

Prioritizing Substances of Genotoxic Concern for In-Depth Safety Evaluation Using Non-Animal Approaches: The Example of Food Contact Materials

Tab. S1: Substance categorization according to (availability of) genotoxicity data from previous European evaluations in a non-FCM context, harmonized classification for mutagenicity under the CLP regulation and the EURL ECVAM and ECHA databases

Results are expressed as positive (+), negative (-), equivocal/insufficient (±) or not available (NA). Training set mutagens are confirmed *in vitro* mutagens although the experimental data may not be publicly available.

CAS number	Chemical name	Final call previous evaluation	Final call EURL ECVAM and/or ECHA database	Harmonized CLP class for mutagenicity	Training set mutagen
Category 1					
57-14-7	N,N-Dimethylhydrazine	NA	+	NA	Yes
74-87-3	Chloromethane	+		NA	Yes
75-55-8	2-Methylaziridine	NA	+	NA	Yes
77-78-1	Dimethyl sulphate	+		Category 2	Yes
101-77-9	4,4'-Methylenedianiline	+		Category 2	Yes
101-80-4	4,4'-Oxydianiline	NA	+	Category 1B	Yes
106-92-3	Allyl 2,3-epoxypropyl ether	NA	+	Category 2	Yes
107-05-1	3-Chloropropene	NA	±	Category 2	Yes
107-07-3	2-Chloroethanol	NA	+	NA	Yes
122-60-1	2,3-Epoxypropyl phenyl ether	NA	±	Category 2	Yes
123-73-9	(E)-crotonaldehyde	NA	±	Category 2	Yes
302-01-2	Hydrazine	NA	+	NA	Yes
556-52-5	2,3-Epoxypropan-1-ol	NA	+	Category 2	Yes
2210-79-9	2,3-Epoxypropyl o-tolyl ether	NA	±	Category 2	Yes
2386-87-0	7-Oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate	+		NA	Yes
2426-08-6	Butyl 2,3-epoxypropyl ether	NA	+	Category 2	Yes
2451-62-9	1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazine-2,4,6-trione	+		Category 1B	Yes
2530-83-8	[3-(2,3-Epoxypropoxy)propyl]trimethoxysilane	NA	+	NA	No
4170-30-3	Crotonaldehyde	NA	+	Category 2	Yes
Category 2					
75-00-3	Chloroethane	NA	±	NA	Yes
78-87-5	1,2-Dichloropropane	NA	±	NA	Yes
78-94-4	Butenone	NA	NA	NA	Yes
80-40-0	Ethyl toluene-4-sulphonate	NA	NA	NA	Yes
80-48-8	Methyl toluene-4-sulphonate	NA	NA	NA	Yes
85-83-6	1-(2-Methyl-4-(2-methylphenylazo)phenylazo)-2-naphthol	±	NA	NA	Yes
85-86-9	1-(4-(Phenylazo)phenylazo)-2-naphthol	±	NA	NA	Yes
96-23-1	1,3-Dichloropropan-2-ol	NA	±	NA	Yes
98-88-4	Benzoyl chloride	NA	±	NA	Yes
100-44-7	α-Chlorotoluene	NA	±	NA	Yes
106-87-6	7-Oxa-3-oxiranyl-bicyclo[4.1.0]heptane	NA	NA	NA	Yes
106-88-7	1,2-Epoxybutane	NA	±	NA	Yes
106-90-1	2,3-Epoxypropyl acrylate	NA	NA	NA	Yes
107-02-8	Acrylaldehyde	±	±	NA	Yes
111-44-4	Bis(2-chloroethyl) ether	NA	±	NA	Yes
111-64-8	Octanoyl chloride	NA	±	NA	Yes

CAS number	Chemical name	Final call previous evaluation	Final call EURL ECVAM and/or ECHA database	Harmonized CLP class for mutagenicity	Training set mutagen
130-15-4	1,4-Naphthoquinone	NA	NA	NA	Yes
140-95-4	1,3-Bis(hydroxymethyl)urea	NA	NA	NA	Yes
286-20-4	1,2-Epoxy cyclohexane	NA	±	NA	Yes
558-30-5	2,2-Dimethyloxirane	NA	±	NA	Yes
624-65-7	3-Chloropropyne	NA	±	NA	No
1854-26-8	4,5-Dihydroxy-1,3-bis(hydroxymethyl)imidazolidin-2-one	NA	NA	NA	Yes
2224-15-9	2,2'-[Ethylenebis(oxyethylene)]bisoxirane	NA	NA	NA	Yes
2461-15-6	[[[2-Ethylhexyl]oxy]methyl]oxirane	NA	NA	NA	Yes
2478-20-8	6-Amino-2-(2,4-dimethylphenyl)-1H-benz[de]isoquinoline-1,3(2H)-dione	NA	±	NA	No
2602-34-8	[3-(2,3-Epoxypropoxy)propyl]triethoxysilane	NA	±	NA	No
2897-60-1	[3-(2,3-Epoxypropoxy)propyl]diethoxymethylsilane	NA	±	NA	No
3049-71-6	2,9-Bis[4-(phenylazo)phenyl] anthra[2,1,9-def:6,5,10-d'e'f] diisoquinoline-1,3,8,10(2H,9H)-tetrone	NA	±	NA	No
3101-60-8	p-Tert-butylphenyl 1-(2,3-epoxy)propyl ether	NA	±	NA	Yes
3252-43-5	Dibromoacetonitrile	NA	±	NA	Yes
3266-23-7	2,3-Epoxybutane	NA	±	NA	Yes
4016-14-2	2,3-Epoxypropyl isopropyl ether	NA	NA	NA	Yes
5026-74-4	p-(2,3-Epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline	NA	±	NA	No
6471-49-4	3-Hydroxy-4-[(2-methoxy-5-nitrophenyl)azo]-N-(3-nitrophenyl)naphthalene-2-carboxamide	NA	±	NA	Yes
7665-72-7	(Tert-butoxymethyl)oxirane	NA	NA	NA	Yes
17557-23-2	1,3-Bis(2,3-epoxypropoxy)-2,2-dimethylpropane	NA	NA	NA	Yes
21490-63-1	Trans-2,3-dimethyloxirane	NA	NA	NA	Yes
26249-20-7	Epoxybutane	NA	NA	NA	Yes
68227-78-1	N-(5-chloro-2-methylphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	NA	±	NA	No
857892-58-1	Oxirane	NA	NA	NA	Yes
Category 3A					
82-38-2	1-(Methylamino)anthraquinone	NA	NA	NA	No
136-84-5	1,3-Bis(hydroxymethyl)imidazolidin-2-one	NA	NA	NA	No
938-18-1	2,4,6-Trimethylbenzoyl chloride	NA	NA	NA	No
1208-52-2	2,4'-Methylenedianiline	NA	NA	NA	No
1606-83-3	1,1'-[But-2-yne-1,4-diylbis(oxy)]bis[3-chloropropan-2-ol]	NA	NA	NA	No
1742-95-6	4-Aminonaphthalene-1,8-dicarboximide	NA	NA	NA	No
2095-03-6	2,2'-[Methylenebis(p-phenyleneoxymethylene)]bisoxirane	NA	NA	NA	No
2238-07-5	2,2'-[Oxybis(methylene)]bisoxirane	NA	NA	NA	No
3126-95-2	(Propoxymethyl)oxirane	NA	NA	NA	No
3176-79-2	1-[[3-Methyl-4-[(3-methylphenyl)azo]phenyl]azo]-2-naphthol	NA	NA	NA	No
3271-22-5	2,4-Dimethoxy-6-pyren-1-yl-1,3,5-triazine	NA	NA	NA	No
3454-29-3	1-(2,3-Epoxypropoxy)-2,2-bis[(2,3-epoxypropoxy)methyl]butane	NA	NA	NA	No
4482-25-1	5,5'-[(4-Methyl-1,3-phenylene)bis(azo)]bis[toluene-2,4-diamine]	NA	NA	NA	No
6410-38-4	4-[(2,5-Dichlorophenyl)azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	NA	NA	NA	No
6539-67-9	3-[[2-(Acetylamino)-4-[(4-amino-6-chloro-1,3,5-triazin-2-yl)amino]phenyl]azo] naphthalene-1,5-disulphonic acid	NA	NA	NA	No
6655-84-1	3-Hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide	NA	NA	NA	No
7328-97-4	2,2',2'',2'''-[Ethane-1,2-diylidenetetraakis(p-phenyleneoxymethylene)] tetraoxirane	NA	NA	NA	No

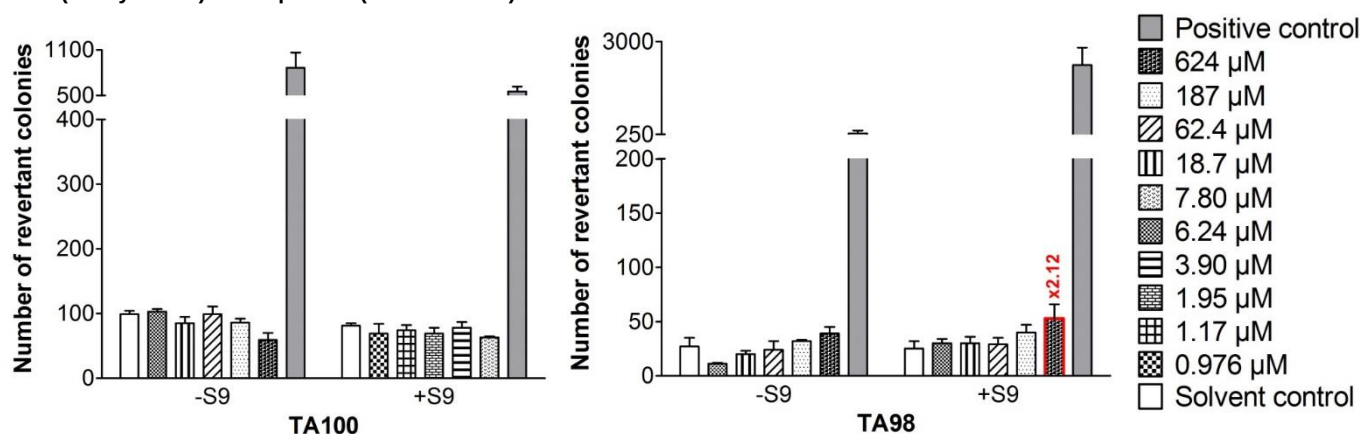
CAS number	Chemical name	Final call previous evaluation	Final call EURL ECVAM and/or ECHA database	Harmonized CLP class for mutagenicity	Training set mutagen
13236-02-7	1,2,3-Tris(2,3-epoxypropoxy)propane	NA	NA	NA	No
14228-73-0	1,4-Bis[(2,3-epoxypropoxy)methyl]cyclohexane	NA	NA	NA	No
16096-30-3	2,2'-[(1-Methylethylene)bis(oxyethylene)]bisoxirane	NA	NA	NA	No
16096-31-4	1,6-Bis(2,3-epoxypropoxy)hexane	NA	NA	NA	No
16403-84-2	4-[(5-Carbamoyl-o-tolyl)azo]-3-hydroxynaphth-2-anilide	NA	NA	NA	No
25188-42-5	7-Benzamido-4-hydroxy-3-[[4-[(4-sulphophenyl)azo]phenyl]azo]naphthalene-2-sulphonic acid	NA	NA	NA	No
28804-47-9	Methyl toluenesulphonate	NA	NA	NA	No
31482-56-1	3-[Ethyl[4-[(4-nitrophenyl)azo]phenyl]amino]propiononitrile	NA	NA	NA	No
36215-07-3	1-Chloro-3-methoxypropane	NA	NA	NA	No
50593-68-5	3-Chloro-6-nitro-1H-indazole	NA	NA	NA	No
52373-93-0	1-Amino-4-(ethylamino)-9,10-dihydro-9,10-dioxoanthracene-2,3-dicarbonitrile	NA	NA	NA	No
56396-10-2	4-[[5-(Anilino)carbonyl-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide	NA	NA	NA	No
62570-50-7	1-Amino-4-(ethylamino)-9,10-dihydro-9,10-dioxoanthracene-2-carbonitrile	NA	NA	NA	No
68818-86-0	9,10-Diethoxyanthracene	NA	NA	NA	No
Category 3B					
1719-57-9	Chloro(chloromethyl)dimethylsilane	NA	±	NA	No
6471-50-7	4-[(4-Chloro-2-nitrophenyl)azo]-3-hydroxy-N-(2-methylphenyl)naphthalene-2-carboxamide	NA	±	NA	No
12236-64-5	N-[4-(acetyl amino)phenyl]-4-[[5-(aminocarbonyl)-2-chloro phenyl]azo]-3-hydroxynaphthalene-2-carboxamide	NA	±	NA	No
36968-27-1	4-[[4-(Aminocarbonyl)phenyl] azo]-3-hydroxy-N-(2-methoxyphenyl) naphthalene-2-carboxamide	NA	±	NA	No
51920-12-8	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[5-methoxy-2-methyl-4-[(methylamino)sulphonyl]phenyl]azo]naphthalene-2-carboxamide	NA	±	NA	No
59487-23-9	4-[[5-[[[4-(aminocarbonyl)phenyl]amino]carbonyl]-2-methoxyphenyl]azo]-N-(5-chloro-2,4-dimethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	NA	±	NA	No
61847-48-1	Methyl 4-[[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[2-hydroxy-3-[[[(2-methoxy phenyl)amino]carbonyl]-1-naphthyl]azo]benzoate	NA	±	NA	No
67990-05-0	N-(5-chloro-2-methoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino) carbonyl]phenyl]azo]naphthalene-2-carboxamide	NA	±	NA	No
68516-75-6	N,N'-naphthalene-1,5-diyl bis[4-[(2,3-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	NA	±	NA	No
Category 4					
4378-61-4	4,10-Dibromodibenzo[def, mno]chrysene-6,12-dione	NA	-	NA	No
6448-95-9	3-Hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-phenyl naphthalene-2-carboxamide	NA	-	NA	No
12225-06-8	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-[(phenyl amino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	NA	-	NA	No
74336-59-7	3-[(4-Chloro-2-nitrophenyl) azo]-2-methylpyrazolo[5,1-b]quinazolin-9(1H)-one	NA	-	NA	No
Category 5					
106-50-3	p-Phenylenediamine	-		NA	Yes
128-95-0	1,4-Diaminoanthraquinone	-		NA	Yes

CAS number	Chemical name	Final call previous evaluation	Final call EURL ECVAM and/or ECHA database	Harmonized CLP class for mutagenicity	Training set mutagen
39817-09-9	2,2'-[Methylenebis(phenyleneoxymethylene)]bisoxirane	-		NA	No

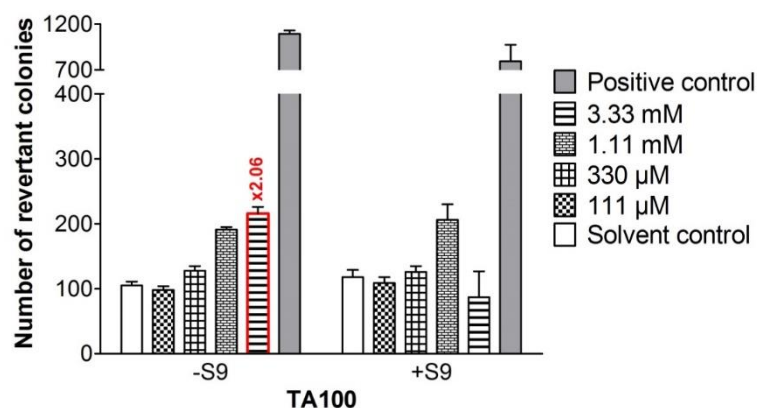
Fig. S1: Ames test results for the study substances in category 3A

Mean and standard deviation of revertant colonies counted in triplicate plates are expressed as a function of tester strain and metabolic condition. Substances were tested in the absence and presence of S9 metabolization mix, first on *Salmonella typhimurium* strain TA100 and, if negative in the former, on strain TA98. In the absence of S9 metabolization mix, sodium azide (11 µM) and 4-nitroquinoline-n-oxide (0.39 µM) served as the positive control in strain TA100 and TA98, respectively, whereas 2-aminoanthracene (1.9 µM) was used in both strains in the presence of S9 metabolization mix. Aromatic (di)azo substances were tested directly in strain TA98 under the modified conditions recommended for this type of compounds, with Congo red (53 µM) as a positive control. For positive results, the induction factor (i.e., multiple of solvent control) is indicated in red.

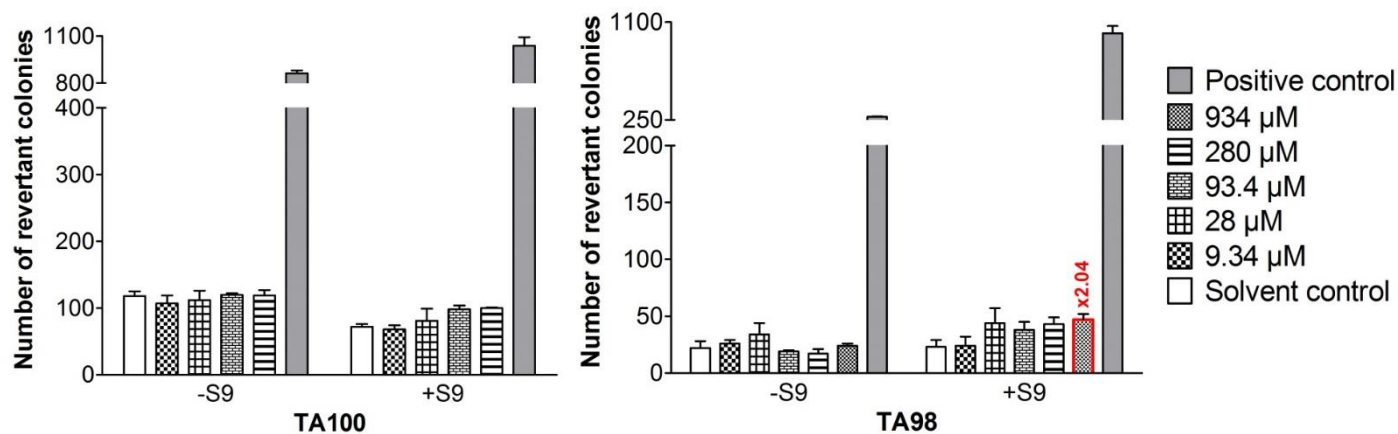
1-(Methylamino)anthraquinone (CAS # 82-38-2)



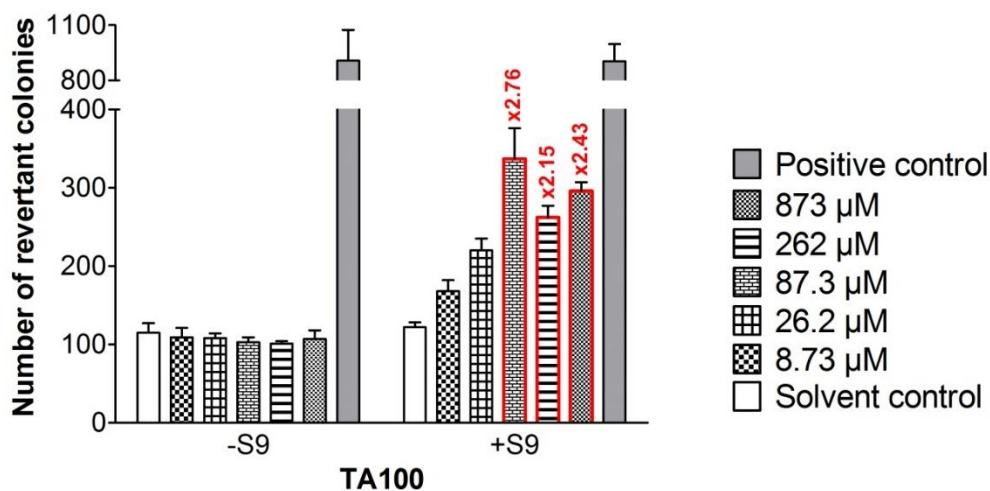
2,4,6-Trimethylbenzoyl chloride (CAS # 938-18-1)



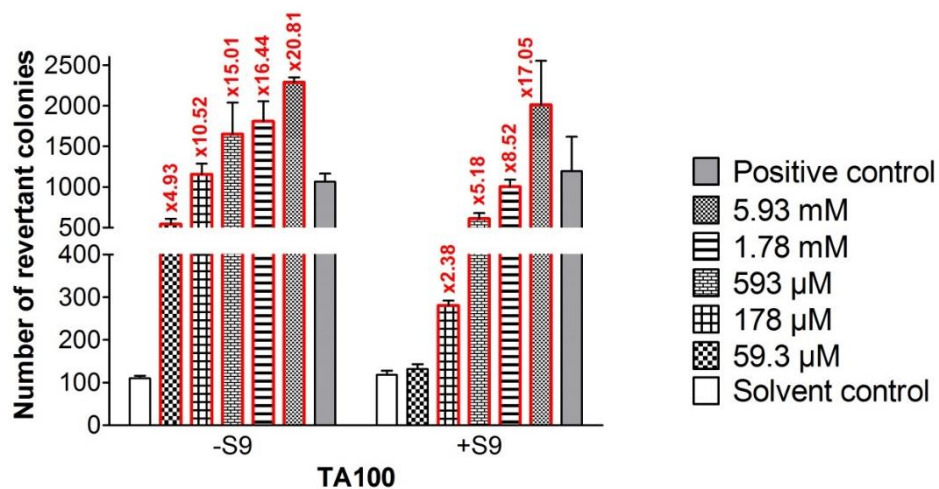
2,4'-Methylenedianiline (CAS # 1208-52-2)



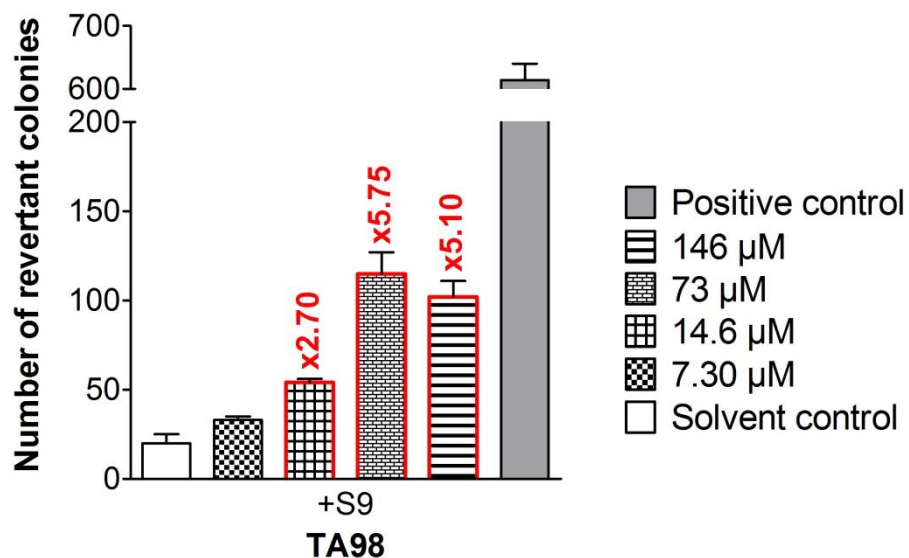
4-Aminonaphthalene-1,8-dicarboximide (CAS # 1742-95-6)



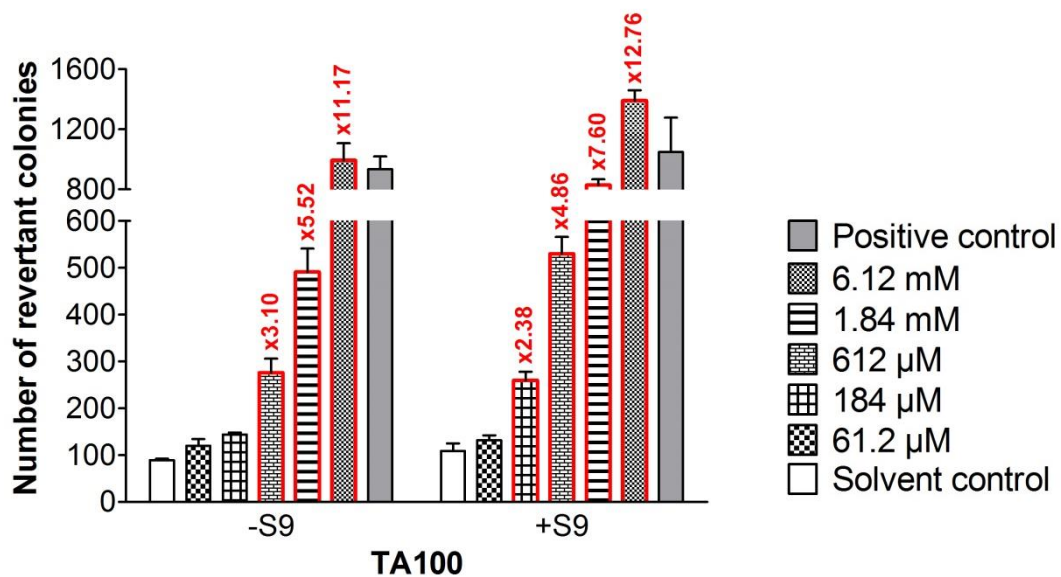
2,2'-[Methylenebis(p-phenyleneoxymethylene)]bisoxirane (CAS # 2095-03-6)



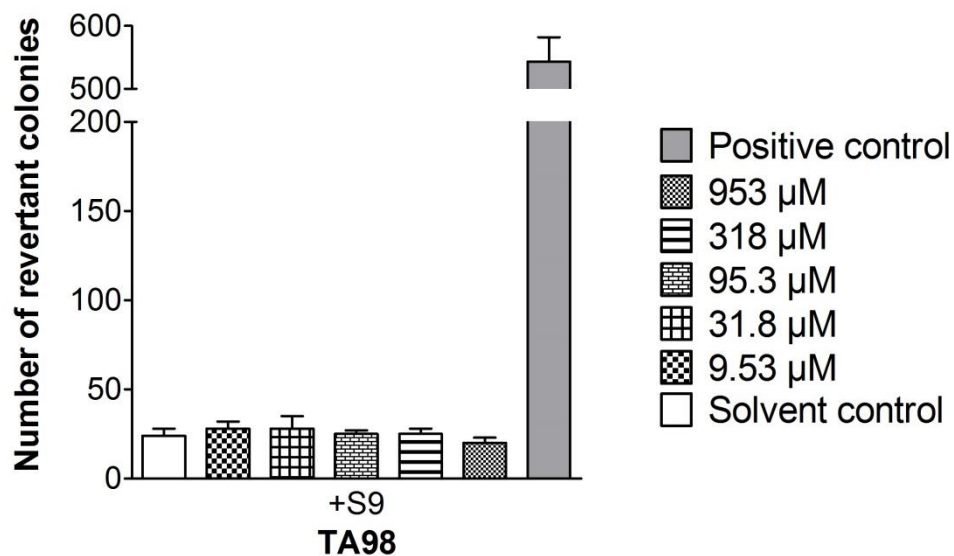
1-[[3-Methyl-4-[(3-methylphenyl)azo]phenyl]azo]-2-naphthol (CAS # 3176-79-2)



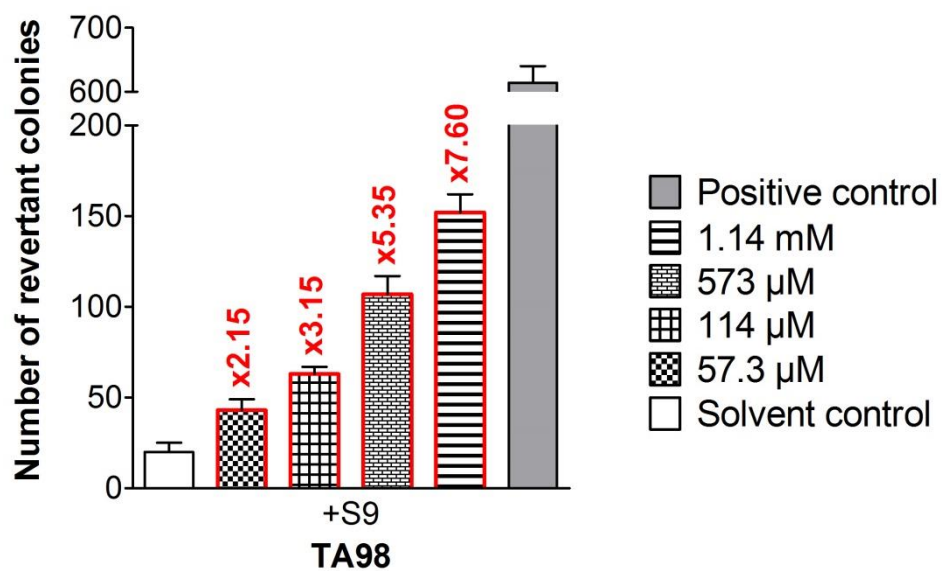
1-(2,3-Epoxypropoxy)-2,2-bis[(2,3-epoxypropoxy)methyl]butane (CAS # 3454-29-3)



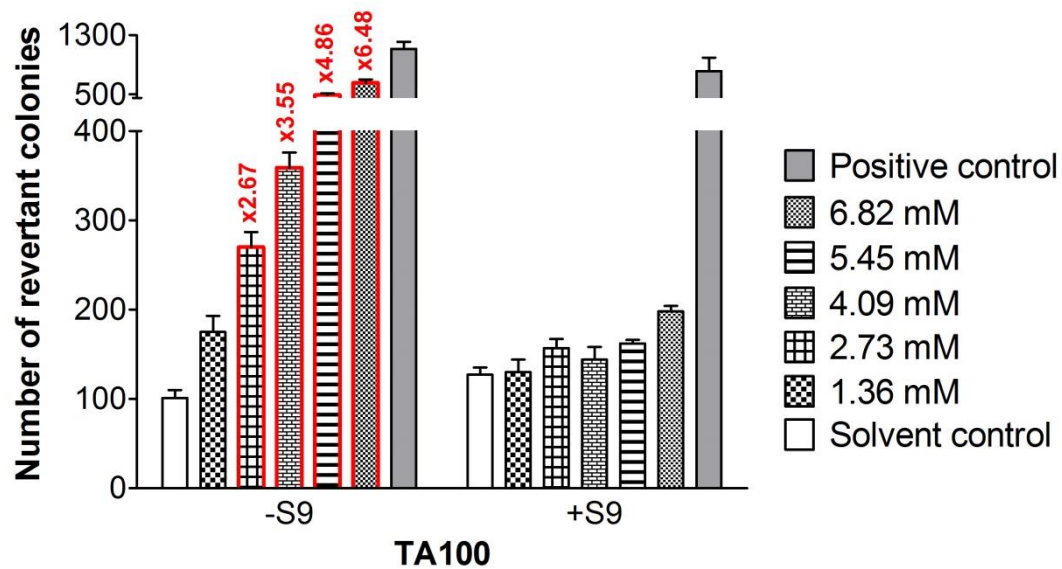
4-[(2,5-Dichlorophenyl)azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide (CAS # 6410-38-4)



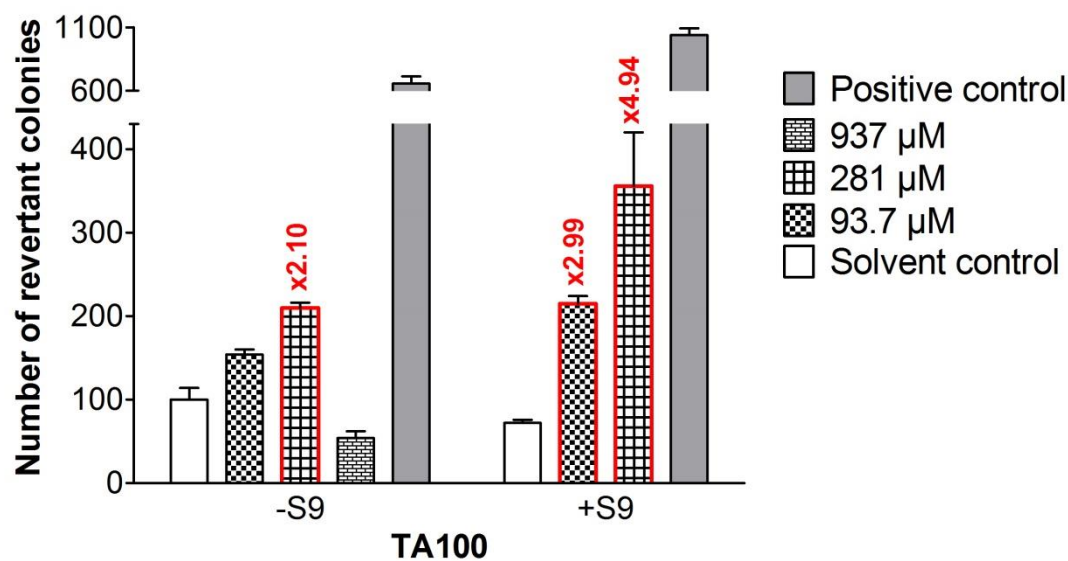
3-[Ethyl[4-[(4-nitrophenyl)azo]phenyl]amino] propiononitrile (CAS # 31482-56-1)



1-Chloro-3-methoxypropane (CAS # 36215-07-3)



3-Chloro-6-nitro-1H-indazole (CAS # 50593-68-5)



9,10-Diethoxyanthracene (CAS # 68818-86-0)

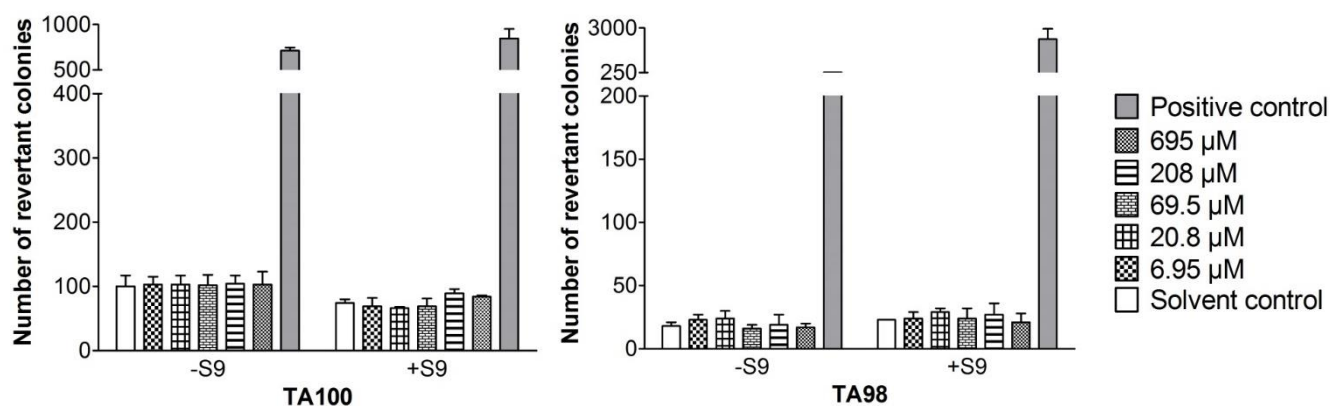
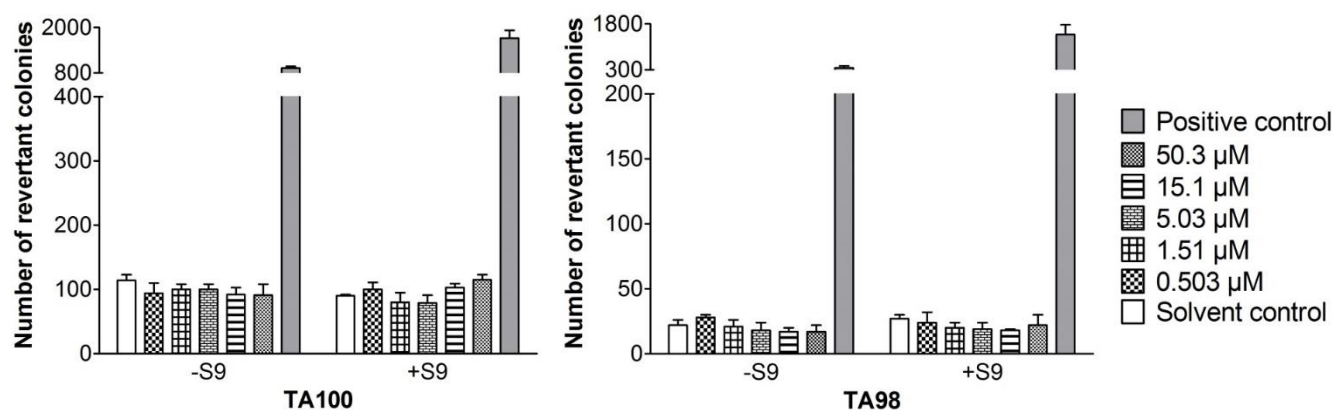


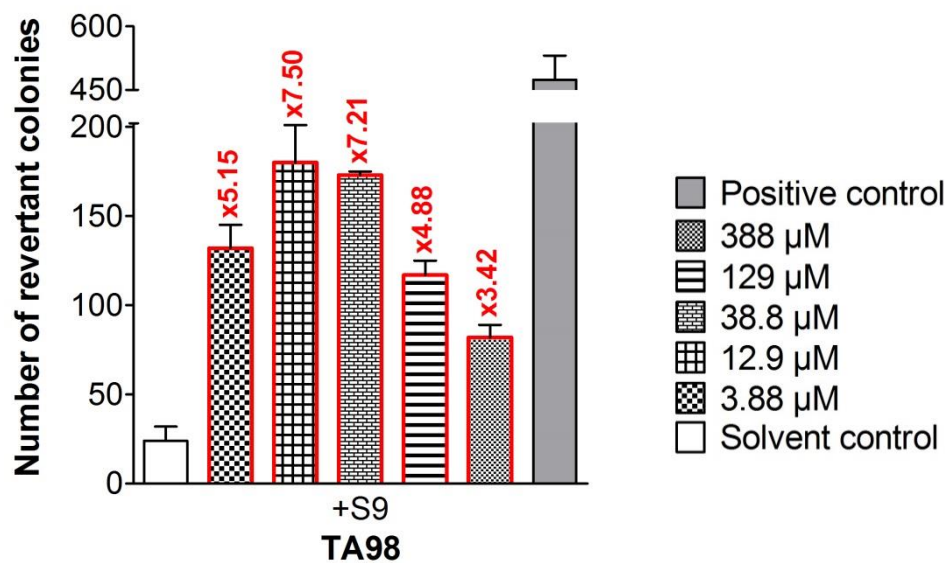
Fig. S2: Ames test results for the study substances in category 3B and 4

Mean and standard deviation of revertant colonies counted in triplicate plates are expressed as a function of tester strain and metabolic condition. Substances were tested in the absence and presence of S9 metabolism mix, first on *Salmonella typhimurium* strain TA100 and, if negative in the former, on strain TA98. In the absence of S9 metabolism mix, sodium azide (11 μ M) and 4-nitroquinoline-n-oxide (0.39 μ M) served as the positive control in strain TA100 and TA98, respectively, whereas 2-aminoanthracene (1.9 μ M) was used in both strains in the presence of S9 metabolism mix. Aromatic (di)azo substances were tested directly in strain TA98 under the modified conditions recommended for this type of compounds, with Congo red (53 μ M) as a positive control. For positive results, the induction factor (i.e., multiple of solvent control) is indicated in red.

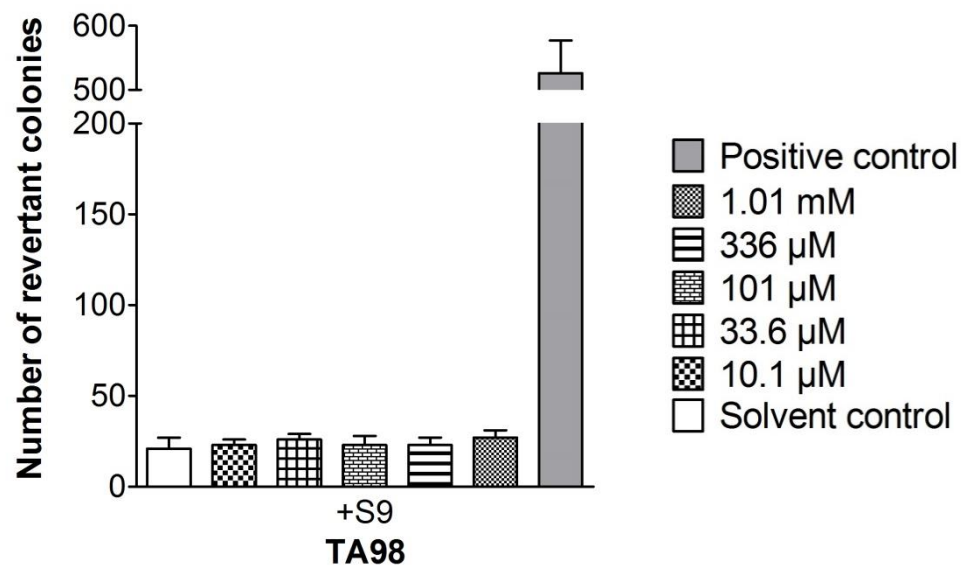
4,10-Dibromodibenzo[def,mno]chrysene-6,12-dione (CAS # 4378-61-4)



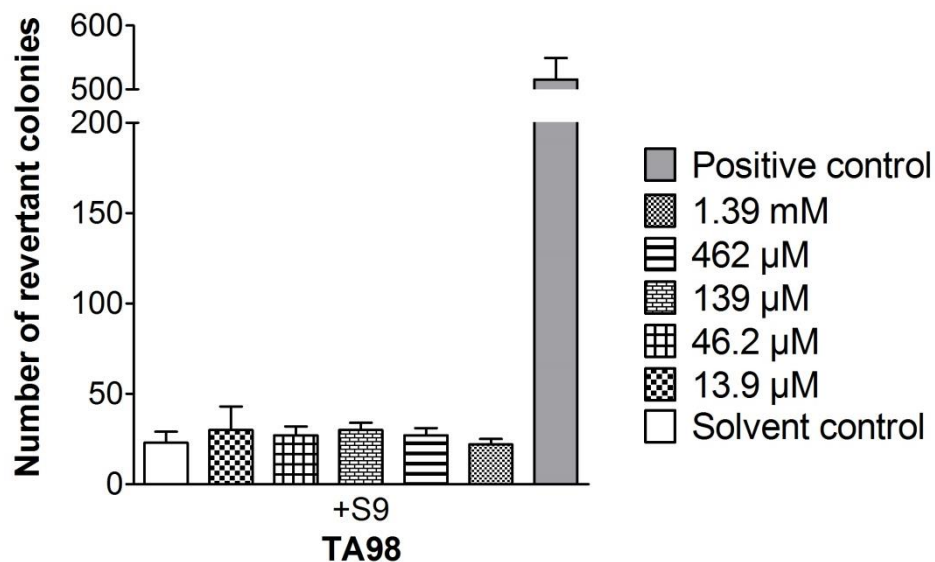
N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino) carbonyl]phenyl]azo]naphthalene-2-carboxamide (CAS # 12225-06-8)



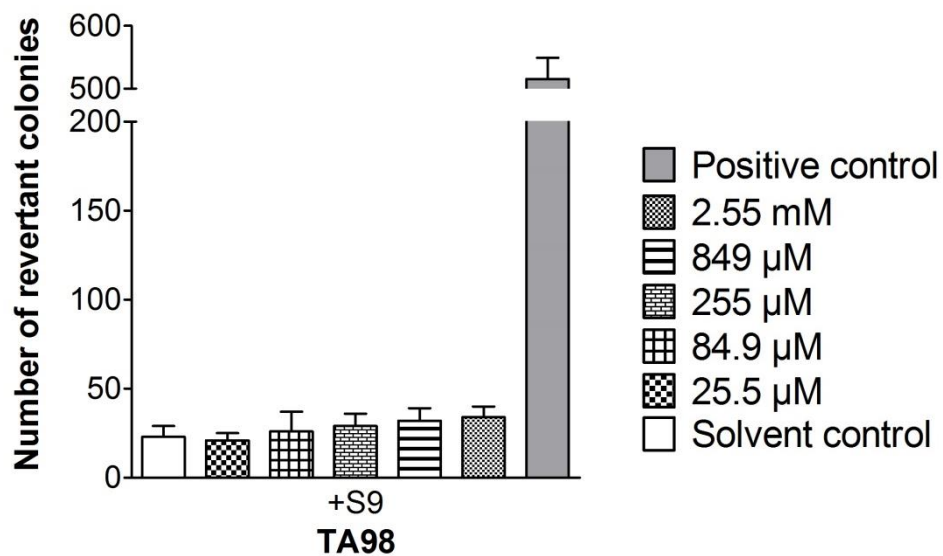
4-[[4-(Aminocarbonyl)phenyl]azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide (CAS # 36968-27-1)



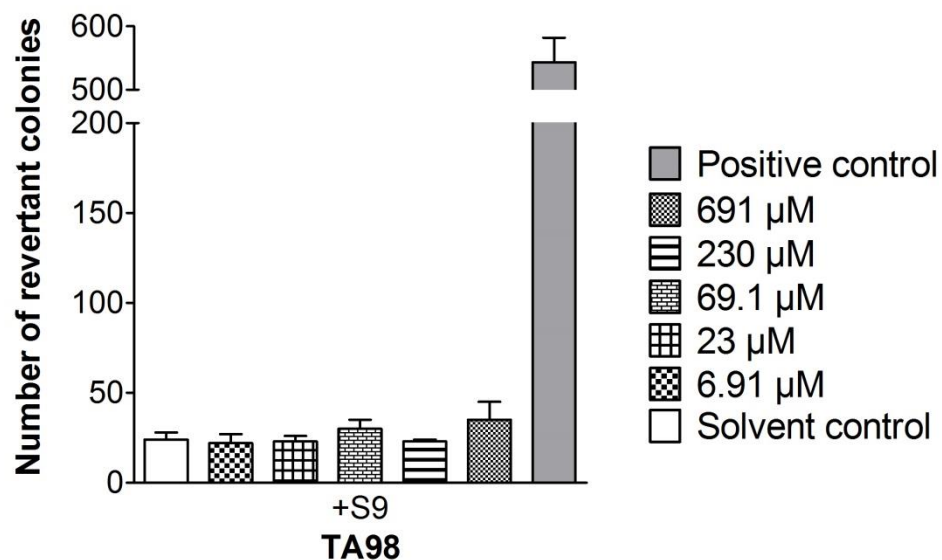
N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[5-methoxy-2-methyl-4-[(methylamino)sulphonyl]phenyl]azo] naphthalene-2-carboxamide (CAS # 51920-12-8)



4-[[5-[[[4-(aminocarbonyl)phenyl]amino] carbonyl]-2-methoxyphenyl]azo]-N-(5-chloro-2,4-dimethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide (CAS # 59487-23-9)



Methyl 4-[[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[[2-hydroxy-3-[[[(2-methoxyphenyl)amino] carbonyl]-1-naphthyl]azo]benzoate (CAS # 61847-48-1)



N,N'-naphthalene-1,5-diylbis[4-[[[(2,3-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide] (CAS # 68516-75-6)

