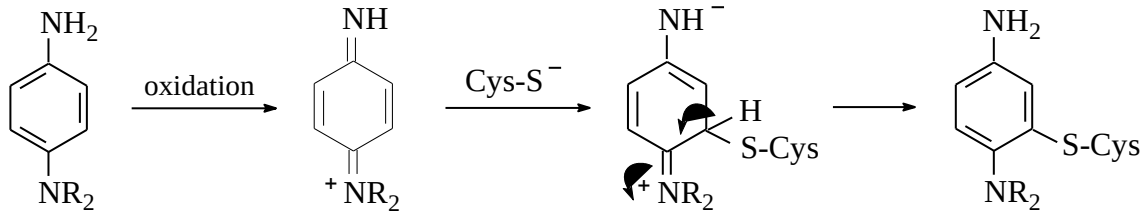
	R = Csp^3 (acy), OH, $OCsp^3$ (acy), Csp^3-Csp^2 (aryl), NH_2, $N(Csp^3)_2$, C(=O)- Csp^3, etc.
Mechanism	AN2, Michael addition to the quinoid type structures such as quinone-imines, quinone-diimines, etc.

I. Abiotic and biotic oxidation of para- and ortho-substituted anilines

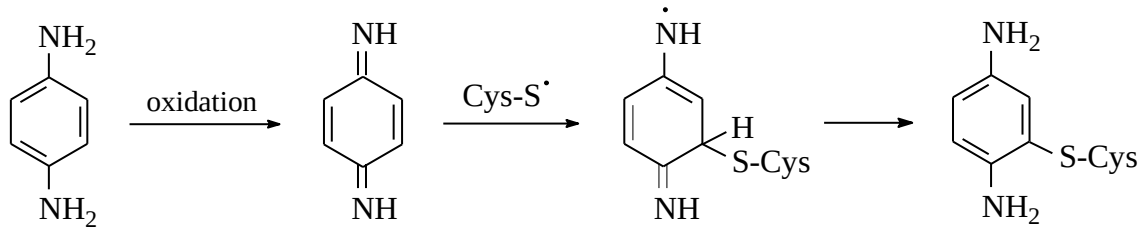
Substituted para- and ortho-aminophenols, phenylenediamines and alkylanilines are susceptible toward oxidation in the presence of air oxygen or peroxidases in the cellular systems [4-7]. The rate of their oxidation were shown to increase when solution pH increased from 6 to 8 [4]. The formation of the corresponding quinoid structures suggests the interaction mainly with protein thiolates [5-7]. The overall mechanism of this reaction can be summarized as shown in Scheme 1.



If one of the amino groups in benzene ring is tertiary, the corresponding diamine can be oxidized to a charged analogue (Scheme 2). This derivative would be more reactive as a Michael acceptor than the uncharged oxidized products [6].

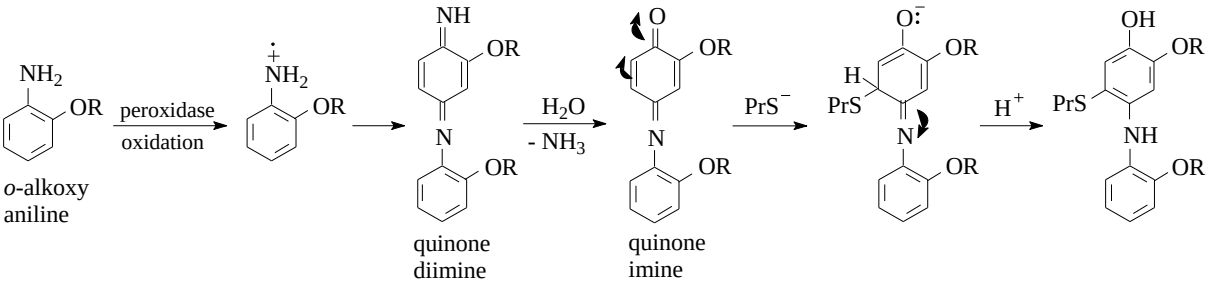


The quinone-imines and quinone-diimines can also react with protein-associated sulfhydryl radicals at a ring carbon atom in a radical analogue of the Michael addition (Scheme 3) [8].



The clastogenicity of *para*- and *ortho*-substituted alkoxyanilines (i.e. anizidines, phenetidines) can be explained by the possibility to undergo one-electron oxidation in the presence of cellular peroxidases. Aromatic amines generally form nitrogen-centered cation-radicals when oxidized by peroxidases [9]. It was suggested that the protein reactive species could be *o*- and *p*-anisidine or phenetidine quinone-imine and quinone-diimine dimers formed as a result of peroxidative oxidation of the initial alkoxyanilines [8,9]. The putative mechanism of the oxidation and protein binding of

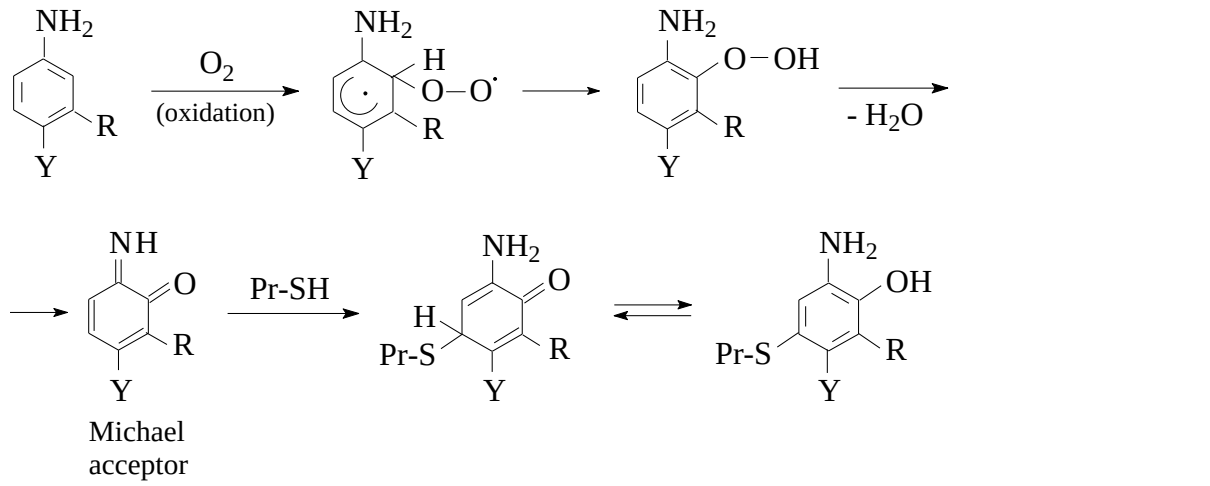
ortho-isomers is shown in Scheme 4.



The reactivity of the p- and o-di-substituted aromatic compounds towards oxidative formation of quinoid structures at physiological pH can be arranged in the following manner: benzenediols > aminophenols > phenylenediamines [10]. The introduction of additional (1 to 3 in number) electron-donating substituents of pronounced positive inductive and/or resonance effects (such as alkyl, alkoxy, hydroxy, and amino groups) into the aromatic structure results in an enhanced reactivity towards oxidation, as expected. However, the rate of the next step of Michael-type cysteine (Cys-SH) conjugation can be decreased, due to the combination of such electronic factors. On the other hand, the presence of additional electron-withdrawing substituents such as -Cl, -Br, -NO2, -COOH, etc. in the aromatic structures can reduce the reactivity towards oxidation but increase the rate of the next nucleophilic addition of Cys-SH.

II. Perhydroxylation of meta-substituted anilines

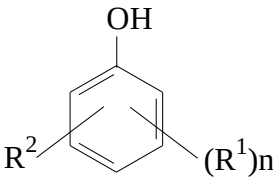
Aromatic amines can function as reducing cofactors for peroxidases, being oxidized in the process to free radicals [9]. The perhydroxylation of meta-substituted anilines is able to occur mainly in ortho- or para-positions towards amino group due to the strong stabilizing effect of a nitrogen atom in the formation of free radicals [8,11]. The ortho- and para-quinones or quinine-imines formed behave as electrophilic intermediates and may undergo Michael-type addition reaction in the presence of cellular nucleophiles (proteins, DNA). The process is shown as an abiotic perhydroxylation reaction sequence resulting from the attack of molecular oxygen in 2-position [8,11]. The protein binding of meta-substituted anilines is presented in Scheme 5.



R = OH, NH_2, Csp^3 (acy), $OCsp^3$ (acy); Y = H, Cl, $OCsp^3$ (acy), etc.

Set of chemicals used for profile development	Substituted Anilines
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances, vol. 73, WHO, Lyon, **1999**, pp. 49, 52–55. 2. S.M. Galloway, M.J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A.D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B.H. Margolin, M.A. Resnick, B. Anderson, E. Zeiger, Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.*, **1987**, 10 (Suppl. 10), 1-175. 3. T. Sofuni, A. Matsuoka, M. Sawada, M. Ishidate Jr, E. Zeiger, M.D. Shelby, A comparison of chromosome aberration induction by 25 compounds tested by two Chinese hamster cell (CHL and CHO) systems in culture. *Mutat. Res.*, **1990**, 241(2), 175-213. 4. M. Uchimiya, A.T. Stone, Reversible redox chemistry of quinones: Impact on biogeochemical cycles. *Chemosphere*, **2009**, 77(4), 451-458. 5. A.O. Aptula, G. Patlewicz, D.W. Roberts, Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol*., **2005**, 18(9), 1420-1426. 6. D.W. Roberts, G. Patlewicz, P.S. Kern, F. Gerberick, I. Kimber, R.J. Dearman, C.A. Ryan, D.A. Basketter, A.O. Aptula, Mechanistic applicability domain classification of a Local Lymph Node Assay dataset for skin sensitization. *Chem. Res. Toxicol*., **2007**, 20(7), 1019-1030. 7. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.D. Cronin, A review of the elecrtophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol*., **2011**, 41(9), 783-802. 8. A.O. Aptula, S.J Enoch, D.W. Roberts, Chemical mechanisms for skin sensitization by aromatic compounds with hydroxyl and amino groups. *Chem. Res. Toxicol*., **2009**, 22(9), 1541-1547. 9. D.C. Thompson, T.E. Eling, Reactive intermediates formed during the peroxidative oxidation of anisidine isomers. *Chem. Res. Toxicol.*, **1991**, 4(4), 474-481. 10. A. Brunmark, E. Cadenas, Redox and addition chemistry of quinoid compounds and its biological implications, *Free Radic. Biol. Med.*, **1989, 7**(4), 435-477. 11. D.W. Roberts, A.O. Aptula, G. Patlewicz, Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability

	domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol.*, **2007**, 20(1), 44-60.

Individual profile/alert	
Name	Substituted Phenols
Type of profile	Structural alert
Description/applicability domain	 where: (R1)n = (Csp3(acy))n alkyl groups at n = 1–4 and with linear or branched chains, containing between one and four carbons, but without tert-butyl groups in ortho-position toward OH group; (R1)n = Csp3(acy)-Csp2(vinyl or aryl) at n = 1 and located preferably in the ortho or para positions toward OH group; (R1)n = Hal (F, Cl, Br) atoms at n = 1–4; (R1)n can also represents CH=CH-Y at Y = C(O)-Csp3(acy) or N3+; (R1)n = Csp2 (aryl), mainly phenyl group; n = 1; (R1)n = para-NH-C(=O) group at n = 1 or ortho-C(=O)Y groups, where Y = H, OH, NH2 at n = 1; R2 should be at least one hydrogen atom located in appropriate position(s) of the corresponding compound.
Mechanism	AN2, Michael-type addition to quinoid structures

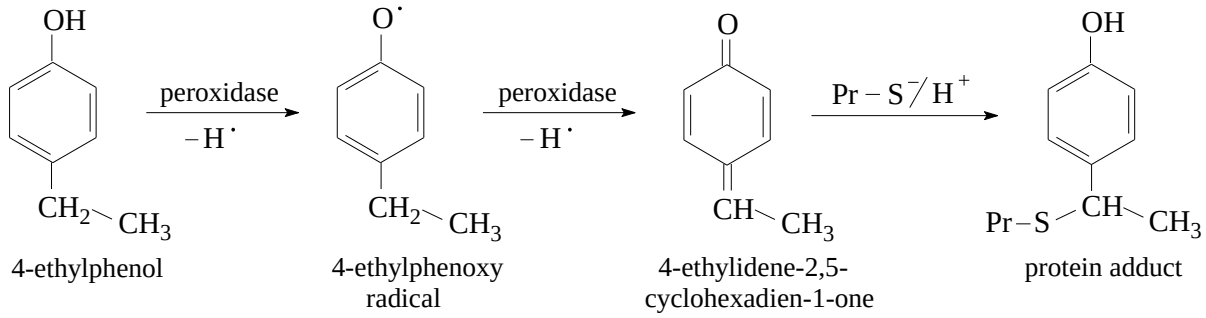
I. Alkylated and alkenylated phenols

1. ortho- and para-Alkylated phenols containing primary and secondary alkyl groups

The quinone methides are able to be formed if alkyl groups possess at least one hydrogen atom in alpha-position relative to the arene ring and are also located in ortho- or para-positions toward hydroxyl group [7]. The observed in the in vitro clastogenicity in CHO and CHL cells without S9 mix is probably due to abiotic oxidation of alkylated phenols in an aqueous medium and in the presence of peroxidases and air oxygen [3].

For example, the one- and two-electron oxidative pathways of some ortho- and para-alkyl and arylalkyl phenols (2- and 4-ethylphenols, 2-sec-butylphenol, 2,3,6-trimethylphenol, bisphenol F, etc.) can lead to quinone methide formation (Scheme 1). The quinone methides are class of reactive

electrophilic compounds which are capable of alkylating cellular proteins such as sulfur nucleophiles and also other nucleophilic sites in proteins [3,4] via an Michael-type addition reaction (Scheme 1).



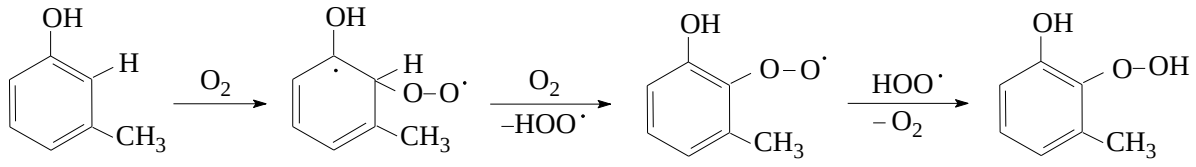
4-ethylphenol | 4-ethylphenoxy radical | 4-ethylidene-2,5-cyclohexadien-1-one | protein adduct

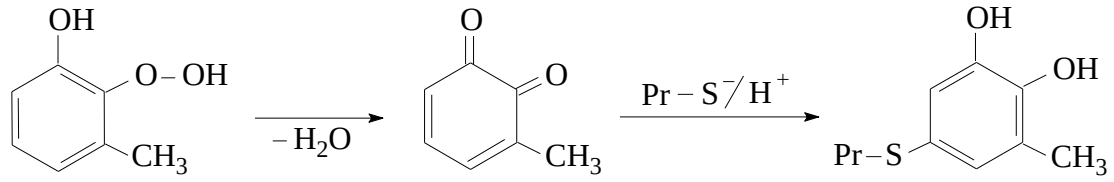
2. meta- and para-Alkylated phenols

Another group of substituted phenols are ***meta***- and ***para***-alkylated phenols such as *m*-cresol, *p*-*tert*-butyl phenol, bisphenol A, *p*-cumylphenol, etc., which can not be oxidized directly to quinone methides or quinones.

➢ *meta*-Alkylated phenols

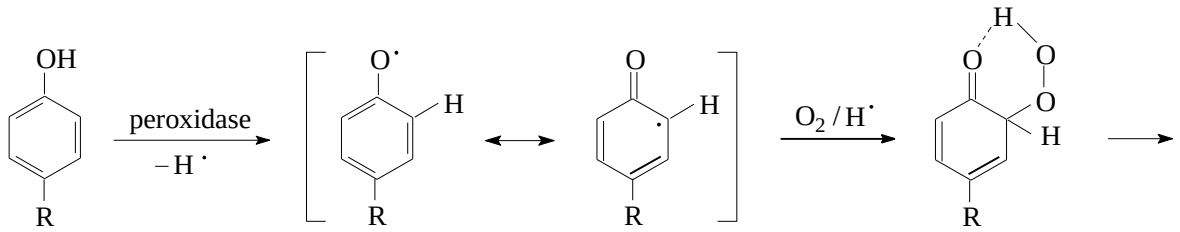
It may be suggested that the interaction of *m*-cresol with protein nucleophiles takes place similarly to that of 1,3-phenylenediamines, 3-aminophenols and 1,3-benzenediols. This process includes an abiotic perhydroxylation reaction sequence resulting from the attack of molecular oxygen in *ortho*- or *para*-positions and the formation of a free peroxyl radical which is converted into the corresponding perhydroxylated derivative [8,9]. The reactivity depends on the ability of the *meta* substituents to stabilize a free radical intermediate. The corresponding *ortho*- and *para*-benzoquinones are likely to be formed as a result of an intramolecular dehydration (Scheme 2).





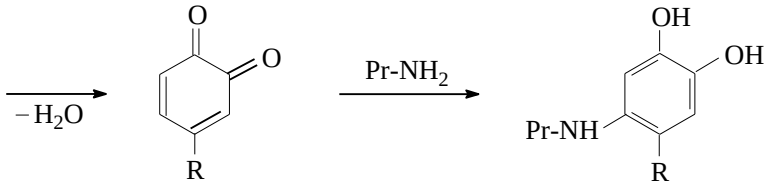
➢ *para*-Alkylated phenols

para-Alkylated phenols containing tertiary alkyl groups such as *p*-*tert*-butylphenol, bisphenol A, *p*-cumylphenol, etc. cannot be oxidized directly to quinones. The possible pathway of their activation in the absence of S9 fraction may be associated with an abiotic perhydroxylation under the influence of enzymes found in mammalian cells. The perhydroxylated phenol intermediate is easily dehydrated to the corresponding *ortho*-benzoquinone, which undergoes Michael type addition reaction (Scheme 3).



resonance-stabilized phenoxyl radical

hydroperoxide formation



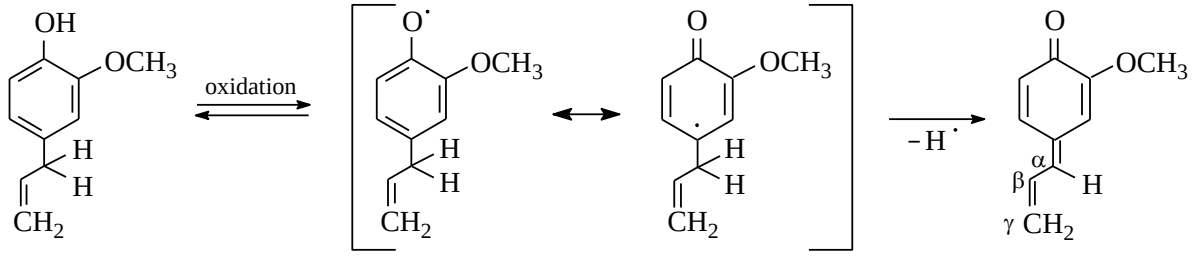
o-quinone-type fragment

protein adduct

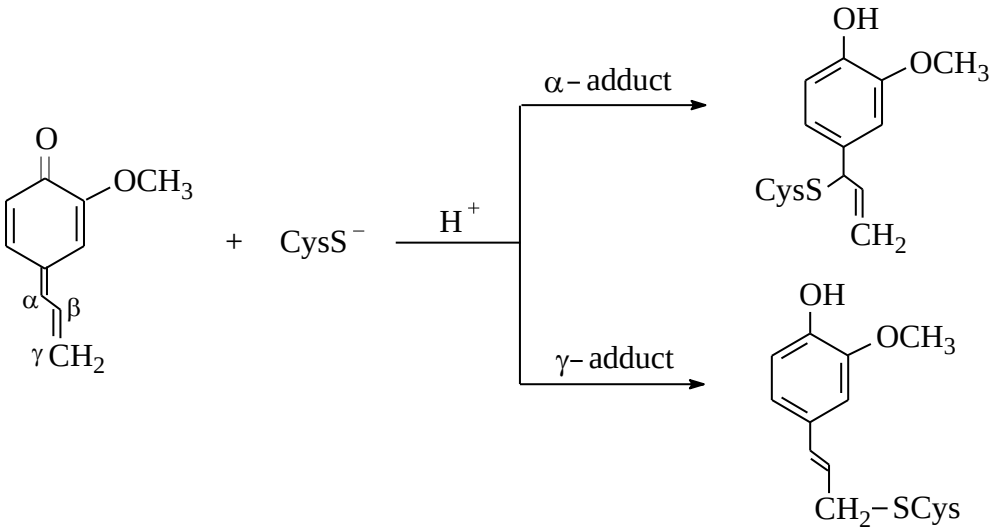
3. *Alkenyl-substituted phenols*

➢ Eugenol and eugenol-related compounds

Eugenol and eugenol-related compounds can also be oxidized in Chinese hamster cells. The reaction mechanism includes the formation of a phenoxyl radical via one-electron oxidation, which subsequently can change into a eugenol quinone methide that is able to bind covalently to thiol groups in proteins (Scheme 4) [11,12].



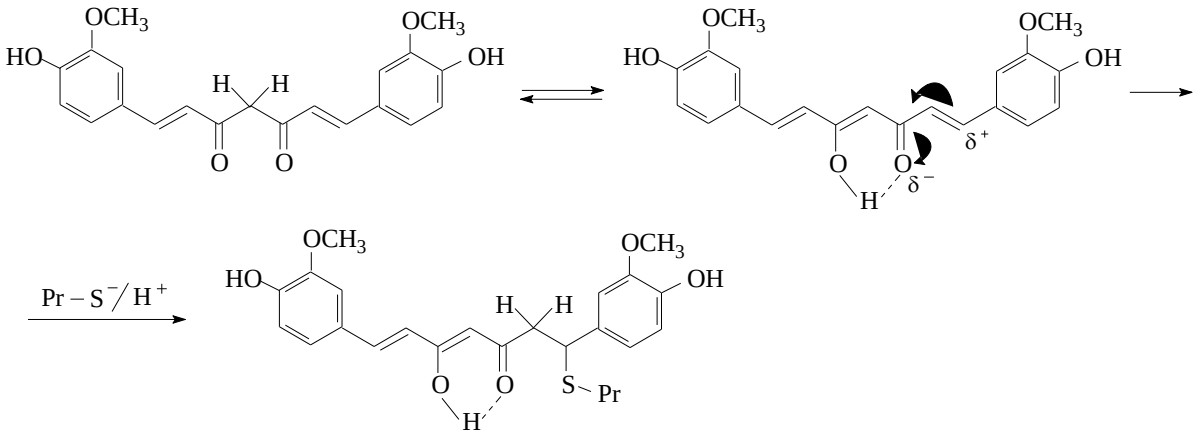
The so obtained conjugated quinone methide was found to give two types of adducts. The product resulting from the attack of a protein nucleophile at the alpha-position was produced under kinetic control, while the product of gamma-attack was formed under thermodynamic control (Scheme 5) [12].



➢ Curcumin reactivity

The equivocal results for chromosomal aberrations were found for alkenylated phenol curcumin in Chinese hamster ovary cells without metabolic activation [13,14]. The EFSA Panel considered that the indications provided by the positive results for curcumin in several in vitro and in vivo tests for genotoxicity, especially those detecting chromosomal aberrations and DNA adducts should not be disregarded, and that the available in vivo genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity.

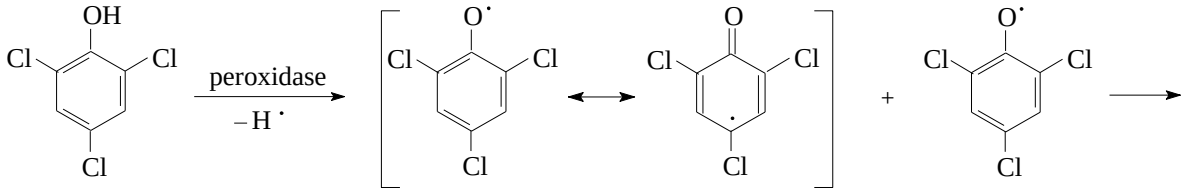
Curcumin can be regarded as a direct acting clastogen due to the presence of an α,β-unsaturated 1,3-dicarbonyl moiety in para-position against the hydroxyl group. Taking into account that it can be converted into a stable enol form, the mechanism of protein binding in the in vitro assay without metabolic activation can be presented as a Michael-type nucleophilic addition to the α,β-unsaturated carbonyls (Scheme 6).



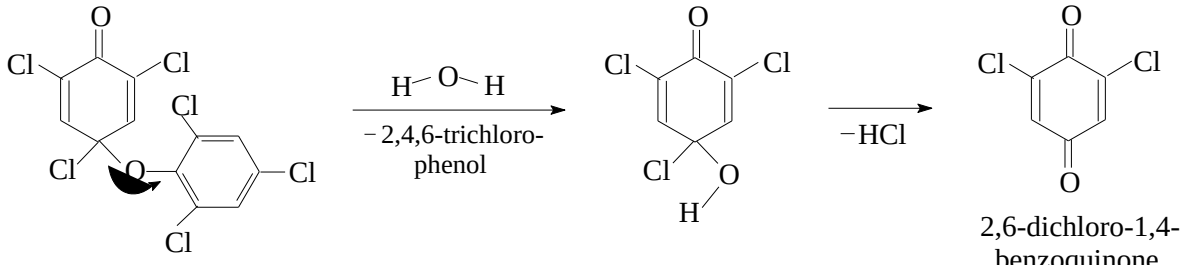
II. Halogenated phenols

The halogenated phenols are able to undergo one- or two-electron oxidation under the influence of different peroxidases and/or dihydrogen peroxide [15,16]. Sturgeon et al. [15] established that the transient 2,4,6-trichlorophenoxyl radical intermediate can exist free in solution as a result of one-electron peroxidase oxidation (Scheme 7). Then, 2,4,6-trichlorophenoxyl radical intermediate undergoes enzyme-independent reactions, such as radical-radical (carbon-oxygen) coupling, leading to the formation of a two-electron oxidized 2,6-dichloro-1,4-benzoquinone product [15]. The latter

can bind to protein nucleophiles via a Michael-type addition reaction (Scheme 7).



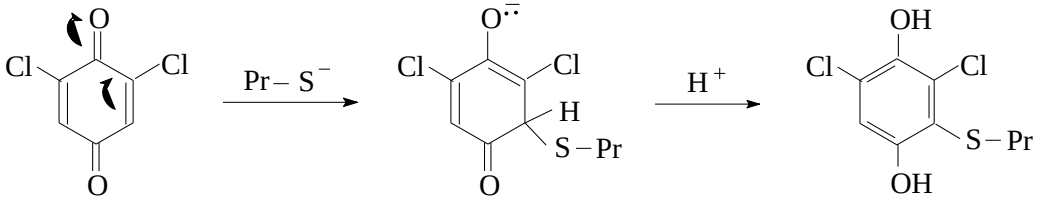
resonance-stabilized phenoxyl radical



dimer formation by radical-radical coupling

2,6-dichloro-1,4-benzoquinone

Michael-type addition reaction



protein-adduct formation

Depending on the substrate, peroxidases are thought to carry out both one- and two-electron oxidations [15]. The condensation products arising from coupling reactions of resonance-stabilized halophenol radicals could be formed in the non-irradiated systems. Different types of condensation products were found such as chlorinated hydroxylated biphenyls, hydroxylated diphenyl ethers, etc. [16]. However, the presence of halogens in positions 3, 4 and 5 against hydroxyl group will impede the access of the phenoxyl radical to the carbon-centered radical, limiting the formation of the intermediate dimer and the corresponding quinone (Scheme 7 above).

The mechanism of action of triclosan, a chlorinated aryloxyphenol, has been considered to be similar to the other halogenated phenols, targeting proteins and leading to their coagulation and precipitation. These effects clearly include the cell surface (cell wall and cell membrane) [17]. However, more recent studies have shown that triclosan specifically binds to an enzyme (enoyl reductase) and causes conformational changes in its protein structure [18,19].

III. Hydroxylated biphenyls

ortho-Phenylphenol (OPP, 2-hydroxybiphenyl) and its sodium salt (SOPP, sodium o-phenylphenate) are examples of biphenyl derivatives with a low degree of hydroxyl substitution. OPP and SOPP are broad spectrum antimicrobials with a variety of applications [20-24].

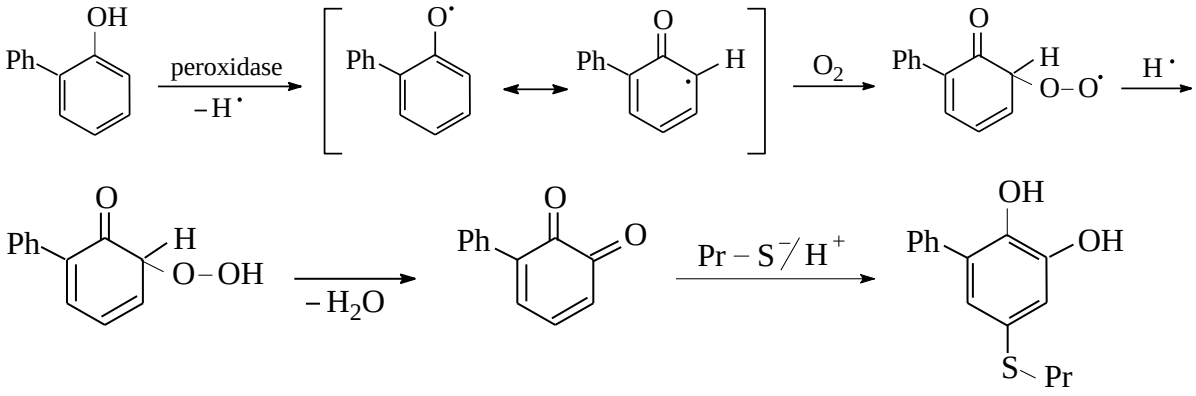
OPP and SOPP are believed to be oxidized by mixed function oxidases (CYP enzymes) to 2,5-dihydroxybiphenyl. Oxidation of dihydroxybiphenyl leads to 2-phenyl-1,4-benzoquinone via reactive semiquinone radicals. These semiquinone intermediates and/or the quinone obviously are responsible

for the tumor initiating activity of OPP and SOPP observed [26].

The microsomal oxidative metabolism of OPP is an essential prerequisite for the covalent binding of this substance to proteins [25]. In addition, OPP was found to bind covalently to calf thymus DNA in the presence but not in the absence of microsomes, indicating that its conversion to an activated metabolite is required [21].

Generally, the investigators concluded that two different processes may have caused the clastogenicity of OPP: the direct effect of OPP in the absence of metabolic activation and electrophilic reaction of OPP metabolite(s) (e.g., phenylhydroquinone, phenylbenzoquinone) in the presence of metabolic activation [27]. According to another study of Tayama and Nakagawa [28], the involvement of reactive oxygen species (such as H2O2 and $O2^{\cdot -}$) in the presence of metabolic activation was minor. However, Li et al. [24] suggested that the disruption of lysosomal membrane integrity and the oxidative stress, leading to DNA fragmentation, may be the mechanism of DNA damage induced by OPP.

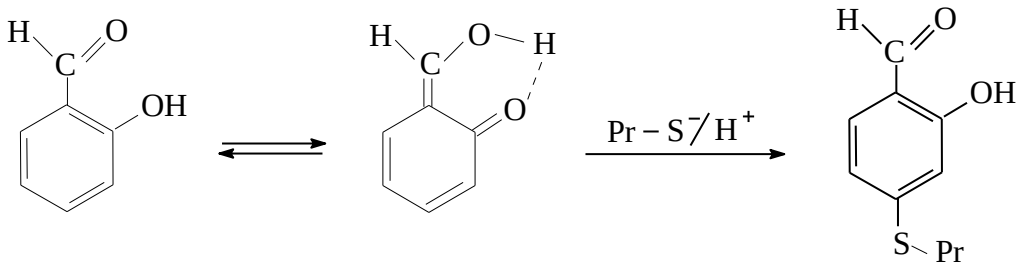
Then, it could be assumed that in the absence of metabolic activation oxygen-derived free radicals may be formed by normal cellular metabolism (peroxidase availability) and/or in aerobic conditions and by exogenous sources such as ionizing radiations, UV radiation, etc. The possible perhydroxylation of resonance stabilized ortho- and para-phenoxy radicals may consistently lead to the formation of the ortho- or para-quinone moieties that take part in Michael-type addition reaction with protein nucleophiles (Scheme 8).



IV. Salicylic acid derivatives

Among the salicylic acid derivatives (such as o-, m-, and p-hydroxybenzaldehydes, salicylic acid and salicylamide), positive results in Chinese hamster cell lines without metabolic activation have been found for salicylaldehyde (o-hydroxybenzaldehyde), salicylic acid and salicylamide.

It may be hypothesized that salicylaldehyde can become reactive via the formation of the corresponding tautomeric oxo-enol form in analogy with 2,6-dihydroxy-4-methylbenzaldehyde [29]. Regardless of the disturbed aromaticity of benzene ring, oxo-enol forms are expected to be partially stabilized by intramolecular hydrogen bond. The ortho-quinone methide structure of enol form may exhibit certain reactivity against protein nucleophiles according to Michael-type addition reaction, as shown for salicylaldehyde in Scheme 9.



keto form oxo-enol form protein-adduct formation

meta- and para-Hydroxybenzaldehydes cannot form an intramolecular hydrogen bond and are more susceptible to air oxidation being converted to the corresponding meta- and para-hydroxybenzenecarboxylic acids.

As with salicylic acid, Reszka et al. [30] were found that it may form phenoxyl radicals and subsequently a biphenol quinone dimmer in the presence of myeloperoxidase and lactoperoxidase systems together with dihydrogen peroxide. This process takes place effectively in slightly acidic to near neutral medium. The reactive intermediate biphenol quinone can form adducts with protein nucleophiles.

Set of chemicals used for profile development	Substituted Phenols
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Brooke, D., Mitchell, R., Watts, C., Dungey, S., Indans, I., Environmental Risk Evaluation Report: *para*-C_{12}-Alkylphenols (Dodecylphenol and Tetrapropenylphenol). Science Report, Environmental Agency, UK, May **2007**. 2. Alkylphenols Category, Screening-Level Hazard Characterization. U.S. Environmental Protection Agency, September, **2009**. 3. Thompson, D.C., Thompson, J.A., Sugumaran, M., Moldeus, P., Biological and toxicological consequences of quinone methide formation. *Chem.-Biol. Interact*., **1992**, 86(2), 129-162. 4. Thompson, D.C., Perera, K., Krol, E.S., Bolton, J.L., *o*-Methoxy-4-alkylphenols that form quinone methides of intermediate reactivity are the most toxic in rat liver slices. *Chem. Res. Toxicol*., **1995**, 8(3), 323-327. 5. Okuda, K., Fukuuchi, T., Takigushi, M., Yoshihara, S., Novel pathway of metabolic activation of bisphenol A-related compounds for estrogenic activity. *Drug. Metab. Dispos*., **2011**, 39(9), 1696-1703. 6. Kolšek, K., Mavri, J., Dolenc, M.S., Reactivity of bisphenol A-3,4-quinone with DNA. A quantum chemical study. *Toxicol. In Vitro*, **2012**, 26(1), 102-106. 7. S.J. Enoch, C.M. Ellison, T.W. Schultz, M.D. Cronin, A review of the elecrtophilic reaction chemistry involved in covalent protein binding relevant to toxicity, *Crit. Rev. Toxicol*., **2011**, 41(9), 783-802.

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protein interface inhibition. *J. Biol. Chem*., **2014**, 289(48), 33287-33295.
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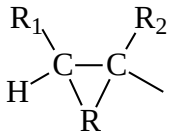
Individual profile/alert

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

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

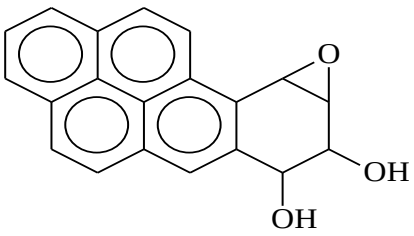
- Primary, mono-substituted, terminal epoxides, with -CH2- group are more reactive than secondary ones;

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

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

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

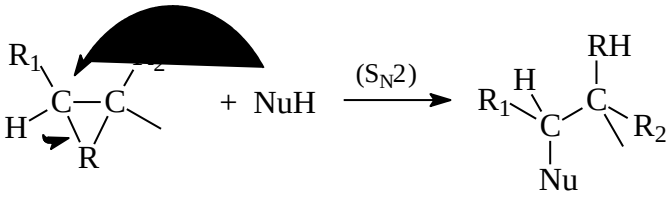
An example can be presented, which indicates the positive in vitro CA results of epoxides, which are mainly determined by covalent protein binding. The majority of the mode of binding a characteristic benzo[a] pyrene metabolite to nuclear macromolecules, which occurs in intact hamster embryo cells was reported to be due to formation of adducts with various classes of nuclear proteins. Thus benzopyrene diol epoxide:



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

On the basis of the above discussions, and the fact that the cyclic carbon in the three-

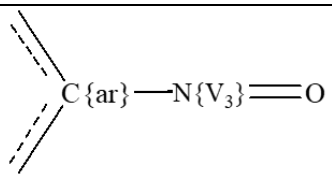
membered heterocycles may interact as both the “soft” and “hard” electrophile, the following SN2-type reaction mechanistic schemes which may elicit CA by protein binding can be expertly proposed [3, 5, 9]:



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

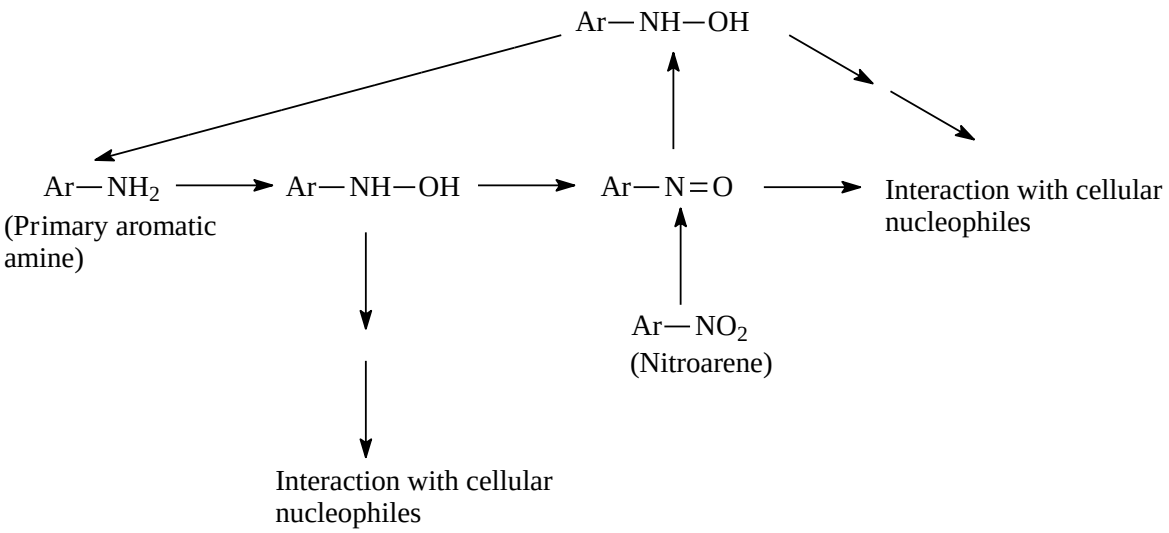
Set of chemicals used for profile development	Epoxides, Aziridines and Sulfuranes
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Roberts, D. W., A. M. Api, R. J. Safford, J. F. Lalko, Principles for identification of High Potency Category Chemicals for which the Dermal Sensitisation Threshold (DST) approach should not be applied, Regulatory Toxicology and Pharmacology, 2015, 72, 683 – 693. 4. Schramm, Fr., A. Muller, H. Hammer, A. Paschke, G. Schuurmann, Epoxide and Thiirane Toxicity In vitro with the Ciliates Tetrahymena pyriformis: Structural Alerts Indicating Excess Toxicity, Environ. Sci. Technol. 2011, 45, 5812 – 5819. 5. Roberts, D. W., Gr. Patlewitz, P. S. Kern, Fr. Gerberick, I. Kimber, R. Dearmann, C. A. Ryan, D. Basketter, A. O. Aptula, Mechanistic Applicability Domain Classification of a Local Lymph Node Assay Dataset for Skin Sensitization, Chem. Res. Toxicol. 2007, 20, 1019 – 1030. 6. Buback, V., M. Mladenovic, B. Engels, T. Schirmeister, Rational Design of Improved Aziridine-Based Inhibitors of Cysteine Proteases, J. Phys. Chem. B 2009, 113, 5282 –5289. 7. McLeod, M. C., A. Kootstra, B. K. Mansfield, T. J. Slaga, J. K. Selkirk, Specificity in interaction of benzo[a]pyrene with nuclear macromolecules: Implication of derivatives of two

	dihydrodiols in protein binding, Proc. Natl. Acad. Sci (USA), 1980, 77(11), 6396 – 6400. 8. Wei, Q., J. Gu, L. Cheng, M. L. Bondy, H. Jiano, W. K. Hong, M. R. Spitz, Benzo[a]pyrene Diol Epoxide Induced Chromosomal Aberrationas and Risk of Lung Cancer, Canc. Res. 1996, 56, 3975 – 3979. 9. Enoch, S. J., C. M. Ellison, T. W. Schultz, M. T. D. Cronin, A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity, Crit. Rev. Toxicol. 2011, 41(9), 783 – 802.

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

A number of chemicals, which show positive in vitro CA effects after S9 metabolic activation to C-nitroso compounds belong to the sub-class of aromatic amines, and, to a lesser extent, to that of nitroarenes. Aromatic amines exert their toxic effects usually after oxidative biotransformation, primarily in liver. As a result, aromatic N-hydroxylamines are generated, which can further undergo oxidative activation by two-electron oxidation to nitrosoarenes. Nitrosoarenes can also be formed by reduction of nitroarenes [3].

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

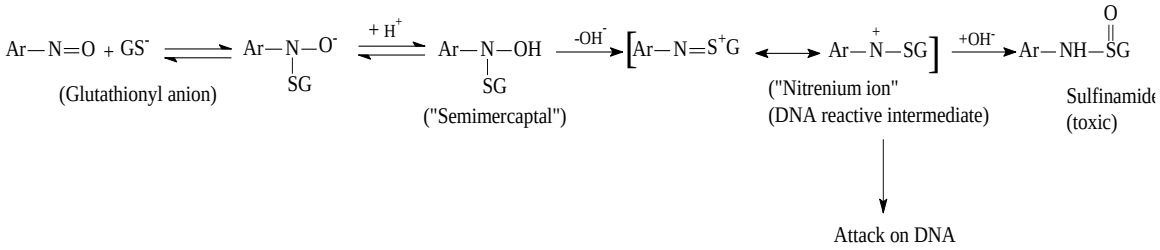


Such metabolic activation, with the contribution of nitroreductase, CYP isoenzymes and other

enzymatic systems may produce electrophilic intermediates, responsible for toxic, allergic, mutagenic, and carcinogenic effects.

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

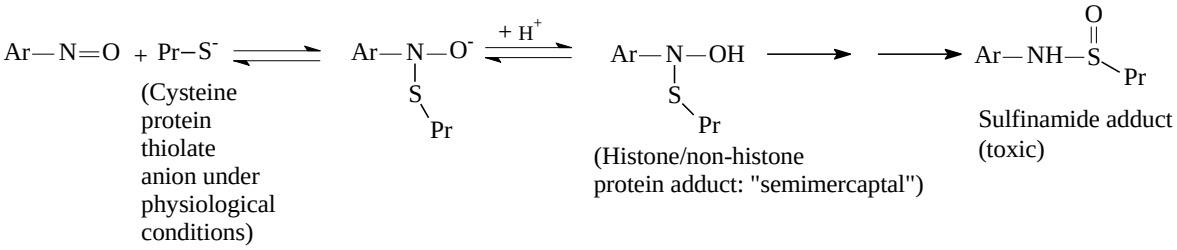
Formation of products of interaction of C-nitroso compounds with thiols such as glutathione (GSH) has been proposed for chemicals such as Nitrosobenzene, 4-Nitrosophenetol, 4-Nitroso-N,N-dimethylaniline, some nitrosoheterocyclic compounds such as nitroso-substituted pyridoindoles and pyridoimidazoles,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



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

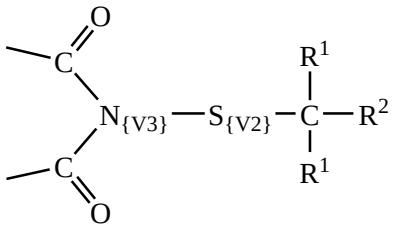
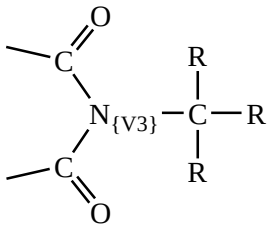
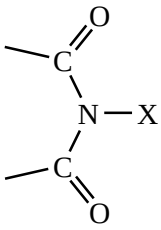
Covalent binding to proteins can inactivate vital enzymes and may lead to haptenization, followed by an immune response and skin sensitization [3]. Moreover, formation of protein adducts, derived from N-hydroxylamine metabolites oxidized to nitrosoarenes in erythrocytes, has been proposed for 2-Nitrotoluene,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

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

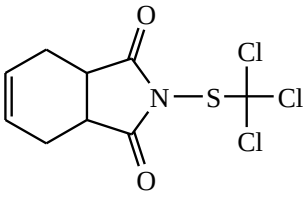


Set of chemicals used for profile development	C-Nitroso compounds protein binding
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Eyer, P., Reactions of Oxidatively Activated Arylamines with Thiols: Reaction Mechanisms and Biological Implications. An Overview, Environ. Health Persp. 1994, 102, Suppl. 6, 123 – 132. 4. Kalgutkar, A. S., I. Gardner, R. S. Obach, C. L. Shaffer, E. Callegari, K. R. Henne, A. E. Mutlib, D. K. Dalvie, J. S. Lee, Y. Nakai, J. P. O, Donnell, J. Boer, S. P. Harriman, A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups, Current Drug Metabol., 2005, 6, 161 – 225. 5. Shamovsky, I., L. Ripa, L. Borjesson, Chr. Mee, B. Norden, P. Hansen, C. Hasselgren, M. O, Donovan, P. Sjo, Explanation for Main Features of Structure-Genotoxicity Relationships of Aromatic Amines by Theoretical Studies of Their Activation Pathways in CYP1A2, JACS, 2011, 133, 16168 – 16185. 6. Sabbioni, G., Chr. R. Jones, O. Sepai, et al. Biomarkers of Exposure, Effect, and Susceptibility in Workers Exposed to Nitrotoluenes, Cancer Epidemiol Biomarkers Prev. 2006, 15(3), 559 – 566.

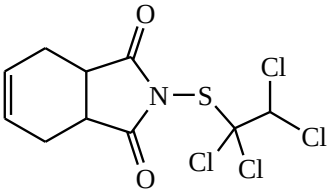
Individual profile/alert	
	N-Haloacylamides

Type of profile	Structural alert
Description/applicability domain	 R^1 = F, Cl, Br, I; R^2 = R^1 or $CH(R^1)_2$   R = F, Cl, Br, I; X = F, Cl, Br, I;
Mechanism	Acylation, Direct acylation involving a leaving group

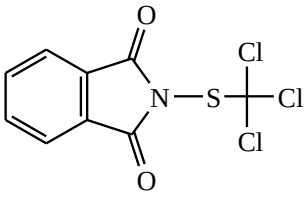
Agricultural fungicides as captan, captafol and folpet were studied in vitro for induction of chromosomal damage in various types of Chinese hamster cells [1-3].



Captan

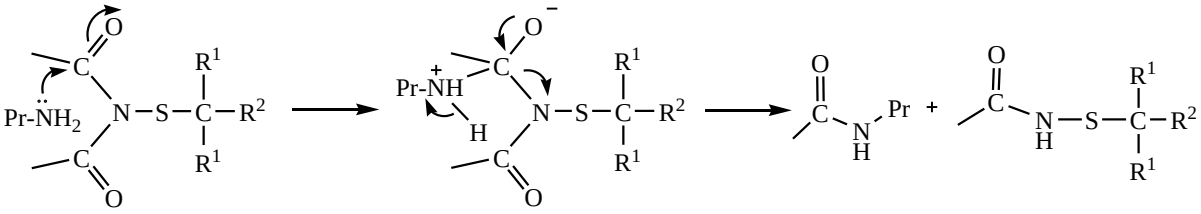


Captafol



Folpet

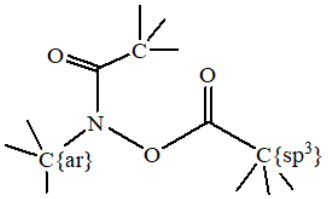
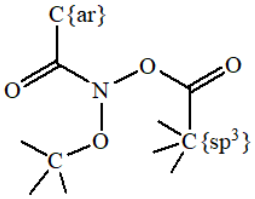
The test results show positive responses in the absence of S9 and little or no activity when S9 is present [1]. These imides as acylating agents in vitro are considered to be hard electrophiles and could bind to Lysine residues in proteins (Pr-NH2) [4]. For imide structures such as RCO.NYCOR1 and YNHCOR1 which are not sufficiently acidic, when Y = H, but becomes more acidic and reactive, when Y is a strongly electronegative group (for example trichloromethylthio functional group SCCl3) an acylation mechanism is shown below:



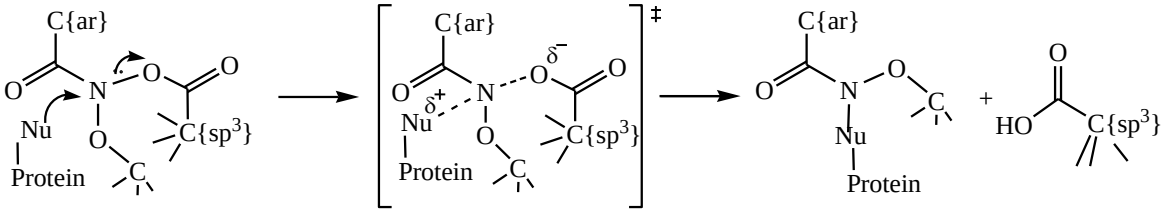
R1 = F, Cl, Br, I; R2 = R1 or CH(R1)2;

Set of chemicals used for profile development	N-Haloacylamides

Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	Arce, G.T., Gordon, E.B., Cohen, S.M., Singh, P., Genetic toxicology of folpet and captan. Crit. Rev. Toxicol., 2010, 40(6), 546–574. 2. Tezuka, H., Ando, N., Suzuki, R., Terahata, M., Moriya, M., Shirasu, Y., Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. Mutat. Res., 1980, 78(2), 177–191. 3. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutat. Res., 1988, 195(2), 151–213. 4. Aptula, A.O., Roberts, D.W., Mechanistic applicability domains for nonanimal-based prediction of toxicological end points: general principles and application to reactive toxicity. Chem. Res. Toxicol., 2006, 19(8), 1097–1105.

Individual profile/alert	
	N-Oxicarbonyl amides, N-Acyloxy-N-alkoxyamides
Type of profile	Structural alert
Description/applicability domain	 *N*-Oxycarbonyl amides  *N*-Acyloxy-*N*-alkoxyamides
Mechanism	SN2, Nucleophilic substitution at a Nitrogen atom
It was found that N-acyloxy-N-alkoxyamides are anomeric amide electrophiles that are capable of direct interaction with DNA, inducing DNA damage [3, 4]. The term "anomeric" is rather used to describe all systems, bearing two heteroatoms bound to the nitrogen that are thus capable of displaying anomeric effects. Amides, which are geminally substituted with two heteroatoms at the central nitrogen atom can support anomeric effects in much the same way as their carbon-containing analogues, e.g., acetals and aminals [4]. Based on their chemical electrophilicity in SN2 reactions with nitrogen and sulfur nucleophiles such as ammonia, N-methylaniline [3,5] and alkylthiols [4, 6], it is expertly assumed that N-acyloxy-N-alkoxyamides will be active in chromosomal aberration assay via covalent binding to lysine and cysteine residues in the chromosomal proteins. A common SN2	

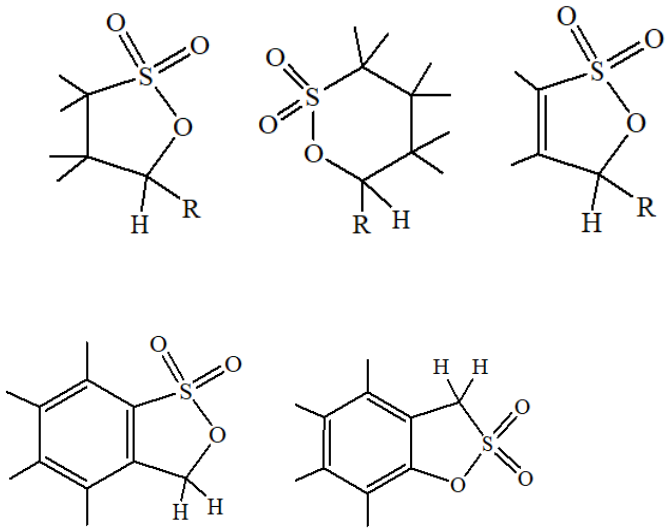
mechanism for N-acyloxy-N-alkoxyamides with nucleophilic groups of proteins is shown below:



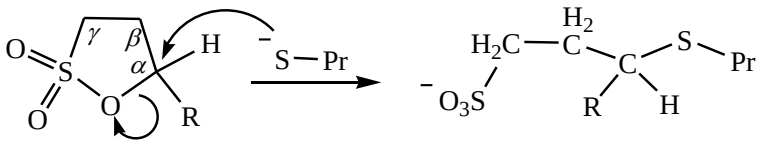
Nu = NH_2, SH

Set of chemicals used for profile development	N-Oxicarbonyl amides, N-Acyloxy-N-alkoxyamides
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Roberts, D.W., Aptula, A.O., Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. Chem. Res. Toxicol., 2007, 20(1), 44–60. 2. Tates, A.D., Kriek, E., Induction of chromosomal aberrations and sister-chromatid exchanges in Chinese hamster cells in vitro by some proximate and ultimate carcinogenic arylamide derivatives. Mutat. Res., 1981, 88(4), 397–410. 3. Banks, T.M., Bonin, A.M., Glover, S.A., Prakash, A.S., Mutagenicity and DNA damage studies of N-acyloxy-N-alkoxyamides--the role of electrophilic nitrogen. Org. Biomol. Chem., 2003, 1(13), 2238–2246. 4. Glover, S.A., Adams, M., Reaction of N-acyloxy-N-alkoxyamides with biological thiols. Aust. J. Chem., 2011, 64(4), 443–453. 5. Glover, S.A., Anomeric amides—Structure, properties and reactivity. Tetrahedron, 1998, 54(26), 7229–7271. 6. Glover, S.A., Rosser, A.A., Heteroatom substitution at amide nitrogen-resonance reduction and HERON reactions of anomeric amides. Molecules, 2018, 23(11), pii: E2834. doi: 10.3390/molecules23112834.

Individual profile/alert	
	Sultones protein binding
Type of profile	Structural alert

Description/applicability domain	 R = H, C{sp3, acy}
Mechanism	SN2, Ring opening SN2 reaction

The correlations between the biological action pattern and chemical reactivity of sultones as alkylating agents and rate constants for the reactions of 1,3-propane sultone and 1,4-butane sultone with a number of nucleophiles at physiological temperature have been determined [2]. The comparison degrees of alkylation of alkane sultones and some sulfonate and sulfate open-chain esters (for example methyl methanesulfonate and dimethyl sulfate) shown similarity in their reactivity as alkylating agents [2]. The activity of these compounds in chromosomal aberration assay is expertly assumed to be result of alkylation of nuclear proteins associated with DNA. The formation of a protein adduct of alkane 1,3-sultones by ring opening SN2 mechanism with nucleophilic residues in proteins [3,4] is shown below:



Sultone

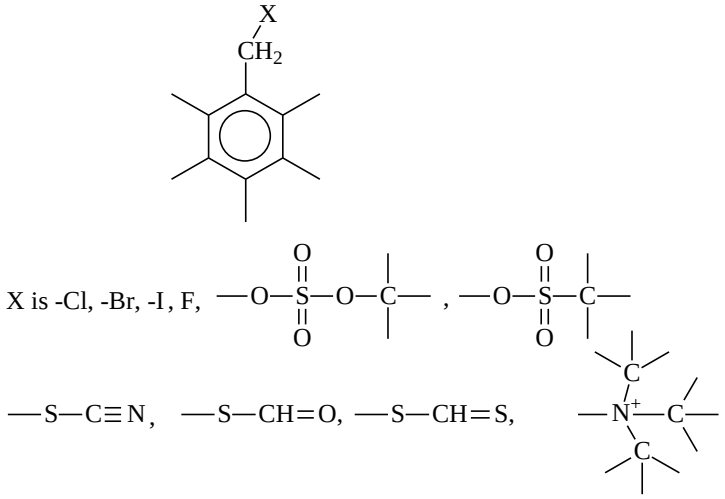
R = H, alkyl chain

Alkylated protein

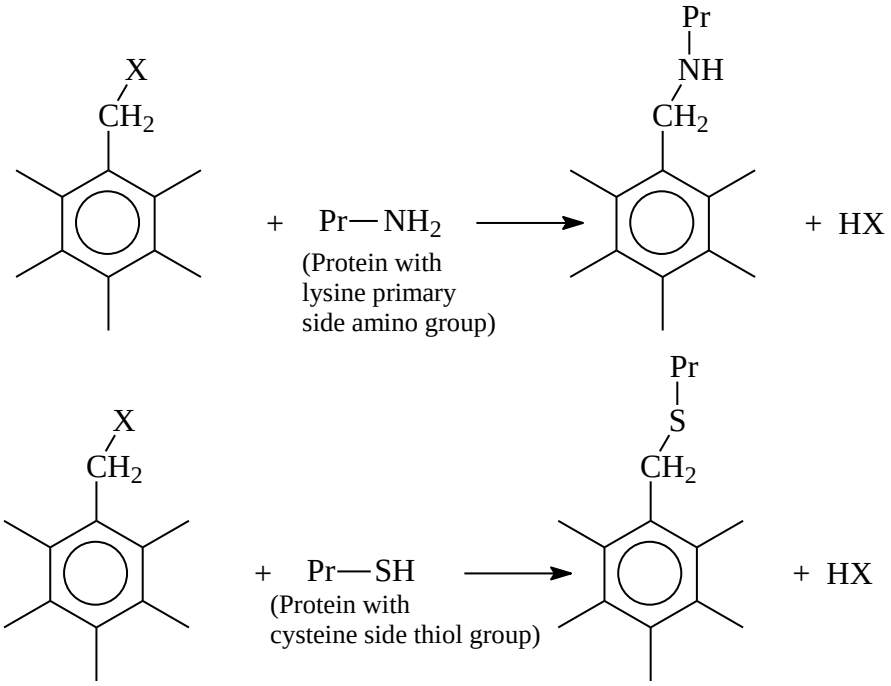
The presence of the alkyl group (R) in the □-position of alkane sultones leads to marked reduction in their electrophilicity [3].

Set of chemicals used for profile development	Sultones protein binding
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Ishidate, M. Jr, Odashima, S., Chromosome tests with 134 compounds on Chinese hamster cells in vitro--a screening for

	chemical carcinogens. Mutat. Res., 1977, 48(3-4), 337-353. 2. Osterman-Golkar, S., Wachtmeister, C.A., On the reaction kinetics in water of 1,3-propane sultone and 1,4-butane sultone: a comparison of reaction rates and mutagenic activities of some alkylating agents. Chem. Biol. Interact., 1976, 14(1-2), 195-202. 3. Lepoittevin, J.-P., Basketter, D.A., Goosens, A., and Karlberg, A.-T., Eds. Allergic Contact Dermatitis The Molecular Basis. Springer, Heidelberg. 1998, pp. 100-102. 4. Rüegg, U.T., Rudinger, J., Alkylation of cysteine thiols with 1,3-propane sultone. Int. J. Pept. Protein Res., 1974, 6(6), 447-456. 5. Roberts, D.W., Williams, D.L., Bethell, D., Electrophilic reactions of skin-sensitizing sultones. Chem. Res. Toxicol., 2007, 20(1), 61-71.

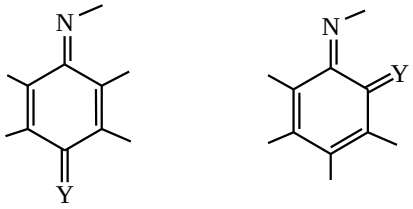
Individual profile/alert	
	alpha-Activated benzyls
Type of profile	Structural alert
Description/applicability domain	 *Note:* Where applicable, benzylic carbon is bound to the other functionalities via their O-atom
Mechanism	SN2, Nucleophilic subsitution on benzilyc carbon atom
The genotoxic activity of various alkyl nitrites has been documented. Nitrite functionality enhances the reactivity by increasing the electrophilicity of the benzylic carbon atom, and acting as a good leaving group (Chemical 5, Table 1) [6]. In addition, the biological activity of organic nitrites is likely to be initiated by benzylic-type electrophiles capable of modification of cysteine residues in proteins [7]. According to another publication [8], by analogy with Cl and other halogens attached to the benzylic carbon, functionalities such as –OSO2R, –OSO2OR and –SCN (Table 1, Chemicals (4), (6) and (7)) can be regarded as “pseudohalides”, since their conjugated acids are strong. Therefore, these	

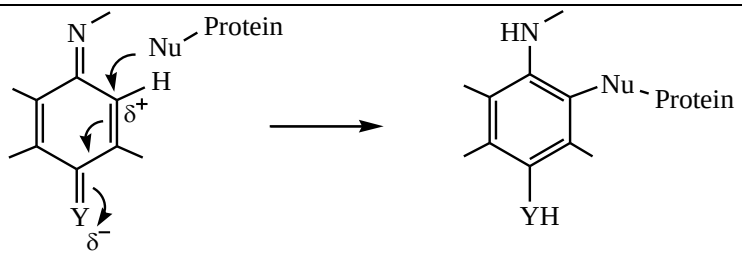
functionalities are assumed to be good leaving groups acting by SN2 mechanism. Such compounds exhibit protein binding capabilities, and are strong skin sensitizers [8].
Therefore, despite the lack of reported experimental data on the in vitro CA for such compounds, it could be expertly assumed that chemicals (4) – (7) may also possess reactivity towards histone/non-histone proteins and may form adducts, thereby acting as in vitro genotoxins.
On the basis of the above discussion, and the fact that benzylic carbon may interact as both the “soft” and “hard” electrophile, the following SN2-type reaction mechanistic schemes which may elicit CA by protein binding can be expertly proposed:



Set of chemicals used for profile development	alpha-Activated benzyls
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 2. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 3. Benzyltrimethylammonium Chloride, ECHA Registration Dossier; https://www.echa.europa.eu/el/web/guest/registration-dossier/-/registered-dossier/13489/7/7/1. 4. Japan Chemical Database; https://dra4.nihs.go.jp/mhlw_data/home/file/file611-19-8.html.

	5. Application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes, EMA/CHMP/ICH/458894/2015, Committee for Human Medicinal Products; 23 July 2015; https://www.ema.europa.eu/en/documents/scientific-guideline/application-principles-ich-m7-guideline-calculation-compound-specific-acceptable-intakes-step-2b_en.pdf. 6. Benigni, R., C. Bossa, Mechanisms of Chemical Carcinogenicity and Mutagenicity: A Review, Chem. Rev. 2011, 111, 2507 – 2536. 7. Dunlap, T., S. Abdul-Hay, R. Esala, P. Chadrasena, et al., Nitrates and NO-NSAIDs in Cancer Chemoprevention & Therapy: In Vitro Evidence Querying the NO Donor Functionality, Nitric Oxide. 2008, 19(2), 115 – 124. 8. Roberts, D. W., A. M. Api, R. J. Safford, J. F. Lalko, Principles for identification of High Potency Category Chemicals for which the Dermal Sensitisation Threshold (DST) approach should not be applied, Regulatory Toxicology and Pharmacology, 2015, 72, 683 – 693.

Individual profile/alert	
	Quinoneimines protein binding alert
Type of profile	Structural alert
Description/applicability domain	 *o*- and *p*-Quinoneimines and quinonediimines Y = O, NH
Mechanism	AN2, Michael addition to the quinoid type structures
The proposed reaction pathways of protein adduct formation between quinone(di)imines and cysteine and lysine residues in proteins are shown below: ➢ Michael addition reaction and formation of covalent bonded protein adduct with para-quinone(di)imines [5]:	

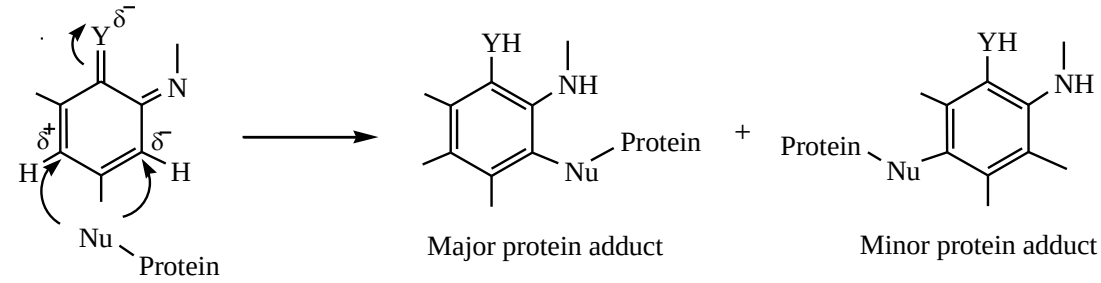


para-Quinone(di)imine

Nu = SH, NH_2; Y = O, NH;

Protein adduct

- Michael addition reaction and formation of covalent bonded protein adducts with ortho-quinone(di)imines [5] :



ortho-Quinone(di)imine

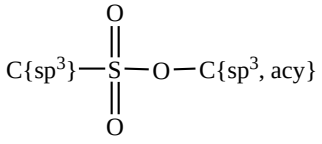
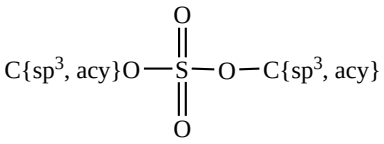
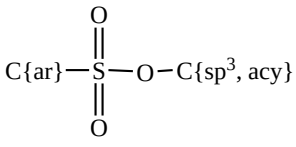
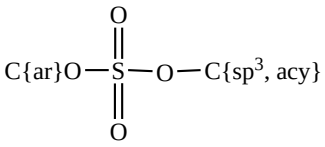
Nu = SH, NH_2; Y = O, NH;

Major protein adduct

Minor protein adduct

Set of chemicals used for profile development	Quinoneimines protein binding alert
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Klopčič, I., Dolenc, M.S., Chemicals and drugs forming reactive quinone and quinone imine metabolites. Chem. Res. Toxicol., 2019, 32(1), 1-34. 2. Gaulden, M.E., Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, 1987, 2(5), 357-365. 3. Galligan, J.J., Marnett, L.J., Histone adduction and its functional impact on epigenetics. Chem. Res. Toxicol., 2017, 30(1), 376-387. 4. Powis, G., Hodnett, E.M., Santone, K.S., See, K.L., Melder, D.C., Role of metabolism and oxidation-reduction cycling in the cytotoxicity of antitumor quinoneimines and quinonediimines. Cancer Res., 1987, 47(9), 2363-2370. 5. Bolton, J.L., Dunlap, T., Formation and biological targets of quinones: cytotoxic versus cytoprotective effects. Chem. Res. Toxicol., 2017, 30(1), 13-37.

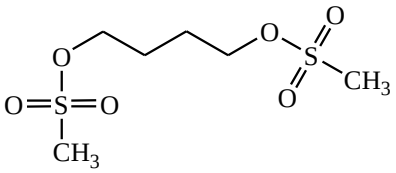
	6. Kovacic, P., Jacintho, J.D., Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. Curr. Med. Chem., 2001, 8(7), 773-796. 7. Monks, T.J., Jones, D.C., The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. Curr. Drug Metab., 2002, 3(4), 425-438. 8. Penning, T.M., Genotoxicity of ortho-quinones: reactive oxygen species versus covalent modification. Toxicol. Res., 2017, 6(6), 740-754. 9. Turchi, G., Glatt, H.R., Seidel, A., Puliti A, Sbrana I. Structure-activity relationship in the induction of chromosomal aberrations and spindle disturbances in Chinese hamster epithelial liver cells by regioisomeric phenanthrene quinones. Cell Biol. Toxicol., 1997, 13(3), 155-165. 10. Enescu, M., Gardey, B., Mechanism of cysteine oxidation by a hydroxyl radical: a theoretical study. ChemPhysChem., 2006, 7(4), 912-919. 11. Chung, K.T., Murdock, C.A., Stevens, S.E. Jr, Li, Y.S., Wei, C.I., Huang, T.S., Chou, M.W., Mutagenicity and toxicity studies of p-phenylenediamine and its derivatives. Toxicol. Lett., 1995, 81(1), 23-32.

Individual profile/alert	
Name	Sulfonates and sulfates protein binding alert
Type of profile	Structural alert
Description/applicability domain	 Alkyl alkanesulphonates  Dialkyl sulfates  Alkyl arenesulfonates  Alkyl aryl sulfates
Mechanism	SN2, Nucleophilic substitution at sp3-carbon atom (alkylation)

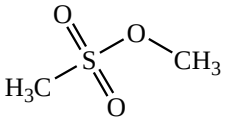
<u>Mechanism of protein binding by sulfonates</u>

The sulfonate group in alkyl alkane- and arenesulfonates and sulfate group in dialkyl and

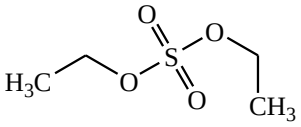
alkyl aryl sulfates are excellent leaving groups therefore compounds with active structural fragments as well as shown above are alkylating agents via bi-molecular nucleophilic substitution (SN2) mechanism. Busulfan (myleran), methyl methanesulfonate, and diethyl sulfate were found to be active in test for chromosomal aberration in mammalian cells without metabolic activation [4,5].



Busulfan

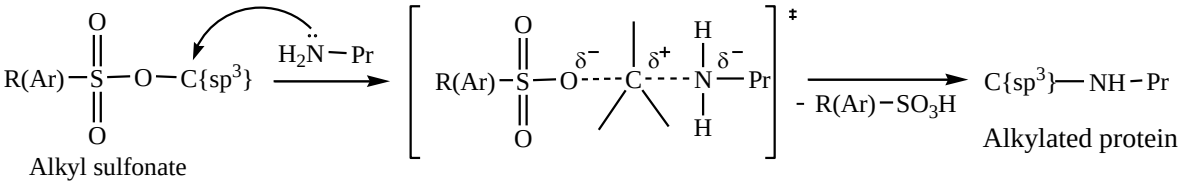


Methyl methanesulfonate



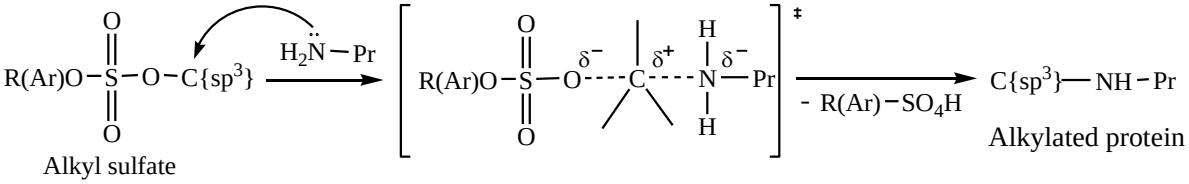
Diethyl sulfate

The activity of these compounds in chromosomal aberration assay is assumed to be result of alkylation of nuclear proteins associated with DNA. The formation of protein adducts via SN2 reaction of sulfonates and sulfates [6] with nucleophilic residues in proteins are shown below:



Alkyl sulfonate

Alkylated protein



Alkyl sulfate

Alkylated protein

Set of chemicals used for profile development	Sulfonates and sulfates protein binding alert
Data/Knowledge used for profile development	An extensive review of the literature was performed enabling the chemistry associated with DNA binding to be defined and encoded in this profiler according to the references listed below.
References	1. Mekenyan, O., Todorov, M., Serafimova, R., Stoeva, S., Aptula, A., Finking, R., Jacob, E., Identifying the structural requirements for chromosomal aberration by incorporating molecular flexibility and metabolic activation of chemicals. *Chem. Res. Toxicol.*, **2007**, 20(12), 1927-1941. 2. Puyo, S., Montaudon, D., Pourquier, P., From old alkylating agents to new minor groove binders. *Crit. Rev. Oncol. Hematol.*, **2014**, 89(1), 43-61. 3. Estrada, E., Molina, E., Automatic extraction of structural alerts for predicting chromosome aberrations of organic compounds. *J. Mol. Graph. Model.*, **2006**, 25(3), 275-288. 4. Ishidate, M. Jr, Harnois, M.C., Sofuni, T., A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat. Res.*, **1988**, 195(2), 151-213. 5. CCRIS: Diethyl Sulfate, CAS No 64-67-5. Last visited November

	26, 2019 on https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+ccris:@term+@rn+64-67-5 6. Enoch, S.J., Ellison, C.M., Schultz, T.W., Cronin, M.T., A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol.*, **2011**, 41(9), 783-802.