Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

EC Number: 203-750-9

CAS Number: 110-26-9

Index Number: -

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CONTENTS

1 PHYSICAL HAZARDS

Not evaluated in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No studies available.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated.

3.2 Acute toxicity - dermal route

Not evaluated.

3.3 Acute toxicity - inhalation route

Not evaluated.

3.4 Skin corrosion/irritation

Not evaluated.

3.5 Serious eye damage/eye irritation

Not evaluated.

3.6 Respiratory sensitisation

Not evaluated.

3.7 Skin sensitisation

Not evaluated.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 Study 1: Gene mutation test in Bacteria

Study reference:

Hashimoto K and Tanii H. Mutagenicity of acrylamide and its analogues in Salmonella typhimurium. Mutation Research 1985 Dec; 158(3):129-33.

Detailed study summary and results:

Test type

OECD TG 471

Test substance

MBA (>95% purity) dissolved in water.

Administration/exposure

Strains: S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100

With and without metabolic activation (Aroclor S9)

Concentrations: 0, 5, 50, 500, 1000 and 5000 µg/plate.

Negative and positive controls included.

Results

Not mutagenic (with or without metabolic activation). Cytotoxicity was not specified. No detail on the experimental design or on specific or detailed results for MBA are given in the report.

Genotoxic effect: negative

3.8.1.2 Study 2: Gene mutation test in Bacteria

Study reference:

Zeiger et al. 1988 (NTP study number 613268)

Detailed study summary and results:

Test type

Bacterial mutagenicity Ames, similar to OECD TG 471

Test substance

MBA dissolved in DMSO (purity not indicated)

Administration/exposure

Salmonella typhimurium strains TA100, TA1535, TA97, TA98

With and without metabolic activation (S9)

Doses: 0, 100, 333, 1000, 3333, 10000 µg/plate.

Vehicle control: DMSO

Positive controls were 2-aminoanthracene, sodium azide, 9-aminoacridine, 4-nitro-O-phenylenediamine.

Results

Strain TA100: equivocal results with 10% Hamster S9, positive and weakly positive with 30% Hamster S9, for other conditions results were negative. Strain TA1535: positive with 10% Hamster S9, for other conditions results were negative. Strain TA97: negative. Strain TA98: negative. Results for positive strains are summarised below.

Table:1. Detailed results summarised for positive S. Typhimurium strains.

Strain	**TA100**			**TA1535**	
Dose (µg/Plate)	With 10% Hamster S9	With 30% Hamster S9	With 30% Hamster S)	With 10% Hamster S9	With 10% Hamster S9
0	117 ± 7.8	126 ± 8.0	105 ± 2.0	12 ± 2.9	8 ± 0.7
100	131 ± 17.5	133 ± 11.0	119 ± 3.1	11 ± 1.5	10 ± 2.6
333	146 ± 13.5	121 ± 10.8	138 ± 6.7	17 ± 0.9	9 ± 1.9
1000	134 ± 5.0	143 ± 1.0	146 ± 7.2	29 ± 0.6	18 ± 2.6
3333	172 ± 0.7	172 ± 12.2	185 ± 2.7	52 ± 8.9	27 ± 3.5
10000	155 ± 10.9	195 ± 6.5	198 ± 4.3	49 ± 2.6	30 ± 1.9
Trial summary	Equivocal	Weakly Positive	Positive	Positive	Positive
Positive Control (2-aminoanthracene)	1860 ± 46.6	540 ± 5.2	569 ± 27.5	562 ± 18.0	398 ± 6.7

Values given as Mean or Mean ± Standard Error Mean. The number of samples = 3, unless samples marked toxic or contaminated were excluded from mean and SEM calculations.

Genotoxic effect: positive

3.8.2 Animal data

3.8.2.1 Study 1: In vivo micronucleus test

Study reference:

NTP study number 710269

Detailed study summary and results:

Test type

In vivo micronucleus test (in bone marrow of male mice)

Test substance

MBA given by i.p. injection. No information on degree of purity, impurities or batch number.

Test animals

Male mice, B6C3F1. 5 animals per dose group.

Administration/exposure

0, 25, 50, 100 mg/kg given by intraperitoneal injection. Vehicle: Phosphate buffered saline.

Number of treatments: 2. Time interval between final treatment and cell sampling: 24 hrs.

Positive control used: 1.0 mg/kg Mitomycin-C

Statistical method:

Cochran-Armitage trend test

Results and discussion

MN PCE/1000 (mean ± SEM): 0.50 ± 0.16 (controls), 8.30 ± 2.89 (25 mg/kg bw), 8.20 ± 2.87 (50 mg/kg bw), 20.00 ± 2.93 (100 mg/kg bw). All MBA doses resulted in statistically significant increase in MN in PCE in bone marrow ($p < 0.001$). Mean percent PCE were 45.12 ± 1.54 (control), 38.92 ± 3.53 (25 mg/kg bw), 31.08 ± 3.61 (50 mg/kg bw), 32.36 ± 1.69 (100 mg/kg bw).

Positive control MN PCE/1000 mean 20.40 ± 2.13 (% PCE, mean: 37.96 ± 5.35).

Genotoxic effect: positive.

3.8.2.2 Study 2: In vivo comet assay

Study reference:

Hansen MK, Sharma AK, Dybdahl M, Boberg J and Kulahci M. In vivo Comet assay – statistical analysis and power calculations of mice testicular cells. Mutation Research 774 (2014) 29–40.

Detailed study summary and results:

Test type

In vivo comet assay in mice testicular cells. No guideline. The study was performed under conditions approved by The Danish Agency of Protection of Experimental Animals and the in-house Animal Welfare Committee.

Test substance

MBA CAS 110-26-9 (Acrylamide, N,N′-methylenebis-) obtained from Sigma–Aldrich, Brondby, Denmark. Purity not indicated. No information on impurities or batch number.

Test animals

CD-1 male mice, 5 weeks old at study initiation, weight 29.8 ± 1.2 g. 5 animals per group.

Administration/exposure

0, 50, 100 and 190 mg/kg dissolved in water. The dose was chosen based on data from the published literature of in vivo genotoxicity test data and experimental LD50 values after oral exposure of mice. The maximum dose was 50% of the LD50-value.
A positive control group of 5 mice given 300 mg/kg bw EMS (dissolved in water) was included.
Mice were dosed orally by gavage twice 24 hrs apart. Dosing preparations were given in a volume of 1 ml/100 g bw. 2-4 hrs after second dosing animals were anaesthetized and killed.

Testicles were excised and weighed. After removing the capsule, the right testicles were put in cryotubes and used later in the Comet assay. The left testicles were processed for paraffin fixation. One section per testis was evaluated by an experienced pathologist. The DNA isolated from the testicular tissue origins from a mixture of different cell types.
Statistical methods are described in detail in the original publication. One of the main aims of the study was to identify a statistic suitably summarizing the % tail DNA of mice testicular samples in Comet assay studies. A second aim was to provide curves for this statistic outlining the number of animals and gels to use.

Results and discussion
Histopathological examination and the incidence of highly damaged cells: No treatment-related effects were observed in the histological examination of testes.
MBA induced an effect in testicular cells. The % tail DNA increased with increasing dose, mean 7.1 (0 mg/kg bw), 6.7 (50 mg/kg bw), 8.3 (100 mg/kg bw) and 14 (190 mg/kg bw). The highest dose demonstrated statistically significant difference with the control at $p>0.001$. The positive control had a mean obtained % tail DNA at 12.4 (raw data). In addition to MBA, ten other compounds were evaluated in this study.

Genotoxic effect: positive.

3.8.2.3 Study 3: Rodent dominant lethal test

Study reference:

Rutledge JC, Cain KT, Kyle J, Cornett CV, Cacheiro NLA, Witt K, Shelby MD and Generoso WM. Increased incidence of developmental anomalies among descendants of carders of methylenebisacrylamide-induced balanced reciprocal translocations. Mutation Research, 229 (1990) 161-172.

Detailed study summary and results:

Test type

Rodent Dominant Lethal Test. Similar to OECD TG 478 with modifications.

Test substance

Electrophoresis grade (99.9%) N, N'-methylenebisacrylamide (CAS No. 110-26-9) was obtained from Bethesda Research Laboratories, Gaithersburg, MD. No information on batch number.

Test animals and doses

Dominant lethals I; male mice (C3H × 101)F1, 0 and 225 mg/kg, 36 treated males/group. Females: T-stock

Dominant lethals II; male mice (C3H × 101)F1, 0 and 225 mg/kg, 30 treated males/group. Females: (SEC x C57BL)F1

Dominant lethals III; male mice (C3H × 101)F1, 0 and 90 mg/kg (daily doses for 5 days), 60-65 treated males/group. Females: (SEC x C57BL)F1 and (C3H × 101)F1

Heritable translocations; male mice (C3H × 101)F1, 0 and 90 mg/kg (daily doses for 5 days), 97 treated males/group and 25 controls. Females: (SEC x C57BL)F1

Administration/Exposure

MBA solutions were made in Hanks' balanced salt solutions and injected intraperitoneally in a maximum volume of 1.0 ml. Control mice were given 0.8 ml of HBSS. A 30-day acute toxicity study for single and multiple doses was performed in order to determine the maximum tolerated dose. Doses used in the experiment were 225 mg/kg bw (single dose) or 90 mg/kg bw (given daily for 5 days). Fisher’s exact test was used to compared incidences in fetal death, phenotypic anomalies and total gestational anomalies between MBA semisteriles and normal fertile animals.

Dominant lethals were calculated using the following equation: % dominant lethals = (1 – living embryos/pregnant female (treated)/living embryos/pregnant female (control)) x 100.

Results

No information on clinical signs, general toxicity, body weight changes, food/water consumption. In the dominant lethal study male mice were injected with a single dose of 225 mg/kg bw (i.p.) in the afternoon; and the following morning, each male was caged with two females. Females were checked for vaginal plugs each morning and those that mated were replaced by fresh ones. Mated females were killed for uterine analysis at around 17 days gestation.

Study I: The aim was to determine the effect of MBA at various stages in spermatogenesis. Males were mated for 50 days. Dominant lethal effects were observed only in matings during the first 4 days after treatment. The number of implantations per pregnant female was statistically significantly decreased compared to control (7.4 vs. 9.7, respectively). In addition, the average number of living embryos was statistically significantly decreased (5.1 per pregnant female vs 8.1 for controls). The percent of dead implants was 31.1, vs. 15.8 for the corresponding controls. In addition, pregnancies were reduced, and a statistically significantly decrease in the number of and living embryos were observed in females mated 36.5-43.5 days post-treatment to MBA-exposed males.

Study II: The aim was to verify the dominant-lethal effects in maturing spermatozoa. Males were mated for 12 days. Dominant lethal effects (10 percent dominant lethals) were seen only in matings for 0.5-3.5 days post-treatment. The study showed statistically significantly higher percentage of dead implants in the MBA-treated group during this time interval, 14 percent, vs. 2 percent in controls.

Study III: The aim was to study the effect after increasing the level of dominant-lethal response of the maturing spermatozoa by 5 consecutive daily injections of MBA (450 mg/kg bw cumulative) and by using 2 different stocks of female mice. Clear dominant-lethal effects of MBA in maturing sperm (matings between 0.5 and 3.5 days after the last dose) were observed; 22-26 percent in one stock of females, and 34-46 percent in the other stock of females.

In the heritable translocation study, each male was caged with two females for 5 days beginning the day after the last injection. After this period, females were separated and caged individually. Only male offspring was kept for further study.

A sequential method of identifying translocation carriers among first-generation male offspring was employed, which screens the sterile and partially sterile presumptive carriers of balanced reciprocal translocations. To confirm that translocation heterozygosity was the basis for partial sterility, 10 randomly selected semi-steriles were cytogenetically analysed for evidence of chromosome rearrangement in diakinesis metaphase I spermatocytes. All sterile offspring were analysed cytogenetically.

24 partially sterile and 13 normal, fertile male offspring from MBA-treated males and 1 partially sterile and 8 normal, fertile male offspring from the corresponding control group were mated with females to produce fetuses analysed in the anatomical study. All pregnant females were killed at gestational day 17. Their uteri were analysed for the incidence of resorption bodies (representing embryonic death at peri-implantation stages), midgestational embryonic death (presence of placenta and embryonic mass that lacks eye pigment), late gestation fetal death (at least some eye pigment visible) and living fetuses. Fisher's exact test was used to compare the incidences of fetal death, phenotypic anomalies, and total gestational anomalies between MBA semisterile males and normal fertile stocks.

MBA induced a marked increase in the incidence of semisterile offspring (36/350 vs. 1/127 in the control and 1/8095 in the laboratory's historical controls). Semi sterility was indicated by reduced number of living embryos (less than half of the normal), primarily due to an increased incidence of peri implantation embryonic death. Multivalent chromosomes were present in the meiocytes of all 10 randomly selected semisteriles, indicating that most, or all of the semisterile male offspring produced were translocation heterozygotes. None of the 4 steriles in the control group were carriers, while 11 out of 14 steriles in the MBA group were confirmed cytogenetically to be carriers. The incidence of dead implants in at least 6 females mated to semisterile males ranged from 35 to 75% compared to 0 to 8% in at least 3 females mated to males of normal fertility. One spontaneously semisterile male was found in the control group.

Anatomical analysis of embryos conceived by semi sterile and normal fertile males:

Of 36 semisteriles in the MBA group, 10 were killed for cytogenetic analysis and 2 did not conceive sufficient number of fetuses for anatomical analysis. The remaining 24 MBA-induced and 1 spontaneously occurring semisterile translocation carriers were compared with males of normal fertility from the MBA group (13) and from the control group (8) with respect to the incidence of anomalies among mid- to late gestation conceptuses. The incidence of all gestational anomalies in all conceptuses conceived by the 24 MBA-induced semisteriles was 10.8%, which was statistically significantly higher than for the two normal, fertile groups (1.0% and 2.2%, respectively).

Each male and all the conceptuses he conceived represent a specific chromosomal rearrangement. Chromosomal rearrangements were grouped according to the percentage of total gestational anomalies. Results showed that the anomalies in the MBA semisterile group were not randomly distributed among rearrangements. Only certain rearrangements produced statistically significantly high numbers of anomalies. 13 out of the 24 rearrangements produced statistically significantly increased rates of gestational anomalies (mid- and late-gestation deaths plus abnormal live fetuses). 19 of the 21 males from the 2 normal, fertile

MBA rearrangements that produced statistically significantly higher incidences of gestational anomalies and the single rearrangement from the control group were analysed separately. 10 of the MBA rearrangements exhibited excess gestational deaths (statistically significant by Fisher's exact test compared with pooled control). Of these, 3 also had excess defects among living fetuses including small size, abnormal eyes, exencephaly, and cleft palate.

Table 2: Dominant-lethal effects in MBA-treated male mice (table modified from Rutledge et al. 1990).

Treatment[a]	Treatment to fertilization interval (days)	No of mated females	No of pregnant females	Implantations/ pregnant female	Living embryos/pregnant female	Percent dead implants
MBA	0.5-3.5	49	37	7.4*	5.1*	31.1
	4.5-7.5	58	51	9.1	7.1	22.2
	8.5-11.5	66	65	8.7	7.5	14.0
	12.5-15.5	54	51	8.5	6.9	19.1
	16.5-19.5	58	54	8.3	7.2	12.9
	20.5-23.5	61	42	7.6	6.7	11.3
	24.5-27.5	60	45	8.2	6.9	16.2
	28.5-31.5	58	57	8.2	6.8	16.5
	32.5-35.5	62	45	6.8	5.7	16.1
	36.5-39.5	72	23	4.7*	4.4*	6.4
	40.5-43.5	66	27	5.5*	4.6*	17.4
	44.5-47.5	53	44	8.1	7.1	12.9
	48.5-49.5	29	26	6.9	6.2	12.5
Control	0.5-3.5	66	61	9.7	8.1	15.8
	4.5-7.5	69	63	9.1	7.8	13.9
	8.5-11.5	63	58	9.6	8.3	13.5
	12.5-15.5	65	58	9.2	7.9	14.9
	16.5-19.5	65	58	8.4	7.3	13.5
	20.5-23.5	76	70	8.9	7.6	15.1
	24.5-27.5	67	59	8.8	7.8	11.9
	28.5-31.5	67	63	9.1	7.7	15.7
	32.5-35.5	73	63	9.0	7.7	13.9
	36.5-39.5	63	56	8.8	7.4	15.1
	40.5-43.5	69	63	9.2	8.1	11.6
	44.5-47.5	73	59	7.9	6.2	21.6
	48.5-49.5	35	31	8.5	7.2	15.6

[a]Male mice were given a single i.p. injection of 225 mg/kg MBA. * Significantly different from control ($p<0.01$).

Table 3: Dominant-lethal effects of MBA on maturing spermatozoa (table modified from Rutledge et al. 1990).

Treatment[a]	Treatment to fertilization interval (days)	No of mated females	No of pregnant females	Implantations/ pregnant female	Living embryos/ pregnant female	Percent dead implants	Percent dominant lethals[b]
MBA	0.5-3.5	50	33	9.9	8.5	14*	10
	4.5-8.5	44	35	10.9	9.9	9	-3
Control	0.5-3.5	64	56	9.7	9.4	2	
	4.5-8.5	46	35	9.9	9.6	3	

[a]30 male mice were given a single i.p. injection of 225 mg/kg MBA. Control mice (n=30) were given Hanks' balanced salt solution. [b]Dominant lethals = (1- (living embryos/pregnant female (treated)/living embryos/pregnant female (control))) x 100. * No of dead implants per female is significantly higher than corresponding control ($p<0.01$ by Student's t-test).

Table 4: Dominant-lethal response to multiple MBA injections (table modified from Rutledge et al. 1990).

Treatment [a]	**Stock of females**	**Treatment to fertilization interval /days)**	**No of mated females**	**No of pregnant females**	**Implantations/ pregnant female**	**Living embryos / pregnant female**	**Percent dead implants**	**Percent dominant lethals**
MBA	(C3Hx101)F_1	0.5-1.5	21	18	6.4*	4.9**	23	26
		2.5-3.5	27	25	7.0	5.3**	25	22
		4.5-5.5	17	15	7.3	5.9	19	8
		6.5-7.5	11	11	6.6	5.7	14	16
		8.5-9.5	16	15	7.4	6.9	7	-5
		10.5-11.5	25	21	7.3	6.8	8	-5
	(SECxC57BL) F_1	0.5-1.5	38	31	9.7	5.2**	46	46
		2.5-3.5	21	17	8.2*	6.1**	26	34
		4.5-5.5	21	18	9.7	8.5	12	11
		6.5-7.5	21	14	9.6	9.0	6	11
		8.5-9.5	27	23	9.4	9.0	4	-10
		10.5-11.5	26	16	8.8	8.1	7	10
Control	(C3Hx101)F_1	0.5-1.5	16	15	7.7	6.6	14	
		2.5-3.5	32	30	7.4	6.8	8	
		4.5-5.5	19	18	7.3	6.4	12	
		6.5-7.5	13	10	7.9	6.8	14	
		8.5-9.5	15	14	7.1	6.6	7	
		10.5-11.5	33	29	7.0	6.5	7	
	(SECxC57BL) F_1	0.5-1.5	35	29	9.9	9.7	2	
		2.5-3.5	29	21	9.9	9.2	6	
		4.5-5.5	20	16	9.9	9.5	4	
		6.5-7.5	15	12	10.3	10.1	2	
		8.5-9.5	28	22	8.5	8.2	4	
		10.5-11.5	26	19	9.4	9.0	5	

[a]65 male mice were given i.p. injections of 90 mg/kg MBA daily for 5 days. 60 control males were treated with Hanks balanced salt solution each day. *Significantly different from corresponding control $p<0.05$. ** Significantly different from corresponding control $p<0.01$.

Table 5: MBA-induced heritable translocations in mice (table modified from Rutledge et al. 1990).

Treatment[a]	No of male progeny scored	No of semi-sterile males	No of sterile males	Frequency of translocations (%)
MBA	350	36	14[b]	13.4
Contemporary control	127	1[c]	4[d]	0.8
Historical control	8095	1	16[e]	0.04

[a]97 male mice were given 90 mg/kg MBA by i.p. injection daily for 5 days. Males were mated to females on days 1-5 after the last dose. Progeny were derived from germ cells treated as sperm. 25 males in control groups were treated with Hanks' balanced salt solution. [b]11 were confirmed cytogenetically as carriers of translocations, including one with 2 translocations. [c]this rearrangement involved 3 chromosomes. [d]Cytogenetic analysis showed no translocations in the steriles. [e]two were confirmed cytogenetically as translocation carriers.

Table 6: Uterine contents of females mated to semisterile or normal fertile males[a] (table modified from Rutledge et al. 1990).

Experimental group	No of males mated	No of pregnant females	Implantations/ female	Living fetuses/ female	Resorption bodies[b] (%)	Midgestation and late deaths[c] (%)	Abnormal live fetuses[d] (%)	Death and fetal anomalies during gestation[c,e] (%)
MBA semisterile	24	239	10.3 (0.81)	4.5 (1.44)	52 (16)	7.4	3.7 (10.8)	10.8
MBA normal, fertile	13	57	10.5 (1.13)	10.1 (1.07)	4 (0.03)	0.2	0.90 (0.01)	1.0
Control semisterile	1	11	10.7	2.0	79	12.0	0	12.0
Control normal, fertile	8	35	10.4 (1.16)	10.0 (1.19)	2 (0.03)	1.4	0.90 (0.01)	2.2

[a]Standard deviations in parentheses. [b]Percent of all implants, [c]Percent of gestational embryos (living embryos plus midgestation and late deaths). [d]Percent of living embryos. [e]Total anomalies include midgestation and late deaths and abnormal living embryos.

Table 7:. Frequency distribution based on incidence of anomalies within each rearrangement and normal stock[a] (table modified from Rutledge et al. 1990).

Percentage of anomalies[b]	MBA semisterile	MBA normal fertile	Control normal fertile
0-4	9	13	7
5-9	5	0	1
10-14	4	0	0
15-19	2	0	0
20-24	2	0	0
25-29	1	0	0
30-34	1	0	0
Total	24	13	8

[a]Each rearrangement and normal stock represent 1 male and all conceptuses he sired. [b]Anomalies included midgestation and late death and live abnormal fetuses. Calculation was based on all implants except resorption bodies.

Table 8: Types of anomalies in various rearrangements and groups (table modified from Rutledge et al. 1990).

Rearrangements or groups	No of dead conceptuses (mid-and late gestation)	Total gestational anomalies
MBA semisterile high-anomaly rearrangements		
104-122	4	9
359-221	12	20
34-80	4	9
160-153	1	5
84-105	12	13
188-178	13	13
212-189	10	10
346-192	5	5
351-192	4	6
37-80	4	5
41-80	2	4
228-198	1	3
285-214	3	2
Total	75	106
Pooled MBA low-anomaly semisterile rearrangements	4	10
Pooled normal fertile stocks	6	14

Number of conceptuses analysed: 42-55 (total 608), Pooled MBA low-anomaly semisterile rearrangements: 475 conceptuses, pooled normal fertile stocks; 943 conceptuses.

Table 9: Detailed types of anomalies in various rearrangements and groups (table modified from Rutledge et al. 1990).

Rearrangements or groups	**Specific types or location of anatomic defects in living fetuses**									
MBA semisterile high-anomaly rearrangements	Growth retardation	Cleft palate	Eye	Exencephaly	Tail	Edema	Mandible	Limb	Abdominal wall	No of fetuses affected
104-122	3	1	2	2	1					5
359-221	3	2	4			1	1			8
34-80	5									5
160-153	2	2	1		1					4
84-105		1								1
188-178										0
212-189										0
346-192										0
351-192	1		1						1	2
37-80					1					1
41-80		2	1							2
228-198	1					1				2
285-214				1	1					1
Total	15	8	9	3	4	2			1	31
Pooled MBA low-anomaly semisterile rearrangements	2	0	0	0	1	0				5
Pooled normal fertile stocks	2	1	1				1		3	8

Genotoxic effect: positive

3.8.2.4 Study 4: Two-generation study, reproductive toxicity and dominant lethal effects

Study reference:

Chapin RC, Fail PA, George JD, Grizzle TB, Heindel JJ, Harry GJ, Collins BJ, Teague J. The Reproductive and Neural Toxicities of Acrylamide and Three Analogues in Swiss Mice, Evaluated Using the Continuous Breeding Protocol, Fundamental and Applied Toxicology 27, 9-24 (1995). NTP: Abstract for RACB90016 (nih.gov)

Detailed study summary and results:

Test type

A modified design of the National Toxicology Program's Reproductive Assessment by Continuous Breeding (RACB) protocol is a two-generation study developed by NTP to identify hazards to toxic effects on male

and/or female reproduction, to characterize that toxicity, and to define the dose-response relationships for each compound. These studies have been performed by laboratories under contract to NIEHS using GLP.

Test substance

Test material: N,N'-methylenebisacrylamide (CAS No.110-26-9). The identity of test article was confirmed through mass spectrometry, ir, uv, and/or NMR spectrometry, as appropriate. Purity: 97-99%.

Test animals

Crl:CD-1 Swiss albino mice from Charles River Laboratories. Animals were 8 weeks old at the start of Task 1 and 11 weeks old at the start of Task 2, and naive females were 11 weeks old at the start of the dominant-lethal segment.

Method and Results

MBA was evaluated using a modified design of the NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol, which divided into 4 tasks. **Task 1** included a 28-day dose-range finding study (endpoints were clinical signs, grip strength, interim and terminal body weights, and food and water consumption in 6 groups, 8 animals/group). During the last week of Task 1, animals in each treatment group were cohabited as mating pairs, and the presence of vaginal plugs was monitored. Data from Task 1 were used to set concentrations for Task 2.

Task 2 included the continuous breeding phase, consisting of a control group (n = 40 mating pairs) and three dose groups (n = 20 mating pairs). Task 2 started with a 7-day period of dosing animals housed separately, followed by a 98-day dosing period in which mice were housed as mating pairs (endpoints were clinical signs, parental body weights, fertility (ability to produce live pups), number of litters/pairs, number of live pups/litter, sex ratio of the pups, the mean pup weights taken at birth, study day of delivery, and parental food and water consumption). After each litter was delivered, the pups were counted, sexed, weighed, and humanely killed. After weaning, the pups were culled to two/sex/litter and maintained on the same dose of test agent as their parents until Day 74 of age (±10 days). These F1 mice were used for Task 4 fertility assessments. During a 6-week separation period, randomly selected control and high dose males were cohabited with 3 untreated females for up to 4 nights to evaluate dominant-lethal effects in the males. Females were killed on gestation Day 16, and uteri were examined for number of live, dead, and resorbing implants.

Task 3, the crossover mating trial, was performed after the 6-week holding period to define the gender affected by treatment. During Task 3, the high-dose and control groups were assigned partners in the other dose group for a 1-week mating period, and some controls were reassigned untreated mating partners, so that the final mating groups were control males x control females, high-dose males x control females, and control males x high-dose females. Reproductive endpoints were the same as those for Task 2. During the crossover mating of the control and high-dose groups, the low- and middle dose groups were housed separately and

continued on treatment. After delivery of the Task 3 litters, the females were subject to daily vaginal lavage for 12 days to assess estrous cyclicity. Finally, all F0 mice were killed and necropsied. Data collected at necropsy were body and selected organ weights, epididymal sperm number, motility, morphology, and testicular spermatid head count (expressed both as heads/g testis and as heads/testis). The F1 mice, under continual exposure to dosed water since weaning at PND 21, were mated at 74 (±10) days of age to nonsibling partners of the same treatment group for 1 week or until a copulatory plug was detected and then were separated. The F1 mice were killed necropsied; endpoints were as for Task 3. Tissues evaluated microscopically were testes and epididymides.

Task 2. Body weights and food consumption for the F0 mice were not affected by MBA exposure at 10, 30, and 60 ppm. During the first week of exposure, water consumption was increased in all dosed male groups and the middle and high concentrations for females, but there were no differences among the groups at Week 16 of Task 2. Low- mid- and high doses were calculated to correspond to 1.6, 4.7, and 9.3 mg/kg/day, respectively. All groups averaged 4.8-4.9 litters/pair. There was a slight but statistically significant decrease in the aggregate mean number of live pups/litter in the mid and high groups. There was no decrease in the proportion of pups born alive. Live pup weight was decreased at the high dose. Cumulative days to deliver each litter were increased (with an average of 4 days per litter) in the high dose for three litters. For the number of live pups in each litter, there was a difference in the first litter, in which the control pairs delivered statistically significantly more live pups than did the middle- and high-dose groups (13.3 ± 0.3, 10.9 ± 0.6*, and 9.6 ± 0.9* pups/litter, respectively). There were no differences across groups for the second and third litters. In the fourth and fifth litters, only the middle-dose group delivered statistically significantly fewer pups/litter than did the controls (control and middle-dose group for the fourth litter, 15.4 ± 0.4 and 13.0 ± 0.9* pups/litter, respectively. The number of live pups/litter increased slightly in all groups over time; there was no progressive reduction in fertility.

MBA was assessed for dominant lethal potential by cohabiting each treated male from the high dose group 60 ppm with three naive females after Task 2's continuous cohabitation. The pregnancy rates for this cohabitation were 73, 82, 75, and 74% (control to high dose). There was a slight but statistically significant increase in early resorptions in the high dose group. As a result, total post implantation loss was increased in this group, compared to controls. There was no corresponding decrease in the number of live implants.

Task 3 (the crossover mating trial) was conducted with controls and the high-dose mice. There was no difference among the groups in the number of offspring produced, although the pup weight adjusted for pup number was statistically significantly smaller for pups from treated dams.

The Task 2/3 mice were killed and necropsied. At necropsy, there was no alteration in F0 male body weights or weights of liver, kidneys/adrenals, seminal vesicles, prostate, or epididymis. There was a slight concentration-related decrease in right testis weight which was statistically significant for the mid- and high dose groups; from controls to the high-dose group, the testis weights were 134.0 ± 5.4, 131.7 ± 5.1, 118.8 ±

4.9*, and 113.7 ± 4.2* mg. There were no adverse effects on epididymal sperm concentration, percentage motile sperm, percentage abnormal sperm, or testicular spermatid head concentration. There was a concentration-related decrease in total number of spermatids/testis; from controls to high dose, these values were 13.6 ± 0.5, 13.7 ± 0.9, 11.1 ± 0.6*, and 9.1 ± 0.7*. MBA exposure did not alter estrous cycle length or progression (control length 4.8 ± 0.2 days). Similarly, at necropsy, F0 female body weight, liver weight, kidneys/adrenals weight, and right ovary weight were not altered by MBA exposure. Histologic evaluation of tissues from the F0 mice found testicular degeneration in one control and three mice in the 60-ppm group.

Exposure of dams to MBA during nursing had no adverse effect on the survival or body weight gain of F1 mice to weaning at Day 21.

Task 4 (fertility assessment, F1 animals) By the week of cohabitation (at 74 ±10 days of age) for the Task 4 mating trial, all treated male mice weighed less than the controls (from control to high dose, in grams, 39.0 ± 0.6, 35.2 ± 0.4*, 34.0 ± 0.8*, and 33.6 ± 0.4*). All MBA-treated female F1 mice also weighed less (95, 90, and 87% of control weights, from low-dose group to high-dose group). Immediately after the mating week, food consumption was increased only in the high-dose males and females (by 16 and 26%, respectively), while water consumption did not differ among the groups. Based on the calculated consumption/kg/day for both sexes, doses of MBA were estimated at 2.6, 9.6, and 16.3 mg/kg bw/day.

The fertility and reproductive performance of second-generation MBA-exposed and control mice were evaluated. MBA did not alter the percentage of fertile pairs, the percentage of females with vaginal plugs, or the percentage of plugged females that delivered a litter. Fewer live pups were delivered in the mice exposed to 60 ppm, and pup weights were decreased in both the middle- and high-dose groups. These Task 4 F1 young adult mice were necropsied after estrous cycle evaluations. At necropsy, the body weights for all treated males were less than controls (by up to 11%). Absolute liver weight and kidney weights were unchanged, so weights for those organs adjusted for body weight were increased in the treated groups. Prostate weights were unchanged, while seminal vesicle weights were decreased slightly in the high-dose group, and testis weight was decreased in the middle- and high-concentration groups. While there were no changes in epididymal sperm concentration, percentage motile sperm, frequency of abnormalities, or number of spermatid heads/g of testis, there was a slight and statistically significant decrease in total spermatids per testis. For F1 female mice necropsied at the end of Task 4, body weight was decreased in all treated groups, and absolute liver weight trended down slightly, so that adjusted liver weight was not different across groups. Relative kidneys/adrenals weight trended up across groups, while absolute and relative ovary weight showed a decreasing trend which reached statistically significance at the top concentration. There was no difference in estrous cycle length between controls and 60 ppm MBA mice (control length 4.5 ± 0.1 days). Microscopically, degeneration was found in the testes of two control mice, one at 30 ppm, and two at 60 ppm.

Table 10: Effects of MBA during continuous breeding (table modified from Chapin et al. 1995).

Dose in ppm	**0**	**10**	**30**	**60**
Live pups/litter	14.4 ± 0.3 (40)	14.9 ± 0.5 (19)	12.9 ± 0.5* (20)	12.4 ± 0.7 (18)*[a]
Live pup weight (g)	1.59 ± 0.02	1.60 ± 0.02	1.55 ± 0.02	1.49 ± 0.02*[a]
Adjusted live pup weight (g)	1.60 ± 0.01	1.61 ± 0.02	1.54 ± 0.02*	1.48 ± 0.042*[a]

Mean ± SEM (n) *Significantly different from control ($p<0.05$). [a] dose-related trend

Table 11: Effects of MBA on fertility and reproductive performance at crossover mating trial (Task 3) (table modified from Chapin et al. 1995).

	Control male x control female	**60 ppm male x control female**	**Control male x 60 ppm female**
Live pups/litter	14.0 ± 1.0 (11)	11.4 ± 1.1 (16)	11.4 ± 1.1 (11)
Live pup weight (g)	1.58 ± 0.03	1.77 ± 0.06	1.49 ± 0.04
Adjusted live pup weight (g)	1.66 ± 0.04	1.74 ± 0.03	1.46 ± 0.04*

Mean ± SEM (n) *Significantly different from control ($p<0.05$).

Table 12: Dominant-lethal data for males exposed to MBA (table modified from Chapin et al. 1995).

Dose in ppm	**0**	**10**	**30**	**60**
Early resorptions	0.83 ± 0.17 (20)	1.19 ± 0.35 (19)	1.28 ± 0.32 (20)	1.34 ± 0.13 (18)*[a]
Dead fetuses	0.12 ± 0.07	0.05 ± 0.03	0.08 ± 0.08	0.12 ± 0.05
Total implantation loss	1.06 ± 0.19	1.26 ± 0.35	1.36 ± 0.35	1.52 ± 0.12 *[a]
Live fetuses	11.2 ± 0.5	12.1 ± 0.4	11.4 ± 0.4	11.3 ± 0.3

Mean ± SEM (n) *Significantly different from control ($p<0.05$). [a] dose-related trend ($p<0.05$).

Table 13: Effects of MBA on reproductive performance in F1 Swiss Mice (Task 4) (table modified from Chapin et al. 1995).

Dose in ppm	**0**	**10**	**30**	**60**
Live pups/litter	13.6 ± 0.4 (17)	12.9 ± 0.5 (15)	12.2 ± 0.8 (18)	9.1 ± 1.0 (15)*[a]
Live pup weight (g)	1.65 ± 0.03	1.58 ± 0.04	1.53 ± 0.03*	1.43 ± 0.03*[a]
Adjusted live pup weight (g)	1.67 ± 0.03	1.59 ± 0.03	1.53 ± 0.03*	1.39 ± 0.04*[a]
Dam body weight (g)	38.4 ± 0.4	35.2 ± 0.7*	34.8 ± 0.8*	32.1 ± 0.8*[a]

Mean ± SEM (n) *Significantly different from control ($p<0.05$). [a] dose-related trend ($p<0.05$).

Table 14: Necropsy data for Task 4 F1 mice after exposure to MBA (table modified from Chapin et al. 1995).

Dose in ppm	**0**	**10**	**30**	**60**
Male				
Terminal body weight (g)	39.2 ± 1.0 (20)	35.8 ± 0.5 (10)*	35.8 ± 1.3 (10)*	34.9 ± 0.7 (10)*[a]
Liver (g)	2.2 ± 0.05	2.2 ± 0.06	2.3 ± 0.09	2.2 ± 0.05
Kidneys/adrenals (g)	0.76 ± 0.03	0.76 ± 0.02	0.87 ± 0.05	0.76 ± 0.03
Right cauda epididymis (mg)	14.5 ± 0.4	16.2 ± 0.8	13.5 ± 0.8	12.4 ± 0.6*[a]
Right testis (mg)	129.7 ± 3.0	134.0 ± 6.4	102.3 ± 3.3*	83.8 ± 3.8*[a]
Relative liver weight	56.4 ± 0.9	61.5 ± 1.4*	65.2 ± 2.3*	62.8 ± 0.8*[a]
Relative kidneys/adrenals weight	19.6 ± 0.6	21.2 ± 0.5	24.4 ± 1.3*	21.8 ± 0.8*[a]
Total spermatid heads x 10^6/testis	15.0 ± 0.4	14.1 ± 0.6	12.5 ± 0.7*	14.0 ± 0.5*[a]
Female				
Terminal body weight (g)	32.9 ± 0.6 (20)	30.6 ± 0.6 (9)*	31.1 ± 1.2 (10)	28.5 ± 1.0 (10)*[a]
Liver (g)	2.0 ± 0.05	1.9 ± 0.06	2.0 ± 0.09	1.8 ± 1.0 (10)*a
Kidneys/adrenals weight	0.55 ± 0.01	0.51 ± 0.02	0.56 ± 0.02	0.50 ± 0.02
Right ovary (mg)	13.0 ± 0.8	11.8 ± 1.0	11.0 ± 1.0	8.7 ± 0.8*[a]
Relative kidneys/adrenals weight	16.7 ± 0.3	16.8 ± 0.5	18.1 ± 0.5*	17.7 ± 0.5[a]
Relative right ovary weight	0.40 ± 0.05	0.38 ± 0.03	0.35 ± 0.03	0.30 ± 0.02*[a]

Mean ± SEM (n) *Significantly different from control ($p<0.05$). [a] dose-related trend ($p<0.05$).

In addition to MBA, the study investigated effects on acrylamide and two other analogues, methacrylamide and N-hydroxymethylacrylamide. Neurotoxicity endpoints were also assessed but are not summarised here. MBA was not neurotoxic in this test.

Genotoxic effect: positive.

3.8.2.5 Study 5: Study on sperm count, morphology and testicular histopathology

Study reference:

Sakamoto J and HashimotoK. Effects of N,N'-methylene-bis-acrylamide (MBA) on mouse germ cells -sperm count and morphology, and testicular pathology. Archives of Toxicology (1988) 62:54-59.

Detailed study summary and results:

Test type

Study of sperm count and morphology, and testicular histopathology. No guideline. Not GLP.

Test substance

MBA of specific reagent grade was purchased from Wako Chemicals, Osaka, Japan. No information on impurities and batch number.

Test animals

Six-week-old male ddY strain mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan).

Administration/exposure

MBA in 0.9% saline solution was administered as a single oral dose of 200, 100 and 50 mg/kg. Control mice received the same volume of the vehicle. 3-4 mice were killed from 1 to 75 days after treatment. Wet weights of the testis and whole epididymis were recorded. Sperm suspension: The right caput epididymis from each mouse was weighed and minced with scissors in 0.8 ml 10% neutral buffered formalin. The resulting suspension was filtered and then stained with 0.5% Eosin Y solution. Sperm counting and scoring: The number of sperm was counted. Sperm (500-1000) was examined for morphological abnormality and scored according to the categories of sperm-head morphology. The results were expressed as percent of abnormal sperm.

Histological preparation: The testis and epididymis were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, and sliced. Sections were stained. The cycle of the seminiferous epithelium was classified into 14 stages (I-XIV). These stages represented series of changes occurring in a given area of the seminiferous epithelium.

Statistical analysis: The differences in means among controls

and treated groups were analysed by one-way analysis of variance followed by a multiple-comparison procedure. The differences between control and the treated group were analysed by either Student's or Welch's t-test.

Results and discussion

Statistically significant testicular weight loss was seen at 25-30 days after dosing of 200 mg/kg bw (weights recovered to control level within 45 days). At 100 mg/kg MBA, testicular weight reached a minimum at 30 and 35 days, although not statistically significant. At 50 mg/kg bw, a slight decrease in testicular weight and testis to body weight ratio was seen at 35 days.

Sperm count and morphology were dose dependently affected. Exposure to 200 mg/kg bw MBA caused a statistically significant decrease in sperm count 30-60 days after treatment. A significant increase in abnormal sperm occurred from 7 to 45 days. High abnormality remained 75 days after treatment.

Exposure to 100 mg/kg bw resulted in sperm count decrease at 30 and 35 days, and a significant increase in sperm abnormality was seen at 30 days. After treatment with 50 mg/kg, no statistically significant decrease in sperm count was seen, but a high rate of sperm abnormality appeared 30-35 days after treatment.

Testicular Histopathology

In mice given 200 mg/kg MBA, round spermatids vacuolated in stages I-III 1 day after treatment, where spermatogonia were reduced. Many tubules in stage VIII contained degenerated cells near the basal membrane where Sertoli cells appeared in place of resting spermatocytes. On day 3, resting spermatocytes and leptotene spermatocytes were missing in stages VIII-XI. On day 7, resting spermatocytes increased in stages VII-VIII, whereas early pachytene spermatocytes decreased, or degenerated in stages I-III. In many tubules in stages IX-XIV, leptotene, or zygotene spermatocytes were absent. Many degenerated round spermatids were extruded into the cauda epididymis. On day 15, leptotene, or zygotene spermatocytes increased in number in stages IX-XIV. Many tubules in stage I lacked round spermatids. In stages I-VIII, early pachytene, or pachytene spermatocytes were reduced. Twenty days later, early pachytene, or pachytene spermatocytes increased in most tubules except in stages X-XII. Round spermatids in stages I-VI decreased, whereas type A and B spermatogonia and resting spermatocytes were frequently seen. On day 30, elongated spermatids at maturation phase and mature sperm became markedly reduced in number. The atrophy of the tubules in stages I-VI progressed, whereas most of the tubules in stages IX-XIII seemed to be active. The exfoliation of Sertoli cells, type A spermatogonia, early pachytene spermatocytes were observed in the lumen. In the epididymis, vacuolated nuclei of round spermatids were extruded. Within 45 days, maturing sperm increased, and there was no tubular atrophy.

In mice receiving 100 mg/kg MBA, early pachytene spermatocytes were reduced in stages I-II 10 days after treatment. On day 15, step 9-15 spermatids were absent, and step 7-8 spermatids rather more scarce. Degenerated nuclei of step 1-8 spermatids were observed frequently. The caput epididymis contained many degenerated nuclei of round spermatids in the lumen. On day 25, round spermatids decreased in stages I-V. On day 30, spermatids at maturation phase were missing in many tubules, but they reappeared 5 days later. On day 45, no histological abnormality was seen compared with control mice.

In mice exposed to 50 mg/kg MBA, degenerated nuclei of the round spermatids were extruded into the epididymis 15 days post-treatment. On day 30, 2-4 necrotic germ cells were seen in most tubules. Round spermatids revealed nuclear degenerations such as pyknosis, swelling, and vacuolation. On day 45, markedly swollen cell bodies were seen at the luminal side in many tubules in all stages.

After exposure to 200 mg/kg MBA sperm abnormality appeared in diphase, which corresponded with early histopathological changes in testis 1-3 days after treatment (including reduction in number of resting, leptotene, and zygotene spermatocytes, and a degeneration of nuclei of round spermatids in stages I-III) . The

marked loss of spermatids at maturation phase at 30 days coincided with the decrease in sperm count in the caput epididymis seen 35 days after treatment with 100 and 200 mg/kg bw of MBA.

Genotoxic effect: positive

3.8.2.6 Study 6: Developmental toxicity study

Study reference:

George JD, Price CJ, Marr MC, Myers CB, Schwetz BA, Heindel JJ. Evaluation of the Developmental Toxicity of Methacrylamide and N,N'-Methylenebisacrylamide in Swiss Mice, TOXICOLOGICAL SCIENCES 46, 124–133 (1998). NTP: Abstract for TER90008 (nih.gov)

Detailed study summary and results:

Test type

Developmental toxicity study (1992). GLP compliant.

Test substance

MBA (CAS No. 100-26-9), 98% pure, was dissolved in distilled water. No information on Batch number.

Test animals

Swiss mice [Crl:CD-1 (ICR) VAF/Plus].

25-26 females/dose group

Female mice weighed from 23 to 33 g on GD 0 (i.e., day of vaginal plug detection).

Administration/exposure

Animals were dosed daily by gavage with MBA solutions or distilled water (vehicle) on GD 6 through 17. The actual volume administered (10 ml/kg) was based on body weight taken daily during the dosing period. Doses were 0, 3, 10 and 30 mg/kg bw.

Results and discussion

Study Abstract (copied)

Timed-pregnant CD-1 outbred albino Swiss mice received either methacrylamide (MAC; 0, 60, 120, or 180 mg/kg/day) or N,N'- methylenebisacrylamide (BAC; 0, 3, 10, or 30 mg/kg/day) po in distilled water on gestational days (GD) 6 through 17. Maternal clinical status was monitored daily. At termination (GD 17), confirmed-pregnant females (27–30 per group, MAC; 24–25 per group, BAC) were evaluated for clinical status and gestational outcome; live fetuses were examined for external, visceral, and skeletal malformations. For MAC, no treatment-related maternal mortality was observed. Maternal body weight on GD 17, maternal weight gain during treatment and gestation, and corrected maternal weight gain were reduced at the high dose. Relative maternal food and water intake was not adversely affected, neurotoxicity

was not observed. Relative maternal liver weight was increased at >120 mg/kg/day; gravid uterine weight was decreased at 180 mg/kg/day. The maternal no-observed adverse effect level (NOAEL) was 60 mg/kg/day. The NOAEL for developmental toxicity was also 60 mg/kg/day. At >120 mg/kg/day, mean fetal body weight was reduced. At 180 mg/kg/day, increased post implantation death per litter was observed. Morphological development was not affected. The maternal NOAEL for BAC was 10 mg/kg/day. At 30 mg/kg/day, decreased maternal body weight on GD 17, maternal body weight change during treatment and gestation, corrected maternal body weight, and gravid uterine weight were observed. Relative maternal liver weight increased at 30 mg/kg/day. The developmental NOAEL was 3 mg/kg/day BAC. Mean fetal body weight was reduced at 30 mg/kg/day. At >10 mg/kg/day, an increased incidence of fetal variations (extra rib) was observed, although fetal malformation rate was unaffected. MAC and BAC were not teratogenic to Swiss mice at the doses tested. BAC was more potent than MAC in causing adverse maternal and developmental effects.

Genotoxic effect: positive

3.8.2.7 Study 7: Sex-linked recessive lethal test in Drosophila

Study reference:

Foureman P, Mason JM, Valencia R, Zimmering S. Chemical Mutagenesis Testing in Drosophila. X. Results of 70 Coded Chemicals Tested for the National Toxicology Program. Environmental and Molecular Mutagenesis 23:208-227 (1994).

Detailed study summary and results:

Test type

Sex-linked recessive lethal test in Drosophila. OECD TG 477 is deleted since 2014.

Test substance

MBA from Aldrich Chemical Co, Batch JJ2626EJ, Purity >99%. Solvent: water.

Test animals

Drosophila melanogaster, Canton-S and Basc stocks.

Administration/exposure

0 and 600 ppm. Toxicity tests were run on a series of exposures and the exposure level was chosen that resulted in 30% mortality after 72 hr of feeding. Exposure: Two or three glass fiber filter discs were saturated with the compound carried in a 5% sucrose solution (or other control solution) at the bottom of a standard glass vial. Solutions were renewed at 24 hr and 48 hr. After 72 hr of exposure, surviving males were mated. Concurrent control males were treated with the solution used to dissolve the test chemical.

Method

For the SLRL test each male was mated individually to three virgin Basc females, then transferred to fresh females every 2 to 3 days to make a total of three broods. F2 cultures were scored as presumptive lethals if the number of wild-type males was 0, 1, or <5% of the number of Basc males (or Basc females). All putative lethals were confirmed through an additional generation. If the initial feeding exposure produced a mutagenic response, a reciprocal translocation test was initiated and concentrating on the brood that proved to be the most sensitive in the SLRL test.

In the reciprocal translocation assay, Canton-S males were mated en masse (10 males and 20 females per vial) to virgin females for 3 days. Fertilized females were kept on regular culture medium and transferred every 3-4 days for as long as they produced eggs.

For the SLRL assay, a minimum of 5000 chromosomes was scored from each of the treated and concurrent control groups unless the mutant frequency exceeded 1%. The corrected treated and control data were compared using a normal approximation to the binomial distribution. In addition, treated data were compared to the historical control. For a compound to be considered mutagenic, the mutant frequency in the treated series (treated frequency) had to exceed 0.15% with a P-value of <0.05, or the treated frequency had to exceed 0.10% with a P-value of <0.01. If the treated frequency was between 0.10% and 0.15% and the P-value was between 0.1 and 0.01, or if the treated frequency was higher than 0.15% and the P-value was between 0.1 and 0.05, the result was considered equivocal. All other results were considered negative. Translocation data for each treated sample were compared to the historical control data for that laboratory using a conditional binomial test. As a rule, at least two translocations are required among 5000 tests in the treated series for a compound to be considered positive. As a comparison the combined historical translocation rate for three laboratories, including 33783 new tests was 4/149 946 (0.0027%).

Results and discussion

MBA 600 ppm caused 26% mortality and 34% sterility. Percent lethals was 0.49 (0.14 in controls) and the substance was considered mutagenic in the SLRL test. Reciprocal translocation experiment was negative for MBA (1 translocation in 5559 tests, according to authors not a statistically significant increase in the frequency of reciprocal translocations).

Genotoxic effect: positive.

3.8.3 Human data

No studies available.

3.9 Carcinogenicity

Not evaluated.

3.10 Reproductive toxicity

Not evaluated.

3.11 Specific target organ toxicity – single exposure

Not evaluated.

3.12 Specific target organ toxicity – repeated exposure

Not evaluated.

3.13 Aspiration hazard

Not evaluated.

4 ENVIRONMENTAL HAZARDS

Not evaluated.