Data Interpretation: Case Studies

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DATA INTERPRETATION CRITICAL FACTORS

Experimental Protocol

- GLP compliance
- Experimental protocol: deviation from the guidelines
- Test compound: characterization, dilution (solvent, culture medium, limits in solubility)
- The solvent/vehicle should not:
  - ✓ react with the test substance,
  - ✓ be incompatible with the survival of the cells or with the maintenance of S9 activity at the concentration used.

Common solvents: water, dimethyl sulfoxide, any other without genotoxic activity. 1% in culture medium.

DATA INTERPRETATION CRITICAL FACTORS

Experimental Protocol

- Controls

  negative (solvent)
  positive (list of known mutagens for each specific endpoint)
  historical controls

- Exposure concentrations

  range of doses
  highest concentration toxic effects

If no cytotoxicity or precipitate is observed, the highest test concentration should correspond to 0.01 M, 5 mg/mL or 5 µl/mL,

For test substances that exhibit a steep concentration-response curve, it may be necessary to more closely space the test substance concentrations so that cultures in the moderate and low toxicity ranges could be considered

DATA INTERPRETATION CRITICAL FACTORS

Experimental Protocol

- Time of treatment
  defined for each specific endpoint

Standard test procedures may have to be modified if substances are not taken up readily and longer incubation times may be required to ensure the intracellular bioavailability of the test substance, as may be the case for **water-insoluble metal compounds.**

Another example is the testing of **nanomaterials** which require careful characterisation of the respective material, not only as added but also in cell culture medium, and may require modification of standard protocols

DATA INTERPRETATION CRITICAL FACTORS

Results

- **Statistical significance/statistical power**

- **Biological relevance**:

  - **extent of effects**
    a positive result from an in vitro test in mammalian cells would be considered of limited or even no relevance if the effect was observed only at highly cytotoxic concentrations.

  - **dose response**

  - **test sensitivity**

  - **reproducibility** If conflicting results that were produced with tests that have similar reliability were observed , it should be judged whether this might be attributable to differences in specific test conditions, e.g. concentrations, animal strains, cell lines, exogenous metabolising systems, etc.

DATA INTERPRETATION CRITICAL FACTORS

Results

- **Comparison of the magnitude of the marginal increase to historical negative control**

- **Target organs/surrogate tissue**
  negative result from an in vivo study would have limited or even no relevance if there was no indication from the study that the test substance reached the target tissue and if there were no other data,e.g. toxicokinetic data, on which such an assumption could be based

- **Other data**: toxic effects
  absorption, distribution, metabolism, excretion (ADME), systemic availability, exposed organs, carcinogenicity tests

Evaluation of the results of genotoxicity tests

Positive results of an in vitro/in vivo test indicate that the tested substance is genotoxic under the conditions of the assay performed

Negative results of an in vitro/in vivo test indicate that the test substance is not genotoxic under the conditions of the assay performed. In the case of in vivo studies, when negative results are obtained, it is important to demonstrate that the substance reaches the target tissue.

Equivocal results of an *in vitro/in vivo test* refers to a situation where not all the requirements for a clear positive result have been met. An example could be where a positive trend was observed, but the dose-response relationship is not statistically significant *Equivocal results are generally less relevant than clearly positive results, however, they may be considered as an indication for a possible genotoxic potential which should be clarified by further testing .*

Inconclusive results of an *in vitro/in vivo test* where no clear result was achieved as a consequence of some limitation of the test or procedure.

Genotoxic risk

Decisions about genotoxic risk are based on qualitative factors that classify an agent as “positive” or “negative” in a test battery and supplementary tests

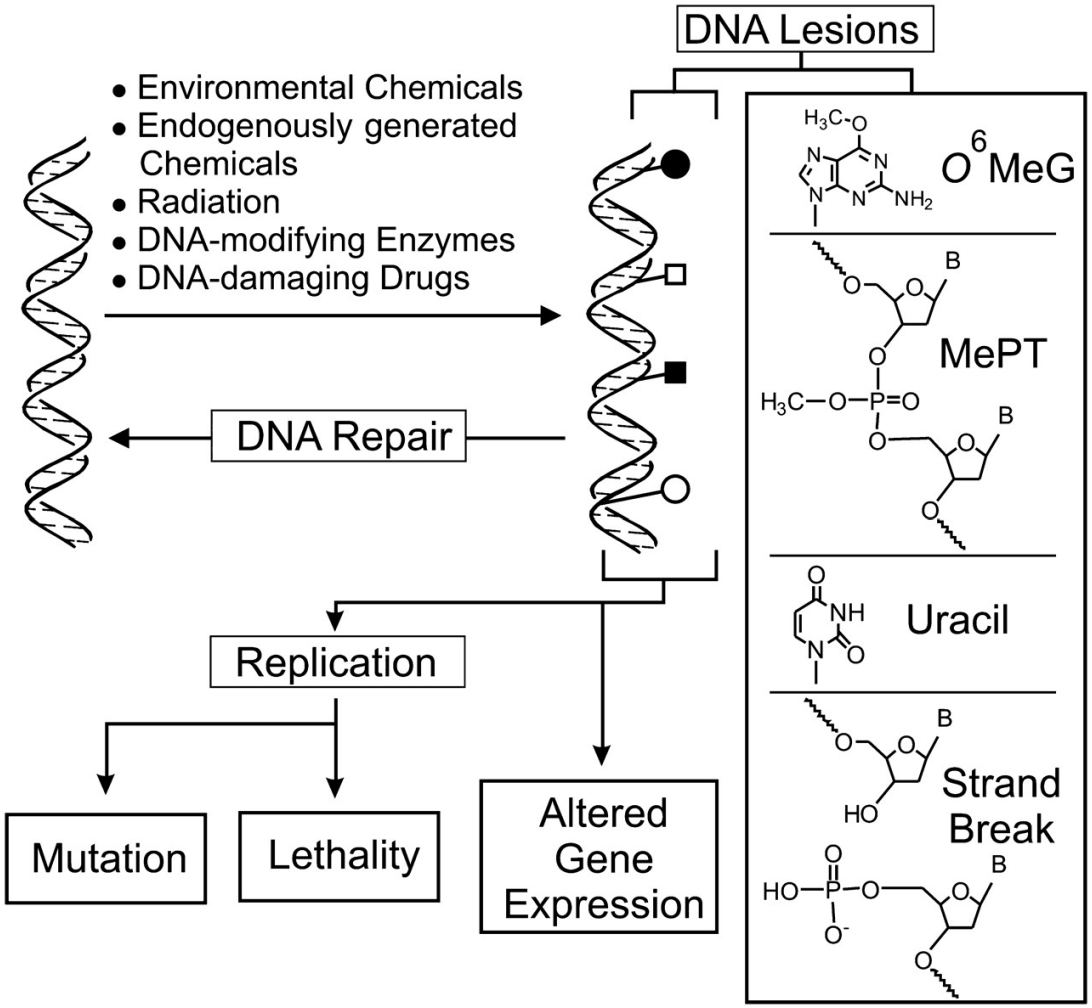
Direct genotoxic agents (DNA interacting compounds) follow linear models when extrapolating low-dose effects from experimental data. Safe exposure for human cannot be established.

Indirect genotoxic agents (interacting with non-DNA targets) are expected to show threshold concentration-effect response curves

Threshold dose: *The actual dose below which there is no increase* in effect over the background level. (Severe practical issues make the unequivocal experimental identification of the threshold dose difficult and a practical threshold is often derived.)

Practical Threshold: *The point where the experimentally derived dose* response relationship first exceeds the background variability in a suitably designed well conducted study.

Pathways by which DNA-damaging agents induce biologically relevant events



miscoding lesion during replication
G ➡ A transition

modifications of the sugar-phosphate backbone, such as MePT, methylphosphotriester which modify the epigenetic program and induce a change in gene expression.

can appear as the enzymatic deamination product of cytosine
miscoding lesion

Block of DNA and RNA polymerase

Shrivastav, N. et al. Carcinogenesis 2010 31:59-70; doi:10.1093/carcin/bgp262

INDIRECT GENOTOXICITY

Indirect mechanisms of genotoxicity can be defined as interactions with non-DNA targets:

lipid peroxidation and protein adducts

•inhibition of repair enzymes (e.g. OGG1, XPD)
Heavy metals

•cell cycle control proteins (e.g. p53, Rb,cyclins)

•apoptosis related gene products (e.g.p53, bax, bcl-2)

•defence proteins against oxidative damage (glutathione)
chloroform, carbon tetrachloride

•metabolization enzymes

•tubulines of the mitotic/meiotic spindle apparatus
methyl benzimidazole carbamates

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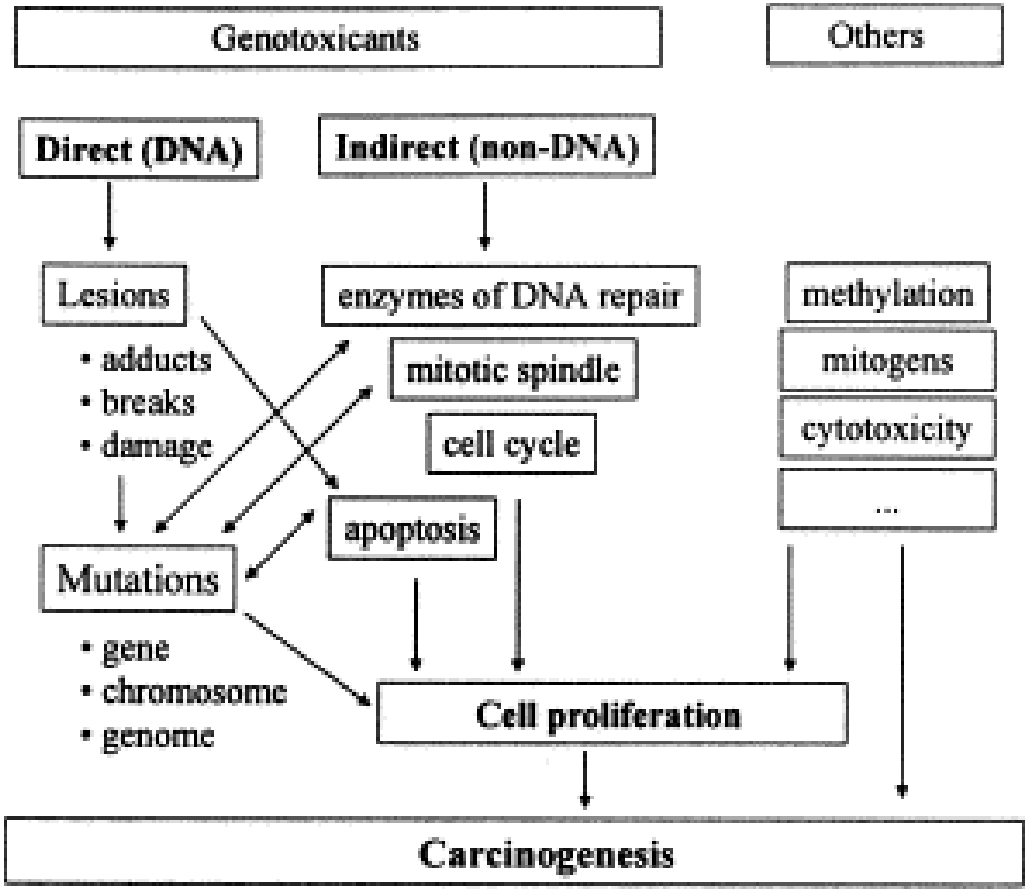
Indirect genotoxic agents



Chemical	Chromosome loss (μM)	Chromosome non-disjunction (μM)
Colchicine	0.033	0.02
Mebendazole	0.29	0.23
Carbendazim	2.47	2.85
Nocodazole	0.053	0.032

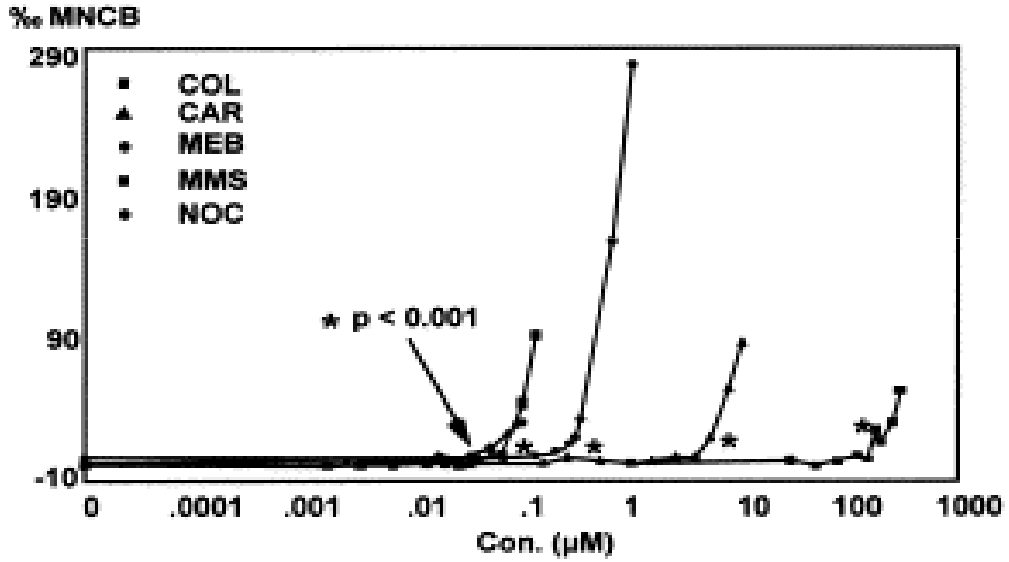


Fig. 4. Thresholds for induction of chromosome loss by aneugens as determined on flow-sorted MN painted by FISH with a general alphoid centromeric probe. COL, colchicine; CAR, carbendazim; MEB, mebendazole; MMS, methanesulfonate; NOC, nocodazole.

Proposals for threshold for in vivo mutagens

Direct-acting small molecular weight alkylating agents

Overload of detoxification pathways Es. paracetamol

Multiple mode of action of genotoxicity Es. acrilamide

Local effects and rapid detoxification Es formaldeyde

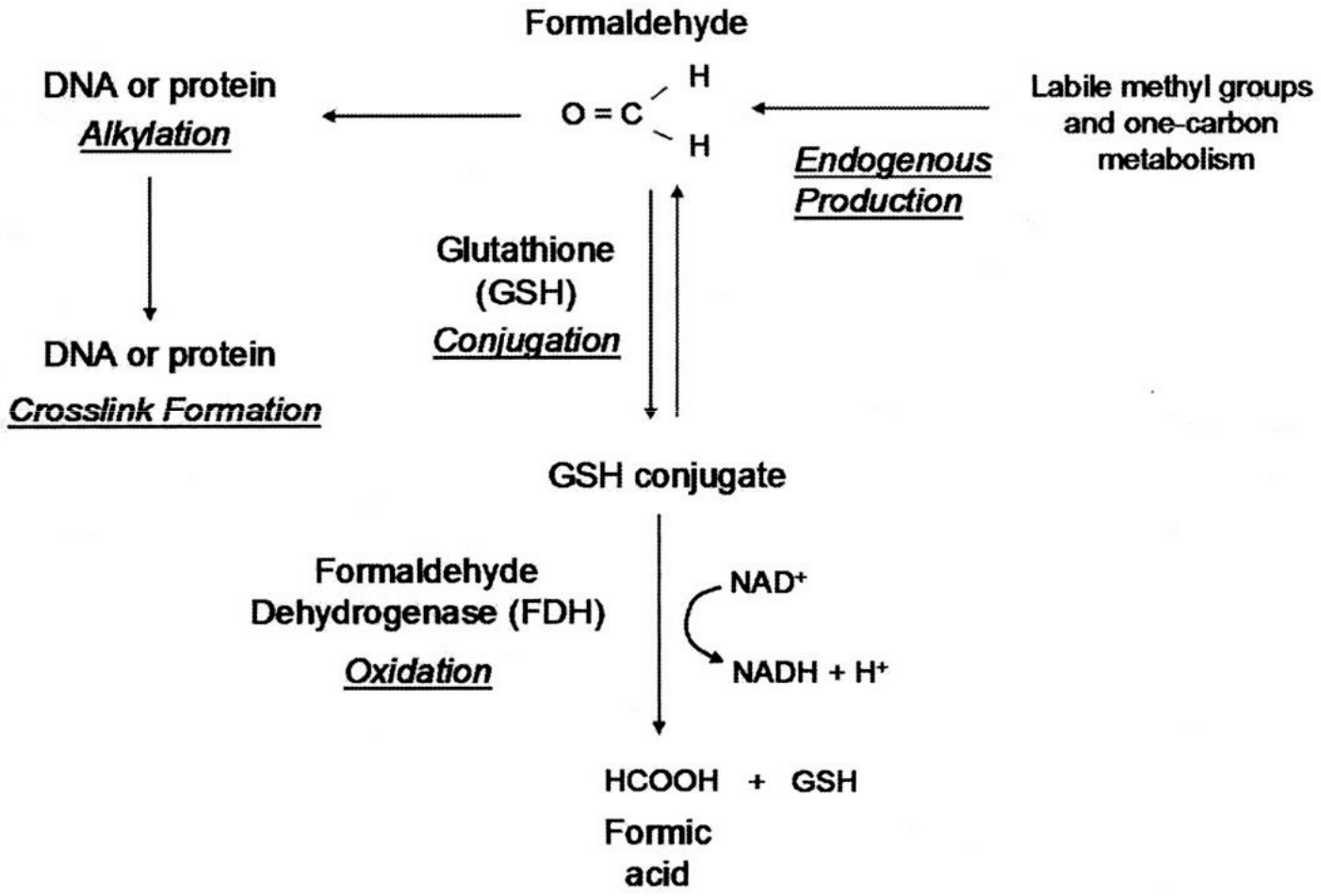


FIG. 1. Primarily metabolic pathway of formaldehyde biotransformation.

Table 37. Genetic and related effects of formaldehyde in experimental systems and animals

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Misincorporation of DNA bases into synthetic polynucleotides *in vitro*	+	NT	30	Snyder & Van Houten (1986)
pUC13 plasmid DNA bound to calf-thymus histones, DNA–protein cross-links	+	NT	0.0075	Kuykendall & Bogdanffy (1992)
Escherichia coli PQ37, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	20	Le Curieux *et al.* (1993)
Escherichia coli K12 (or *E. coli* DNA), DNA strand breaks, cross-links or related damage; DNA repair	+	NT	60	Poverenny *et al.* (1975)
Escherichia coli K12, DNA strand breaks, cross-links or related damage; DNA repair	+	NT	600	Wilkins & MacLeod (1976)
Escherichia coli K12 KS160-KS66 *pol*AI, differential toxicity	+	NT	60	Poverenny *et al.* (1975)
*Escherichia coli pol*A$^+$/W3110 and *pol*A$^-$ p3478, differential toxicity (spot test)	+	NT	10 µL of pure substance	Leifer *et al.* (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	60 µg/plate	Gocke *et al.* (1981)
Salmonella typhimurium TA100, reverse mutation	–	+	10 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	(+)	+	30 µg/plate (toxic above 125 µg/plate)[c]	Connor *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	+	+[d]	9 µg/plate	Pool *et al.* (1984)
Salmonella typhimurium TA100, reverse mutation	(+)	NT	51 µg/plate[c]	Marnett *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	+	NT	6 µg/plate	Takahashi *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	(+)	+	3	Schmid *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation	+	NT	9.3	O'Donovan & Mee (1993)
Salmonella typhimurium TA100, TA104, reverse mutation	+	+	6.25–50 µg/plate	Dillon *et al.* (1998)
Salmonella typhimurium TA102, TA104, reverse mutation	+	NT	21 µg/plate[c]	Marnett *et al.* (1985)

LED, lowest effective dose; HID, highest ineffective dose

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

UK Independent Scientific Advisory Committees

GUIDANCE STATEMENT : THRESHOLDS FOR *IN VIVO MUTAGEN ,2010*

i) The COM reaffirmed the default position that for *in vivo mutagens, in the absence of mechanistic data to* infer a threshold, it is prudent to assume that there is no threshold for mutagenicity.

ii) If there is good reason to consider that a threshold mode of action is appropriate, then it is necessary to investigate the biologically meaningful threshold for all genotoxic effects that have been reported.

iii) An appropriate strategy should be devised for each chemical under consideration to identify threshold dose levels or NOELs for all potential thresholded modes of action of genotoxicity, which may include either *in vitro* or *in vivo studies*

Other mechanisms for carcinogenesis

- receptor-mediated effects,
- cellular toxicity with regenerative cell division
- peroxisome proliferation
- hormonal dysregulation
- immune suppression
- formation of calculi and other deposits that cause chronic irritation

IARC MONOGRAPHS

SUMMARY OF EVALUATIONS – VOLUME 100A

	Agent	Group†	Human evid‡	Sites on which *sufficient* human evidence is based	Other sites with limited human evid	Animal evid‡	Sites on which *sufficient* animal evidence is based	Established mechanistic events	Other likely mechanistic events
ANTI-CANCER DRUGS									
1	**Busulfan**	**1**	**S**	AML		**L**		Genotoxicity, alkylating agent	
2	**Chlorambucil**	**1**	**S**	AML		**S**	M: lympho, lung R: lympho	Genotoxicity, alkylating agent	Immunosuppression
3	**Semustine (Methyl-CCNU)**	**1**	**S**	AML		**L**		Genotoxicity, alkylating agent	
4	**Cyclophosphamide**	**1**	**S**	AML, bladder		**S**	M: mammary, lung, lymph R: bladder, mammary	Genotoxicity, bladder inflammation	Immunosuppression
5	**Etoposide + cisplatin&bleomycin**	**1**	**S**	AML		**ND**		Genotoxicity, translocations in MLL gene	
	Etoposide Group 2A in 2000	**1 NEW**	**L**			**I**		Genotoxicity, translocations in MLL gene	
6	**Melphalan**	**1**	**S**	AML		**S**	M: lung, skin	Genotoxicity, alkylating agent	
7	**MOPP**	**1**	**S**	AML, lung		**ND**		Genotoxicity	
9	**Thiotepa**	**1**	**S**	leukaemia		**S**	M: lymphohaematopoietic R: lymphohaematopoietic	Genotoxicity	
10	**Treosulfan**	**1**	**S**	AML		**ND**		Genotoxicity	

HORMONAL DRUGS

	Agent	Group[†]	Human evid[‡]	Sites on which *sufficient* human evidence is based	Other sites with limited human evid	Animal evid[‡]	Sites on which *sufficient* animal evidence is based	Established mechanistic events	Other likely mechanistic events
8	**Tamoxifen**	**1**	**S**	endometrium ESLC: breast (reduces risk)		**S**	R: liver adenocarc	receptor-mediated (uterus), genotoxicity	
	Estrogens, nonsteroidal	**GR**							
11	**Diethylstilbestrol**	**1**	**S**		Endometrium	**S**	M: uterus, cervix, vagina, ovary, testis, pituitary (female), lymph (female) R: vagina, uterus, mammary H: kidney (male)	Genotoxicity, ER-mediated events, including mitogenesis	
				Breast (exposure during pregnancy)				Genotoxicity	
				Vagina (CCA, exposure in utero) Cervix (CCA, exposure in utero)	Cervix (SCC, exposure in utero) Testis (exposure in utero)			Genotoxicity, ER-mediated events, including mitogenesis	Epigenetic programming (perinatal exposure)
	Estrogens, steroidal	**GR**							
12	**Estrogen-only menopausal therapy**	**1**	**S**	Endometrium, ovary ESLC: colorectum	Breast	**S**	Estradiol M: mammary R: mammary H: kidney Estrone M: mammary	Receptor mediated, tissue specific, agent specific cell prolif (uterus)	Genotoxicity Receptor mediated, tissue specific, agent specific cell prolif (breast)
13	**E-P menopausal therapy, Combined**	**1**	**S**	Breast Endometrium (increased E-induced risk decreases with #days with P) ESLC: colorectum		**L**		Receptor mediated, tissue specific, agent specific cell prolif	Estrogen genotoxicity; stromal paracrine mediated effects

	Agent	Group†	Human evid‡	Sites on which *sufficient* human evidence is based	Other sites with limited human evid	Animal evid‡	Sites on which *sufficient* animal evidence is based	Established mechanistic events	Other likely mechanistic events
4	**E-P contraceptives, Oral combined**	**1**	**S**	Breast, cervix, liver ESLC: endometrium (reduces risk); ovary (reduces risk); colorectum		**S**	R: mammary, liver M: mammary	Receptor mediated, tissue specific, agent specific cell prolif	Estrogen genotoxicity; stromal paracrine mediated effects; Estrogen-stimulated expression of HPV genes
	E-P contraceptives, sequential	**1**							
THERS									
5	**Azathioprine**	**1**	**S**	NHL, skin (SCC)		**S**	M: lymphohaematopoietic	Immunosuppression, DNA damage	
6	**Chlornaphazine**	**1**	**S**	bladder		**L**		metabolism to 2-naphthylamine derivatives, alkylation(?)	
7	**Cyclosporine**	**1**	**S**	NHL, skin (non-melanocytic), multiple other sites		**L**		immunosuppression	DNA damage (ox stress), DNA repair
8	**Plants containing aristolochic acid [new name]**	**1**	**S**	Renal pelvis, ureter		**S(extracts)**	R: renal pelvis	DNA adducts in humans A:T→T:A transversions in human humours in *p*53	
	Aristolochic acid (Group 2A in 2002)	**1 NEW**	**L**			**S**	R: forestomach, renal pelvis	- DNA adducts formed in animals are the same as those found in humans exposed to plants - A:T→T:A transversions in *p*53 - ras activation	

CASE STUDIES

- Technical reports
- Opinions on cosmetic ingredients

Results obtained with *S. typhimurium* TA98: test 1 (range-finding)

Plate No.	Addition		S9 mix + present - absent	Revertant colony counts* and means				
				A	B	C	Mean	sd
1+	None; S9 mix sterility check		+	0	0	0	0	0
1 -	None; buffer sterility check		-	0	0	0	0	0
2	Technical AE 0172747; sterility check	(5000 µg/plate)	-	0	0	0	0	0
3	Technical AE 0172747	(5000 µg/plate)	+	45	42	65	51	13
4	Technical AE 0172747	(1500 µg/plate)	+	51	41	60	51	10
5	Technical AE 0172747	(500 µg/plate)	+	41	45	46	44	3
6	Technical AE 0172747	(150 µg/plate)	+	39	48	49	45	6
7	Technical AE 0172747	(50 µg/plate)	+	52	42	49	48	5
8	Technical AE 0172747	(15 µg/plate)	+	49	38	57	48	10
9	Technical AE 0172747	(5 µg/plate)	+	61	42	49	51	10
10	DMSO	(0.1 ml/plate)	+	53	42	43	46	6
11	Technical AE 0172747	(5000 µg/plate)	-	34	26	49	36	12
12	Technical AE 0172747	(1500 µg/plate)	-	44	42	39	42	3
13	Technical AE 0172747	(500 µg/plate)	-	34	37	49	40	8
14	Technical AE 0172747	(150 µg/plate)	-	26	24	42	31	10
15	Technical AE 0172747	(50 µg/plate)	-	27	34	49	37	11
16	Technical AE 0172747	(15 µg/plate)	-	44	39	38	40	3
17	Technical AE 0172747	(5 µg/plate)	-	46	55	41	47	7
18	DMSO	(0.1 ml/plate)	-	44	41	44	43	2
19	Benzo[a]pyrene	(5 µg/plate)	+	460	753	749	654	168
20	2-Nitrofluorene	(1 µg/plate)	-	2833	2332	2542	2569	252
21	None; 10^{-6} dilution of overnight culture, plated on nutrient agar		-	148	130	135	138	9

TABLE 2

Results obtained with *S. typhimurium* TA98: test 2, with pre-incubation

Plate No.	Addition		S9 mix + present - absent	Revertant colony counts* and means				
				A	B	C	Mean	sd
1+	None; S9 mix sterility check		+	0	0	0	0	0
1 -	None; buffer sterility check		-	0	0	0	0	0
2	Technical AE 0172747; sterility check	(5000 µg/plate)	-	0	0	0	0	0
3	Technical AE 0172747	(5000 µg/plate)	+	34	36	29	33	4
4	Technical AE 0172747	(1500 µg/plate)	+	22	23	44	30	12
5	Technical AE 0172747	(500 µg/plate)	+	22	34	35	30	7
6	Technical AE 0172747	(150 µg/plate)	+	29	32	35	32	3
7	Technical AE 0172747	(50 µg/plate)	+	38	26	37	34	7
8	DMSO	(0.1 ml/plate)	+	30	39	34	34	5
9	Technical AE 0172747	(5000 µg/plate)	-	29	28	30	29	1
10	Technical AE 0172747	(1500 µg/plate)	-	29	30	27	29	2
11	Technical AE 0172747	(500 µg/plate)	-	37	30	30	32	4
12	Technical AE 0172747	(150 µg/plate)	-	22	37	35	31	8
13	Technical AE 0172747	(50 µg/plate)	-	30	22	35	29	7
14	DMSO	(0.1 ml/plate)	-	28	36	36	33	5
15	Benzo[a]pyrene	(5 µg/plate)	+	478	545	571	531	48
16	2-Nitrofluorene	(1 µg/plate)	-	2554	2129	2466	2383	224
17	None; 10^{-6} dilution of overnight culture, plated on nutrient agar		-	97	103	100	100	3

Results obtained with *S. typhimurium* TA100: test 1 (range-finding)

Plate No.	Addition		S9 mix + present - absent	Revertant colony counts* and means				
				A	B	C	Mean	sd
1+	None; S9 mix sterility check		+	0	0	0	0	0
1 -	None; buffer sterility check		-	0	0	0	0	0
2	Technical AE 0172747; sterility check	(5000 µg/plate)	-	0	0	0	0	0
3	Technical AE 0172747	(5000 µg/plate)	+	159	170	153	161	9
4	Technical AE 0172747	(1500 µg/plate)	+	173	154	161	163	10
5	Technical AE 0172747	(500 µg/plate)	+	174	165	170	170	5
6	Technical AE 0172747	(150 µg/plate)	+	173	198	181	184	13
7	Technical AE 0172747	(50 µg/plate)	+	155	159	196	170	23
8	Technical AE 0172747	(15 µg/plate)	+	183	158	203	181	23
9	Technical AE 0172747	(5 µg/plate)	+	205	197	165	189	21
10	DMSO	(0.1 ml/plate)	+	181	165	177	174	8
11	Technical AE 0172747	(5000 µg/plate)	-	187	174	164	175	12
12	Technical AE 0172747	(1500 µg/plate)	-	174	205	179	186	17
13	Technical AE 0172747	(500 µg/plate)	-	125	136	158	140	17
14	Technical AE 0172747	(150 µg/plate)	-	155	147	155	152	5
15	Technical AE 0172747	(50 µg/plate)	-	148	157	131	145	13
16	Technical AE 0172747	(15 µg/plate)	-	144	155	114	138	21
17	Technical AE 0172747	(5 µg/plate)	-	140	168	147	152	15
18	DMSO	(0.1 ml/plate)	-	147	165	158	157	9
19	Benzo[a]pyrene	(5 µg/plate)	+	808	801	876	828	41
20	Sodium azide	(0.5 µg/plate)	-	743	753	705	734	25
21	None; 10^{-6} dilution of overnight culture, plated on nutrient agar		-	168	199	203	190	19

TABLE 4

Results obtained with *S. typhimurium* TA100: test 2, with pre-incubation

Plate No.	Addition		S9 mix + present - absent	Revertant colony counts* and means				
				A	B	C	Mean	sd
1+	None; S9 mix sterility check		+	0	0	0	0	0
1 -	None; buffer sterility check		-	0	0	0	0	0
2	Technical AE 0172747; sterility check	(5000 µg/plate)	-	0	0	0	0	0
3	Technical AE 0172747	(5000 µg/plate)	+	146	148	130	141	10
4	Technical AE 0172747	(1500 µg/plate)	+	160	161	137	153	14
5	Technical AE 0172747	(500 µg/plate)	+	158	166	123	149	23
6	Technical AE 0172747	(150 µg/plate)	+	123	151	160	145	19
7	Technical AE 0172747	(50 µg/plate)	+	153	189	176	173	18
8	DMSO	(0.1 ml/plate)	+	168	143	159	157	13
9	Technical AE 0172747	(5000 µg/plate)	-	125	158	124	136	19
10	Technical AE 0172747	(1500 µg/plate)	-	181	126	143	150	28
11	Technical AE 0172747	(500 µg/plate)	-	146	147	159	151	7
12	Technical AE 0172747	(150 µg/plate)	-	152	133	150	145	10
13	Technical AE 0172747	(50 µg/plate)	-	141	154	119	138	18
14	DMSO	(0.1 ml/plate)	-	145	132	153	143	11
15	Benzo[a]pyrene	(5 µg/plate)	+	843	1025	844	904	105
16	Sodium azide	(0.5 µg/plate)	-	564	510	513	529	30
17	None; 10^{-6} dilution of overnight culture, plated on nutrient agar		-	115	121	147	128	17

Historical control data

Presented below are the historical control data from the period 1 May 1997 to 30 April 2002.

DMSO

	TA100		TA1535		WP2*uvr* A/pKM101 (CM891)		TA98		TA1537	
S9 Mix	-	+	-	+	-	+	-	+	-	+
Maximum	165	179	35	38	177	229	55	79	28	52
Minimum	77	77	9	10	60	78	22	24	6	6
Mean	114	120	19	20	125	145	37	42	12	16
No. of values	591	614	584	608	426	449	593	617	588	611
Standard deviation	17	22	3	3	17	23	4	6	3	6
Upper 99% limit	167	181	35	38	179	232	55	80	28	53
Lower 99% limit	75	75	9	10	58	75	22	23	6	5

Positive Controls

	TA100			TA1535			WP2*uvr* A/pKM101 (CM891)			TA98		TA1537		
S9 Mix	-	-	+	-	-	+	-	-	+	-	+	-	-	+
	(a)	(d)	(i)	(b)	(d)	(j)	(c)	(e)	(k)	(f)	(i)	(g)	(h)	(i)
Maximum	860	1327	1350	927	1130	1145	2312	1533	1704	993	1031	1933	976	543
Minimum	190	208	263	37	49	28	294	244	188	121	123	39	73	67
Mean	379	551	603	196	383	216	1233	662	701	308	532	390	529	244
No. of values	385	608	1013	380	598	998	125	572	719	991	1012	722	6	1006

(a) ENNG 3 µg
(b) ENNG 5 µg
(c) ENNG 2 µg
(d) Sodium azide 0.5µg
(e) AF-2 0.05 µg
(f) 2-Nitrofluorene 1 µg
(g) 9-Aminoacridine 30 µg
(h) 9-Aminoacridine 50 µg
(i) Benzo[a]pyrene 5 µg
(j) 2-Aminoanthracene 2 µg
(k) 2-Aminoanthracene 10 µg

Results obtained with in vitro chromosomal aberration test in human lymphocytes

Mitotic index data - first test

Without S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	[a]Mitotic Index		[a]Relative Mitotic Index (%)	Polyploidy	
	Incidence	% Mean		Incidence	% Mean
0 (DMSO)	139/1000 138/1000	13.9	100	0/500 1/500	0.1
0.08	113/1000 114/1000	11.4	82		
0.16	105/1000 115/1000	11.0	79		
0.31	100/1000 97/1000	9.9	71		
0.63	114/1000 106/1000	11.0	79		
1.25	101/1000 111/1000	10.6	76		
2.5	82/1000 79/1000	8.1	58		
5	78/1000 96/1000	8.7	63		
10	36/1000 43/1000	4.0	29	5/500 4/500	0.9**

[1] Calculations have been made with rounded values

Results obtained with in vitro chromosomal aberration test in human lymphocytes

Mitotic index data - first test (continued)

With S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	[a]Mitotic Index		[a]Relative Mitotic Index (%)	Polyploidy	
	Incidence	% Mean		Incidence	% Mean
0 (DMSO)	101/1000 84/1000	9.3	100	0/500 0/500	0.0
0.08	77/1000 76/1000	7.7	83		
0.16	70/1000 75/1000	7.3	78		
0.31	91/1000 66/1000	7.9	85		
0.63	88/1000 77/1000	8.3	89		
1.25	67/1000 73/1000	7.0	75		
2.5	68/1000 60/1000	6.4	69		
5	60/1000 60/1000	6.0	65		
10	15/1000 221000	1.9	20	1/153 1/114	0.7

Metaphase analysis data - first test

Without S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	No. cells examined	Aberrations				No. of aberrant cells				Relative Mitotic Index %
		Chromatid type	Chromosome type	Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %	
		ctb cte	csb cse		ctg csg					
0	100					0	0.5	0	0.5	100
(DMSO)	100	1				1		1		
2.5	100	1			1	1	1.5	2	2.0	58
	100	1	1			2		2		
5	100		1			1	2.0	1	2.0	63
	100	3				3		3		
10	100	3	3			4	4.5	4	4.5	29
	100	6				5	**	5	**	
0.2 µg/ml	50	10	3			10	20.0	10	20.0	-
(Mitomycin C)	50	9 2	5			10	***	10	***	

Metaphase analysis data - first test (continued)

With S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	No. cells examined	Aberrations						No. of aberrant cells				Relative Mitotic Index %
		Chromatid type		Chromosome type		Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %	
		ctb	cte	csb	cse		ctg csg					
0	100							0	0.5	0	1.5	100
(DMSO)	100	1					2	1		3		
2.5	100	1		1			1	2	1.5	3	2.5	69
	100			1			2	1		2		
5	100	4		1			1	4	2.0	5	2.5	65
	100							0		0		
10	50	11	1	9		2	2	12	22.0	13	23.0	20
	50	8		4		1		10	***	10	***	
10 µg/ml	50	16	1	18				18	34.0	18	36.0	-
(Cyclophosphamide)	50	7		12			2	16	***	18	***	

Results obtained with in vitro chromosomal aberration test in human lymphocytes

TABLE 1

Summary of results

Test 1

Exposure period (hours)	S9 mix	Concentration of Technical AE 0172747 (mM)	Cells with aberrations Excluding gaps			Cells with aberrations Including gaps			Relative Mitotic Index (%)
			Individual values (%)		Mean (%)	Individual values (%)		Mean (%)	
3	-	0 (DMSO)	0	1	0.5	0	1	0.5	100
		2.5	1	2	1.5	2	2	2.0	58
		5	1	3	2.0	1	3	2.0	63
		10	4	5	4.5**	4	5	4.5**	29
		0.2 µg/ml (Mitomycin C)	20	20	[a] 20.0***	20	20	[a] 20.0***	-
3	+	0 (DMSO)	0	1	0.5	0	3	1.5	100
		2.5	2	1	1.5	3	2	2.5	69
		5	4	0	2.0	5	0	2.5	65
		10	24	20	[a] 22.0***	26	20	[a] 23.0***	20
		10 µg/ml (Cyclophosphamide)	36	32	[a] 34.0***	36	36	[a] 36.0***	-

Test 2

Exposure period (hours)	S9 mix	Concentration of Technical AE 0172747 (mM)	Cells with aberrations Excluding gaps			Cells with aberrations Including gaps			Relative Mitotic Index (%)
			Individual values (%)		Mean (%)	Individual values (%)		Mean (%)	
3	-	0 (DMSO)	0	0	0.0	0	0	0.0	100
		2.5	0	0	0.0	0	0	0.0	81
		5	1	0	0.5	1	1	1.0	70
		7.5	6	7	6.5***	6	7	6.5***	26
		0.2 µg/ml (Mitomycin C)	14	16	15.0***	14	16	15.0***	-
3	+	0 (DMSO)	2	0	1.0	2	0	1.0	100
		2.5	0	0	0.0	0	0	0.0	93
		5	0	0	0.0	0	0	0.0	82
		7.5	20	22	[a] 21.0***	20	22	[a] 21.0***	45
		10 µg/ml (Cyclophosphamide)	24	24	[a] 24.0***	24	24	[a] 24.0***	-

*** P<0.001
** P<0.01
Otherwise P≥0.01

[a] 50 cells were analysed for these cultures due to the high levels of aberrations seen.

Mitotic index data - second test

Without S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	[a]Mitotic Index		[a]Relative Mitotic Index (%)	Polyploidy	
	Incidence	% Mean		Incidence	% Mean
0 (DMSO)	127/1000 98/1000	11.3	100	0/500 1/500	0.1
1.25	124/1000 117/1000	12.1	107		
2.5	97/1000 87/1000	9.2	81		
5	77/1000 80/1000	7.9	70		
7.5	34/1000 24/1000	2.9	26	1/288 0/279	0.2
10	11/1000 12/1000	1.2	11		

[a] Calculations have been made with rounded values

TABLE 4

Mitotic index data - second test (continued)

With S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	[a]Mitotic Index		[a]Relative Mitotic Index (%)	Polyploidy	
	Incidence	% Mean		Incidence	% Mean
0 (DMSO)	93/1000 100/1000	9.7	100	0/500 0/500	0.0
1.25	129/1000 118/1000	12.4	128		
2.5	88/1000 91/1000	9.0	93		
5	70/1000 89/1000	8.0	82		
7.5	33/1000 54/1000	4.4	45	4/252 4/285	1.5***
10	5/1000 2/1000	0.4	4		

Metaphase analysis data - second test

Without S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	No. cells examined	Aberrations				No. of aberrant cells				Relative Mitotic Index %
		Chromatid type	Chromosome type	Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %	
		ctb cte	csb cse		ctg csg					
0	100					0	0.0	0	0.0	100
(DMSO)	100					0		0		
2.5	100					0	0.0	0	0.0	81
	100					0		0		
5	100	1				1	0.5	1	1.0	70
	100				1	0		1		
7.5	100	5	1			6	6.5	6	6.5	26
	100	4	2	1		7	***	7	***	
0.2 µg/ml	100	5 3	6			14	15.0	14	15.0	-
(Mitomycin C)	100	12	7			16	***	16	***	

Metaphase analysis data - second test (continued)

With S9 mix, 3 hours treatment and 16 hours recovery

Concentration of Technical AE 0172747 (mM)	No. cells examined	Aberrations				No. of aberrant cells				Relative Mitotic Index %
		Chromatid type	Chromosome type	Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %	
		ctb cte	csb cse		ctg csg					
0	100	2	1			2	1.0	2	1.0	100
(DMSO)	100					0		0		
2.5	100					0	0.0	0	0.0	93
	100					0		0		
5	100					0	0.0	0	0.0	82
	100					0		0		
7.5	50	6 1	3	1		10	21.0	10	21.0	45
	50	8 4	3		1	11	***	11	***	
10 µg/ml	50	8	5		2	12	24.0	12	24.0	-
(Cyclophosphamide)	50	9	5			12	***	12	***	

Scientific Committee on Consumer Safety

SCCS

OPINION ON

HC Blue 17

COLIPA n° C184

The SCCS adopted this opinion at its 18th plenary meeting

of 26 February 2013

Bacterial Reverse Mutation Assay

Guideline: OECD 471 (1997)

Species/Strain: *S. typhimurium TA98, TA100, TA1535, TA1537, and E. coli WP2 uvrA*

Replicates: triplicate cultures in 2 independent experiments

Test substance: Blue 347

Batch: DYBB0847

Purity: 97% (HPLC)

Solvent: DMSO

Concentration: experiment I: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix

experiment II: 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix

Treatment: experiment I: direct plate incorporation with 48 h incubation without and with S9-mix

experiment II: pre-incubation method with 60 minutes pre-incubation and 48 h incubation without and with S9-mix

GLP: in compliance

Study period: 8 September 2008 – 23 September 2008

Bacterial Reverse Mutation Assay

Blue 347 was investigated for the induction of gene mutations in *S. typhimurium and E. coli* (Ames test).
Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system.
Test concentrations were based on the level of toxicity in a pre-experiment for toxicity with all strains both without and with S9-mix.
Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn.
Experiment I was performed with the direct plate incorporation method, experiment II with the pre-incubation method with 60 min pre-incubation.
Negative and positive controls were in accordance with the OECD guideline.

Bacterial Reverse Mutation Assay

Precipitation of Blue 347 was observed in the overlay agar in the test tubes in experiment I from 1000 µg/plate up to 5000 µg/plate and in experiment II from 2500 µg/plate up to 5000 µg/plate. The undissolved particles of Blue 347 had no influence on the data recording.
The plates incubated with Blue 347 showed normal background growth up to 5000 µg/plate without and with S9-mix. Since no toxic effects, evident as a reduction in the number of revertants, were observed in the test groups as well, 5000 µg/plate was chosen as maximum concentration. As in the pre-experiment evaluable plates were obtained for five concentrations or more in the strains used, the pre-experiment is reported as experiment I.
Blue 347 treatments did not result in a biologically relevant increase in the number of revertant colonies in any of the strains tested at any concentration level, neither without nor with S9-mix.
Conclusion
Under the experimental conditions used Blue 347 was not mutagenic in this gene mutation
tests in bacteria.

In vitro Mammalian Cell Gene Mutation Test (tk-locus)

Guideline: OECD 476 (1997)
Cells: mouse lymphoma cell line L5178Y (*tk+/-)*
Replicates: duplicate cultures in a single experiment
Test substance: Blue 347
Batch: DYBB 0539
Purity: 98.5% (HPLC)
Solvent: deionised water
Concentrations: 250, 500, 1000, 2000 and 3000 µg/ml without S9-mix
500, 1000, 2000, 3000 and 4000 µg/ml with S9-mix
Treatment: 4 h treatment both without and with S9-mix; expression period 72 h and a selection period of 10-15 days
GLP: /
Study period: 7 June 2006 – 3 July 2006

In vitro Mammalian Cell Gene Mutation Test (tk-locus)

Blue 347 was assayed for gene mutations at the *tk locus of mouse lymphoma cells both in* the absence and presence of S9 metabolic activation.
Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system.
Test concentrations were based on the results of a pre-test for toxicity with 4 and 24 h exposures to concentrations up to 4000 µg/ml in the absence and presence of S9-mix measuring suspension growth relative to the concurrent vehicle control cell cultures.
In the main tests, cells were treated for 4 h both without and with S9-mix, followed by an expression period of 72 h to fix the DNA damage into a stable *tk mutation and a selection* growth of 10-15 days.
Toxicity was measured in the main experiments as percentage suspension and relative total growth of the treated cultures relative to the concurrent vehicle control cell cultures.
To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed.
controls were in accordance with the OECD guideline.

In vitro Mammalian Cell Gene Mutation Test (tk-locus)

Results

In the pre-experiment no precipitation visible to the unaided eye occurred. Only in culture 2 without S9-mix the appropriate level of toxicity (about 10-20% survival after the highest concentration) was reached.
Without metabolic activation a biologically relevant increase in the mutant frequency was not found; all mutant values found were within the range of the historical control data.
With metabolic activation discriminating results were found between the 2 cultures per concentration.
In culture 1 a dose dependent and statistically significant increase in the mutant frequency outside the range of the negative control data was observed.

In culture 2, however, a biological relevant increase in the mutant frequency was not found which was obviously due to a rather high mutant frequency found for the concurrent control cultures.
Additionally, the mutant frequency for the positive controls was substantially higher (< 6-fold difference) in culture 2 compared to culture 1.
As such the data were interpreted as inconclusive

In vitro Mammalian Cell Gene Mutation Test (hprt locus)

Guideline: OECD 476 (1997)
Cells: mouse lymphoma cell line L5187Y
Replicates: duplicate cultures in 2 independent experiments
Test substance: Cationic Blue 347
Batch: DYBB0847
Purity: 97% (HPLC, 605 and 254 nm)
Solvent: culture medium
Concentrations: experiment I 150, 300, 600, 1200, 1800 µg/ml without S9-mix; 300, 600, 1200, 2400, 3600 µg/ml with S9-mix
experiment II 75, 150, 300, 600 µg/ml without S9-mix
600, 1200, 2400, 3600, 4800 µg/ml with S9-mix
Treatment: experiment I 4 h both without and with S9 mix; expression period 6 days and a selection period of 10-15 days
experiment II 24 h without S9 mix; expression period 6 days and a selection period of 10-15 days
24 h with S9 mix; expression period 6 days and a selection period of 10-15 days
GLP: in compliance
Study period: 29 October 2008 - 30 December 2008

In vitro Mammalian Cell Gene Mutation Test (hprt locus)

Cationic Blue 347 dissolved in culture medium was assayed for mutations at the *hprt locus* of mouse lymphoma cells both in the absence and presence of metabolic activation.

The assay was performed in two independent experiments using duplicate cultures each. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system.

Test concentrations were based on the results of a pre-test for toxicity with 4 and 24 h exposures to concentrations up to 4800 µg/ml, corresponding to the prescribed maximum concentration of 10 mM according to the OECD guideline, in the absence and presence of S9-mix measuring suspension growth relative to the concurrent vehicle control cell cultures.

In the main tests, cells were treated for 4 h (both without and with S9-mix) or 24 h (without S9-mix only, experiment II) followed by an expression period of 6 days, to fix the DNA damage into a stable *hprt mutation and a selection growth of 10-15 days.*

Toxicity was measured in the main experiments as percentage suspension and relative total growth of the treated cultures relative to the concurrent vehicle control cell cultures.

Negative and positive controls were in accordance with the OECD guideline.

Results

In the pre-test no precipitation occurred at 1200 µg/ml without and with S9-mix; at higher concentrations precipitation could not be evaluated due to the intense colour of Cationic Blue 347. No relevant deviations in pH or osmolarity were observed up to the maximum concentration.
Toxic effects were more prominent in the absence than in the presence of metabolic activation.
With S9-mix the appropriate level of toxicity (about 10-20% survival after the highest concentration) was mostly reached, with S9-mix only in experiment II this appropriate level was approached.
In both experiments, a biologically relevant increase in the mutant frequency due to Cationic Blue 347 treatments was not observed at any concentration level, neither without nor with S9-mix.

In vitro Micronucleus Test

Guideline: /
Cells: Chinese hamster V79 cells
Replicates: duplicate cultures in a single experiment
Test substance: Blue 347
Batch: DYBB0539
Purity: /
Solvent: deionised water
Concentrations: 36.7, 73.4, 146.9, 293.8, 587.5, 1175, 2350 and 4700 µg/ml without and with S9-mix
Treatment: 24 h treatment without S9-mix, harvest time immediately after the end of treatment
4 h treatment with S9-mix, harvest time 24 h after the start of treatment
GLP: /
Study period: 19 July 2006 – 4 August 2006

In vitro Micronucleus Test

Blue 347 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in V79 cells.
4700 µg/ml (≈ 10 mM, the prescribed maximum concentration in most OECD guidelines for in vitro genotoxicity tests) in deionised water was applied as maximum concentration.
The concentrations were further chosen based on toxicity data and on the occurrence of precipitation.
In the absence of S9-mix V79 cells were treated for 24 h, in the presence of S9-mix for 4 h; cells were harvested 24 h after the beginning of treatment.
The highest concentration should produce approximately 60% decrease in replication index.
Negative and positive controls were included.

In vitro Micronucleus Test
Results

Precipitation of Blue 347 in culture medium was observed at 2350 µg/ml and above in the absence of S9-mix and at 587.5 µg/ml and above in the presence of S9-mix. In addition no relevant increase in pH or osmolarity occurred. No clear cytotoxic effects indicated by a reduced proliferation index were found both in the absence and the presence of S9-mix.
In the absence of S9-mix biologically relevant and statistically significant increases in the number of V79 cells with micronuclei were found at the 3 highest concentrations tested. The increase did not show a clear concentration dependency.
In the presence of S9-mix a biologically relevant and statistically significant increase in the number of V79 cells with micronuclei was only observed at a mid-concentration.

Comment

Only a short report is available which only contains the “results and discussion” paragraph.
The study was not conducted in compliance with GLP or according to the draft OECD guideline. Purity is not mentioned. Although the same batch was reported to be 98.5% pure in other *in vitro genotoxicity tests available.*
Although rat liver S9 fraction was used, the inducer chemical was not reported.
Despite these shortcomings, the SCCS considers the outcome of this study as relevant.

In vivo Mammalian Erythrocytes Micronucleus Test

Guideline: OECD 474 (1997)
Species/strain: mouse, NMRI
Group size: 5 males and 5 females per test group
Test substance: Blue 347
Batch: DYBB0657
Purity: 100% (HPLC)
Vehicle: 0.9% NaCl
Dose level: 0, 6.25, 12.5 and 25 mg/kg bw
Route: i.p., once
Sacrifice times: 24 h and 48 h (high dose only) after treatment
GLP: in compliance
Study period: 27 September 2006 – 15 November 2006

In vivo Mammalian Erythrocytes Micronucleus Test

Preliminary study on acute toxicity

Male and female mice were treated i.p. with 10 up to 100 mg/kg bw and examined for acute toxic symptoms and/or mortality at 1, 2-4, 6, 24, 30 and 48 h after each treatment.

Mice treated i.p. with 100 mg/kg bw died within 1 h after treatment.
After treatment with 50 mg/kg bw, 1 mouse died after 6 h.
The surviving mice showed reduction in spontaneous activity, abdominal position and ruffled fur at least the first 6 h after treatment.
Mice treated with doses of 25 mg/kg bw and above had blue coloured urine.

I

In vivo Mammalian Erythrocytes Micronucleus Test
Results

In the main experiment mice were exposed orally to 0, 6.25, 12.5 and 25 mg/kg bw.
The mice were examined for acute toxic symptoms and/or mortality at 1, 2-4, 6 and 24 h after treatment.
Bone marrow cells were collected 24 h or 48 h (highest dose only) after dosing.
Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic (PCE/NCE).
Negative and positive controls were in accordance with the OECD guideline.

In the micronucleus test, the mice showed a reduction spontaneous activity and ruffled fur as well as coloured urine even down to the lowest dose of 6.25 mg/kg bw.
A decrease in the PCE/NCE ratio was not observed at both sampling times.
However, the clinical signs reported, particularly the coloured urine, indicated systemic distribution and thus bioavailability of Blue 347.

A biologically relevant increase in the number of cells with micronuclei was not observed at any sampling time and dose level of Blue 347.

Mutagenicity

Overall, the genotoxicity of HC Blue 17 is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity:
gene mutations, chromosome aberrations and aneuploidy.
HC Blue 17 is negative in vitro in:
a gene mutation test in bacteria
a gene mutation tests using the *hprt-locus of mammalian cells.*

Inconclusive results were obtained in:
an in vitro gene mutation test using the tk-locus of mammalian cells
an in vitro micronucleus test with V79 cells.
HC Blue 17 is negative in vivo in:
In vivo mammalian erythrocytes micronucleus test.

Consequently, on the basis of these tests, HC Blue 17 can be considered to have no genotoxic potential and additional tests are unnecessary.

2-nitro-5—glyceryl-methylaniline
Genotoxicity in vitro

End point	Test	Dose	Result
Gene mutation Bacterial cells	Ames Test Study 1	Max conc 5000ug/ml	Negative Purity not available no GLP no Guidelines
	Ames Test Study 2	Max conc 5000ug/ml	Negative
Gene mutation Mammalian cells	MLA tk locus	Max conc 2400ug/ml	Positive
	MLA hprt locus	Max conc 2000ug/ml	Negative
Chromosomal mutation	CHO cells Study 1	Max conc 4000ug/ml	Positive no GLP no Guidelines
	CHO Study 2	Max conc 2420ug/ml	Positive
DNA damage	UDS HeLa cells	Max conc 5000ug/ml	Negative

2-nitro-5—glyceryl-methylaniline
Genotoxicity in vivo

End point	Test	Dose	Result
Chromosomal mutation	MN test Bone marrow	Max conc 550 mg/kg ip	Negative no GLP no Guidelines
	MN test Bone marrow Study 2	Max conc 2000mg/kg ig	Negative No reduction PCE/NCE
	MN test Bone marrow Study 3	Max conc 2000 mg/ml oral	Negative No reduction PCE/NCE
	MN test Bone marrow Study	Max conc 1000 mg/ml by gavage	Negative Plasma analysis confirmed the systemic exposure
DNA damage	UDS Rat Liver	Max conc 1750mg/kg	Negative

THRESHOLD OF TOXICOLOGICAL CONCERN - TTC

The TTC concept is based on establishment of human exposure threshold values for chemicals below which the risk to human health is not appreciable.

The TTC approach allows to identify the threshold values for chemicals without or with very limited toxicity data, **based on their chemical structures and the known toxicity of chemicals which share similar structural characteristics.**

THRESHOLD OF TOXICOLOGICAL CONCERN - TTC

For carcinogens/mutagens

Establishment of the dose giving a 50% tumour incidence (TD50) using data for the most sensitive species and most sensitive site (Cheeseman *et al., 1999).*

- Based on a selected subset of the database containing 730 carcinogenic substances which had adequate estimates of the TD50 following oral dosage.
- Simple linear extrapolation from the TD50 to a 1 in 10 6 incidence.

The approach assumes that all biological processes involved in the generation of tumours at high dosages are linear over a 500,000-fold range of extrapolation

0.05 ug/kg of diet (0,15 ug/person/day or 0.0025 ug/kg bw/day)

"Cohort of Concern": aflatoxin-like compounds, N-nitroso-compounds, azoxy-compounds, and polyhalogenated dibenzo-p-dioxins and-dibenzofurans.

THRESHOLD OF TOXICOLOGICAL CONCERN - TTC

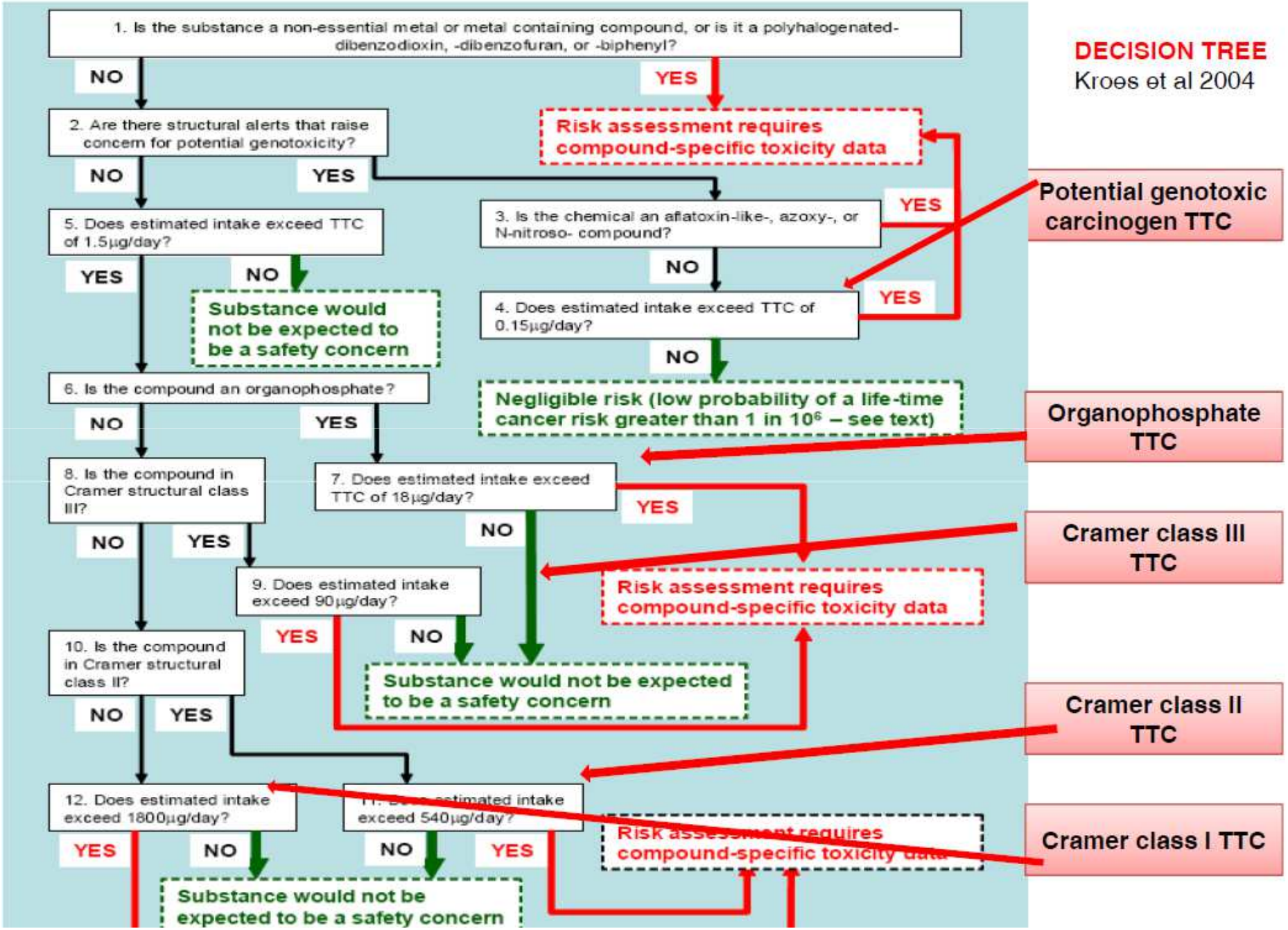
For carcinogens/mutagens

Analysis of dose-response data for carcinogens identified in cancer bioassays.
Determination of daily intake that would give risk of < 1 in a million
Simple linear extrapolation from the TD50 to a 1 in 10 6 incidence.

0.5 ug/kg of diet (1.5 ug/person/day or 0.025 ug/kg bw/day)
Gold Cancer Potency Database (1995)

Threshold of regulation" (TOR) (1.5 ug/person/day) adopted by USFDA for indirect food additives to assess the acceptable exposure of chemicals to which humans are exposed at low levels

The approach assumes that all biological processes involved in the generation of tumours at high dosages are linear over a 500,000-fold range of extrapolation



DECISION TREE

Kroes et al 2004

Threshold of Toxicological Concern (TTC)

The SCCS in 2009 have published an opinion on risk assessment methodologies and approaches for genotoxic and carcinogenic substances. According to this opinion, the “Margin of Exposure” (MOE) and linear extrapolation from the T25 (calculated dose giving a tumour incidence of 25% in an animal experiment) to a risk of 10-5 can be used in risk assessment of genotoxic carcinogens.

The SCCS in 2012 concluded that at present time, the default value of 0.15 µg/person/d corresponding to 2.5 ng/kg bw/d can be used for chemicals with genotoxicity alerts and hence possible DNA reactive carcinogens, but its scientific basis should be strengthened.
This could be achieved by e.g. extending the database, analysing all available carcinogenicity studies, using allometric adjustment factors and/or using the T25 or 1,5 or 10% benchmark dose as points of departure for linear extrapolation. in the MOE-approach, potency is represented by the benchmark dose or the T25 derived from animal carcinogenicity studies. MOEs of > 10 000 when using BMDL10 or 25 000 when using T25 are considered to be of low concern.

(Q)SAR

SARs and QSARs are theoretical models that can be used to predict in a qualitative or quantitative manner the physico-chemical, biological, toxicological properties and environmental fate of compounds from a knowledge of their chemical structure.

The basic assumption for the application of QSAR analyses in risk assessment is that the biological activities of the chemicals depend on its intrinsic nature and can be directly predicted from its molecular structure and inferred from the properties of similar compounds whose activities are known.

Genotoxicity prediction for the classified mutagen dataset

Software (used alone)	ND	EQ	TP	SE	FN	1-SE	No TS
Toxtree (genotoxic carcinogenicity)	0	0	86	0.76	27	0.24	NA
Toxtree (in vivo micronucleus)	0	0	98	0.87	15	0.13	NA
Toxtree (genotoxic carcinogenicity or in vivo micronucleus)	0	0	98	0.87	15	0.13	NA
TOPKAT	1	0	65	0.58	47	0.42	43
CAESAR	1	0	82	0.73	30	0.27	48
HazardExpert	0	5	82	0.77	25	0.23	Not known
Lazar (Kazius/Bursi)	0	0	65	0.58	48	0.42	58*
Lazar (Toxbenchmark)	0	0	56	0.50	57	0.50	60*
Lazar (Kazius/Bursi or Toxbenchmark)	0	0	69	0.61	44	0.39	74*
Derek (mutagenicity or chromosome damage)	0	2	81	0.73	30	0.27	NA
ToxBoxes	0	27	38	0.44	48	0.56	Not known
Software (used in combination)							
Toxtree or CAESAR	0	0	101	0.89	12	0.11	48
Derek or CAESAR	0	0	96	0.85	17	0.15	48
Derek or Lazar	0	0	92	0.81	21	0.19	74*
Derek or TOPKAT	0	0	89	0.79	24	0.21	43
Toxtree or Lazar	0	0	102	0.90	11	0.10	74*
Toxtree or Derek	0	0	104	0.92	9	0.08	NA
HazardExpert or CAESAR	0	0	94	0.83	19	0.17	• 48

Test set of 113 classified mutagens; **ND** – not determined; **EQ** – compounds predicted as equivocal; **TP** – true positives; **SE** – sensitivity; **FN** – false negatives; **1-SE** – false negative rate; **No TS** – number of chemicals already in the training set of the model (where applicable); **NA** – not applicable

* For Lazar it is not important whether a substance is in the dataset used to build the model, since an instance- based prediction is generated by a local model built from data that exclude the query chemical

(Q)SAR approach

More than 100 papers in the scientific literature are devoted to in silico prediction of genotoxicity, comparing performances of different (Q)SAR models, including software models; the large majority of them report the results of evaluation studies for prediction of carcinogenicity. The available models perform better for the prediction of bacterial mutagenicity (the accuracy of Ames test mutagenicity prediction is typically 70-75%) than for in vitro mutagenicity or cytogenetics in mammalian cells.

The present evidence does not justify the application of the (Q)SAR approach alone in predicting the genotoxicity of substances. In cases where limited or no test data are available, the (Q)SAR approach could be useful in a weight-of-evidence approach that includes information from all available sources (e.g. read-across and experimental data).