PART 3

Section 3 Toxicological and Metabolism Studies on the Active Substance

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. Although based on a real submission, the data included in the following summary and evaluation have been amended to protect the commercial interests of the owner of the data.

Applicants should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

IIA 5.1.1- 5.1.3 **Absorption, distribution, metabolism and excretion (ADME) in the rat**

Report: II A 5.1.1/01 Smith H 1996, The absorption, distribution, elimination and metabolism of chemx in Sprague-Dawley rats following oral and intravenous administration Report No.: CCC-14300

Guidelines

The study was conducted according to US EPA FIFRA Guideline § 85-1 and fulfilled the requirements of the OECD Guideline 417 (1983)

GLP: Fully GLP compliant [17].

Executive Summary

In a metabolism study two forms of chemx (98% purity), labelled with ^{14}C at the C-3 or C-5 positions of the chem2 ring were administered to 4 Sprague-Dawley rats/sex/dose in multiple doses by oral gavage or intravenous injection at dose levels of 0, x, xx and xxx mg/kg bw/day (0, xx, xxx and xxxx ppm). The four dose groups were a single low oral dose [Group 1: x mg/kg bw/day (xx ppm)], a single low intravenous dose [Group 2: xx mg/kg bw/day (xxx ppm)], a single high oral dose [(Group 3: xxx mg/kg bw/day (xxxx ppm] and a repeat low oral dose consisting of 14 daily doses of unlabeled chemx followed by a single oral dose of ^{14}C labelled chemx [Group 4 - each dose was x mg/kg bw/day (xx ppm)].

[17] In the US, laboratories are responsible for certifying that they have complied with FIFRA GLP requirements. The EPA (Environmental Protection Agency, Office of Compliance Monitoring) verifies compliance by means of periodic inspections.

Chemx and its metabolites were readily excreted by the rat with urinary excretion being the major route of elimination (77 - 87 % *vs* 4.77 - 13.2 % in faeces) for all animals receiving a low dose, and faecal excretion being the predominant route of elimination (approximately 59 % vs 31.8 - 33.4% in urine) following administration of the high dose. Expiration as carbon dioxide or other volatile compounds was not a significant route of elimination (< 0.04 % radioactivity recovered as CO_2 after 24 hrs). In the low dose groups, absorption was greater than 90 % while at the high dose, absorption averaged approximately 40 %. There was little evidence of retention of chemx or its metabolites - tissue and blood levels were negligible and apart from the liver (< 0.13%), no individual tissue contained more than 0.01 % of the administered dose. Greater than 90 % of the administered dose was excreted in three days. The major component of the excreted radioactivity was unchanged chemx.

Metabolism of chemx in the rat occurred to a limited extent *via* demethylation and chem2 ring hydroxylation. The cleavage of the xxx bond of chemx to form separate chem2 and chem3 metabolites is a minor metabolic pathway in the rat. There was little difference in the metabolic profile regardless of the route of administration, dose level, number of doses or sex. Some slight quantitative differences in minor metabolites were seen between the sexes. The proposed pathway for the metabolism of chemx in rats is outlined in Figure IIA 5.1.1-1.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: chemx -

	chem2 ring labelled:	^{14}C in the C-3 position (label 11 chemx); specific activity 9 mCi/mmol;
	chem3 ring labelled:	^{14}C in the C-5 position (label 12 chemx); specific activity 9 mCi/mmol;
Description:	White powder	
Lot/Batch #:	NPD-9307-5386-T	
Purity:	chem2 ring labelled:	radiochemical purity ≥ 98 % [18]
	chem3 ring labelled:	radiochemical purity ≥ 98 % [18]
CAS #:	16335-17-2	
Stability of test compound:	The test material was stable for at least 7 days at room temperature.	

2. Vehicle and/or positive control: The vehicle used was Emulphor®.

3. Test animals -

Species:	Rat
Strain:	Sprague-Dawley (CD)
Age:	at dosing 46 – 68 days (males); 50 – 70 days (females)
Weight at dosing:	245 - 386 g for males; 162 - 384 for females
Source:	Charles River Laboratories, Raleigh, NC
Acclimation period:	minimum of 10 days quarantine in stainless steel suspension cages, followed by 24 hours acclimation to individual metabolism cages
Diet:	Purina rat chow, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Roth-type glass individual metabolism cages

[18] Details with respect to the purity and content of impurities of the test material are provided in Document J

Husbandry: Husbandry conditions were in accordance with the USPHS-NIH publication *Guide to the Care and Use of Laboratory Animals*.

Environmental conditions -

Temperature: 22 ± 2° C
Humidity: 55 ± 10 %
Air changes: 16 - 20 changes/h
Photoperiod: Alternating 12-hour light and dark cycles

4. Preparation of dosing solutions

The labelled dose consisted of a 1:1 mixture of ^{14}C-Label 11-chemx and ^{14}C-Label 12-chemx. The test substance was in solution at the low dose level and was present as a suspension in the high dose (due to the limited solubility of chemx). The intravenous vehicle used was a mixture of Emulphor® : ethanol : saline (1 : 1 : 8).

B. STUDY DESIGN AND METHODS

1. Pilot study

In the pilot study, separate groups of animals were dosed with one of the two ring-specific labelled ^{14}C-chemx (^{14}C-Label 11-chemx or ^{14}C-Label 12-chemx) at two different dose levels; groups P1 (dosed with ^{14}C-Label 12-chemx) and P3 (dosed with ^{14}C-Label 11-chemx) received xx mg/kg bw (xxx ppm) and groups P2 (dosed with ^{14}C-Label 12-chemx) and P4 (dosed with ^{14}C-Label 12-chemx) received xxx mg/kg bw (xxxx ppm).

2. Main study

The main study consisted of four groups: a single low oral dose [Group 1: xx mg/kg bw (xxx ppm)], a single low intravenous dose [Group 2 : xx mg/kg bw (xxx ppm)], a single high oral dose [Group 3: xxx mg/kg bw (xxxx ppm)] and a repeat low oral dose consisting of 14 daily doses of unlabeled chemx ^{18} followed by a single oral dose of ^{14}C labelled chemx [Group 4 - each dose was xx mg/kg bw (xxx ppm)].

II. RESULTS AND DISCUSSION

1. Pilot study

The results of the pilot phase demonstrated that less than 0.04 % of the dosed radioactivity was recovered as expired $^{14}C0_2$ after 24 hours. For this reason, expired gases were not measured in the main study. The pilot phase also demonstrated that the major component of the excreted radioactivity was the unchanged chemx and minimal cleavage of the xxx bond took place.

2. Storage stability

The storage stability of radiolabelled chemx was measured following storage for 4 months at -20°C. No degradation was observed. In addition, storage stability over a 12-month period in frozen rat excreta, was assessed. Other than a slight quantitative change in the metabolite profiles (minor metabolites), qualitative analysis was unchanged.

3. Main study

Absorption

Comparison of the excreted radioactivity following the low dose oral administration with that obtained following intravenous administration demonstrated that the single oral dose was well absorbed (95 % and 91 % for males and females respectively). Absorption was not affected by repeated doses (93 % and 90 % for males and females respectively). Absorption was considerably lower in the high dose oral group at 36 % for males and 39 % for females.

Distribution

Analysis of tissue distribution at five days after dosing demonstrated minimal amounts of radioactivity associated with tissues for all groups of animals. The liver contained the highest traces of radioactivity (< 0.13 % of the administered dose). No other individual tissue contained more than 0.01 % of the administered dose. The radioactivity associated with the residual carcass ranged from 0.02 - 0.36 % of the administered dose.

Excretion

Urine was the major route of elimination for all animals that received the low dose, and accounted for approximately 77 to 87 % of the dose, while an additional 4.77 % to 13.2 % was excreted in the faeces of these groups. The faeces were the major route of elimination for the high dose treated animals and accounted for approximately 59 % of the dose. Some 31.8 % and 33.4 % of the dose was excreted in the urine of these animals. The rate of excretion of radioactivity in the urine revealed a bi-exponential elimination process. The mean half-life for the initial phase was approximately 2.2 - 5.8 hours and for the terminal elimination phase was 21.4 - 56.7 hours for all groups. Determination of whole body elimination revealed similar elimination rates. Greater than 90 % of the dose was excreted three days after administration. The mean total recovery of the administered dose for all groups of animals ranged between 97 to 101 % of the dose administered.

Table IIA 5.1.1-1 Route of excretion and total recovery of chemx in rat

Group	*Sex of animals*	*Target dose (mg/kg bw)*	*Route of administration*	*Urine*	*Urine + Cage wash*	*Faeces*	*Total recovery*
1	Male	10	Single oral	82.5	87.0	10.2	97.4
1	Female	10	Single oral	77.5	88.7	9.35	98.3
2	Male	10	Intravenous	87.3	89.7	8.96	98.7
2	Female	10	Intravenous	84.9	94.6	4.77	99.4
3	Male	1000	Single oral	31.8	38.2	62.6	100.8
3	Female	1000	Single oral	33.4	43.2	54.7	98.0
4	Male	10	Repeated oral	81.2	85.1	13.2	98.4
4	Female	10	Repeated oral	77.0	90.3	8.41	98.7

The percentage of the dose excreted in urine was compared by route of administration, dose level and sex. Likewise, the percentage of the dose excreted in faeces was compared by route of administration, dose level and sex. Although a number of statistically significant differences were identified, these do not appear from visual inspection to have a biological significance.

Metabolism

Chemx and its metabolites were extracted from pooled urine and cage wash samples and from pooled faeces and identified using HPLC with confirmation using LC/MS. The unextracted fraction was quantified. Extracted samples were used for qualitative and quantitative analysis by HPLC (using reference compounds), mass spectral methods and acid hydrolysis. More than 95 % of the radioactivity was extracted in all dose groups.

There was little difference in the metabolite profile regardless of the route of administration, dose level or sex. Some slight quantitative differences in minor metabolites were seen between the sexes. Repeated dosing did not affect the metabolite profile. The principle radioactive component present in the excreta was un-metabolised chemx. In addition, four other metabolites were identified none of which amounted to more than 5 % of the administered dose; desmethylchemx, 5-hydroxyxchemx, sulphonamide and chem4 sulphate (trace).

Table IIA 5.1.1-2 Proportions of the major metabolites identified in rat excreta

Group	Sex	Excreta	desmethyl-chemx	chem3ide	5-hydroxy-chemx	chemx	% of dose identified
1	Male	Urine	1.41	0.58	0.66	78.56	81.21
		Faeces	1.60	0.16	0.26	1.96	7.18
1	Female	Urine	0.80	2.62	0.32	79.85	83.58
		Faeces	1.13	0.26	0.50	2.67	7.09
2	Male	Urine	1.76	0.84	1.13	82.39	86.11
		Faeces	1.28	ND	0.30	1.57	5.90
2	Female	Urine	1.12	1.77	0.48	88.85	92.22
		Faeces	0.51	0.09	0.10	1.49	3.38
3	Male	Urine	0.35	0.27	0.13	34.76	35.51
		Faeces	3.59	ND	1.26	52.89	60.00
3	Female	Urine	0.17	0.33	ND	40.03	40.53
		Faeces	1.33	0.53	0.31	50.71	53.26
4	Male	Urine	1.74	1.68	0.57	76.55	80.54
		Faeces	1.56	0.15	0.41	3.22	9.21
4	Female	Urine	0.72	ND	1.88	87.18	89.78
		Faeces	0.84	ND	0.84	2.65	5.79

Parent chemx represented 76.55 % to 88.85 % of the dose excreted in the urine at the low dose level groups, while unchanged chemx represented 40 % and 34.76 % at the high dose level (♂ and ♀ respectively). The faeces represented a minor route of excretion at low dose levels; 1.49% to 3.22%. At the high dose level 53% and 51% were eliminated by this route in males and females, respectively.

The proposed metabolic pathways for chemx is as follows: (1) ring hydroxylation of the 5-position carbon of the chem2 ring and (2) demethylation of the methoxy group at either the 4- or 6-position of the chem2 ring, resulting in the formation of the desmethyl and 5-hydroxy-chemx (the most abundant metabolites) followed by demethylation of the methoxy group at either the 4- or 6-position of the chem2 ring. The cleavage of the xxx bridge to form separate chem2 and chem3 metabolites was a minor pathway.

Figure IIA 5.1.1-1 Proposed Pathway for Metabolism of Chemx in Rats

Pathway Omitted

III. CONCLUSIONS

Chemx and its metabolites were readily excreted by the rat with urinary excretion as the major route of elimination for all animals receiving a low dose, and faecal excretion being the predominant route of elimination following a high dose. There was little evidence of retention of chemx or its metabolites; tissue and blood levels were negligible, with no individual tissue containing levels exceeding 0.2 % of the dose. In the low dose groups, absorption was greater than 90 % while at the high dose, absorption averaged approximately 40 %. Metabolism of chemx in the rat occurred to a limited extent via demethylation and chem2 ring hydroxylation. The cleavage of the xxx bond of chemx to form separate chem2 and chem3 metabolites is a minor metabolic pathway in the rat. Expiration as carbon dioxide or other volatile compounds was not a significant route of elimination.

(Smith H 1996)

IIA 5.2.1 **Acute oral toxicity**

Report: II A 5.1/01 Smith H 1993, The acute toxicity of chemx in rats following oral administration, Report No.: CCC-13156

Guidelines

US EPA FIFRA Guideline § 85-1, which is equivalent to OECD 401

GLP

Yes (certified laboratory) with the following exceptions:

1) concentration, homogeneity and stability of the dose were not determined and
2) QA inspection was not carried out during the in-life phase. However, the dose was prepared freshly prior to dosing. These deviations did not compromise the acceptability of the study.

Executive Summary

In an acute oral toxicity study, groups of fasted, young adult Sprague-Dawley rats 5/sex/dose were given a single oral dose (gavage) of chemx (98.9 % purity) in corn oil a single dose of 5,000 mg/kg bw (100,000 ppm) and were observed for 14 days.

Oral LD_{50}	males	=	> 5000 mg/kg bw (> 100,000 ppm)
	females	=	> 5000 mg/kg bw (> 100,000 ppm)
	combined	=	> 5000 mg/kg bw (> 100,000 ppm)

Chemx was found to be of a low order of acute toxicity following exposure of rats. Clinical signs on the day of dosing or within two days after dosing included faecal staining, salivation, mucoid stools and soft stools. All animals except one female had gained weight 7 and 14 days following dosing. The female concerned lost a small amount of weight between post-dosing days 7 and 14. On the basis of this study, chemx does not warrant classification as being harmful or toxic.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9209-4523-T
   - **Purity:** 98.9 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** not determined

2. **Vehicle and/or positive control:** Corn oil

3. **Test animals -**
   - **Species:** Rat
   - **Strain:** Crl:CD(SD)BR, albino
   - **Age:** Young adult
   - **Weight at dosing:** 217 - 286 g males; 215 - 269 females
   - **Source:** Charles River Laboratories, Portage, MI
   - **Acclimation period:** 7 days
   - **Diet:** Chow (#5001), *ad libitum*
   - **Water:** Tap water, *ad libitum*
   - **Housing:** Animals were individually housed in stainless steel suspended cages
   - **Environmental conditions -**
     - **Temperature:** Temperature was not specified
     - **Humidity:** Relative humidity ranged from 35 to 84 %
     - **Air changes:** Not recorded
     - **Photoperiod:** Alternating 12-hour light and d ark cycles

B. STUDY DESIGN AND METHODS:

1. **In life dates:** 15 January to 5 February 1993

2. **Animal assignment and treatment**

   Following a range-finding preliminary test in which no animals died (500 to 5000 mg/kg bw), a single dose of 5000 mg/kg bw (100,000 ppm) was selected for the main study. Animals were assigned to the

test groups listed in Table IIA 5.2.1-1. Following an overnight fast (17 - 22 hours), rats were given a single dose of chemx (99.3 % pure) by gavage. The test substance was administered in corn oil at a volume of 10 ml/kg bw. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 1, 2.5 and 4 hours after dosing and at least once daily for the remainder of the 14-day study. Body weights were recorded at day 0 (prior to dosing), 7 and 14. On day 14, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.1-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Males	Females	Combined
0	0/5	0/5	0/10
5,000	0/5	0/5	0/10

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. Mortality

Details are provided in Table IIA 5.2.1-1. No mortalities occurred at 5,000 mg/kg bw (100,000 ppm), the only dose level tested.

The oral LD_{50} for males was > 5,000 mg/kg bw (> 100,000 ppm)
for females was > 5,000 mg/kg bw (> 100,000 ppm)
combined was > 5,000 mg/kg bw (> 100,000 ppm)

B. CLINICAL OBSERVATIONS

Clinical signs on the day of dosing or within two days after dosing included faecal staining, salivation, mucoid stools and soft stools.

C. BODY WEIGHT

All animals except one female had gained weight 7 and 14 days following dosing. The female concerned lost a small amount of weight between post-dosing days 7 and 14.

D. NECROPSY

No internal abnormalities were observed at gross necropsy.

E. DEFICIENCIES

Despite the concentration, homogeneity and stability of the dose not being determined and despite QA inspection not being carried out during the in-life phase, the study is nevertheless acceptable. The dose was prepared freshly prior to dosing. These deviations did not compromise the acceptability of the study.

III. CONCLUSIONS

The oral LD_{50} of chemx was found to be in excess of 5,000 mg/kg bw (100,000 ppm). Chemx does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Smith H 1993)

IIA 5.2.2 **Acute percutaneous toxicity**

Report: IIA 5.2.2/01 Jones KL 1993, Acute dermal toxicity study in rats chemx, Report: SB-92-480

Guidelines

US EPA FIFRA Guideline § 81-2, which is equivalent to OECD 402

GLP

Yes (certified laboratory) with the following exception: concentration, homogeneity and stability of the dose were not determined. However, the dose was prepared freshly prior to dosing. The deviations were not considered such that they compromised the acceptability of the study.

Executive Summary

In an acute dermal toxicity study, groups of young adult Sprague-Dawley rats, 5/sex were exposed by the dermal route to chemx (98.9% purity). Test material was applied in distilled water for 24 hours to 10 % of each animals body surface at a dose of 5,000 mg/kg bw (100,000 ppm). Animals were observed for the following 15 days.

Dermal LD_{50}	males	=	> 5000 mg/kg bw (> 100,000 ppm)
	females	=	> 5000 mg/kg bw (> 100,000 ppm)
	combined	=	> 5000 mg/kg bw (> 100,000 ppm)

Chemx was found to be of a low order of acute toxicity following exposure of rats *via* the dermal route. Clinical signs included dark material on the facial area, and urine stains. Slight erythema was noted in two rats. All signs were resolved by the fourth day following exposure. On the basis of this study, chemx does not warrant classification as being harmful or toxic.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9209-4523-T
   - **Purity:** 98.9 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** not determined

2. **Vehicle and/or positive control:** test material dosed as received

3. **Test animals -**
   - **Species:** Rat
   - **Strain:** Crl:CD(SD)BR, albino
   - **Age:** Young adult
   - **Weight at dosing:** 240 - 260 g males; 230 - 245 females
   - **Source:** Charles River Laboratories, Portage, MI
   - **Acclimation period:** 5 days
   - **Diet:** Chow (#5002), *ad libitum*
   - **Water:** Tap water, *ad libitum*
   - **Housing:** Animals were individually housed in stainless steel suspended cages
   - **Environmental conditions -**
     - **Temperature:** Temperature was not specified
     - **Humidity:** Relative humidity ranged from 35 to 84 %
     - **Air changes:** Not recorded
     - **Photoperiod:** Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. **In life dates:** 28 October to 16 December 1993

2. **Animal assignment and treatment**

Animals were assigned to the test groups listed in Table IIA 5.2.2-1. On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal. The clipped area accounted for more than 10 % of each animals body surface. The test substance was administered as a single occluded dermal application and was applied moistened with distilled water. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with distilled water. Animals were observed for gross toxicity and behavioural changes on three occasions on the day of dosing and once daily thereafter for the duration of the study. Mortality checks were conducted twice daily. Individual body weights were measured and recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.2-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Males	Females	Combined
0	0/5	0/5	0/10
5,000	0/5	0/5	0/10

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. Mortality

Details are provided in Table IIA 5.2.2-1. No mortalities occurred at 5,000 mg/kg bw (100,000 ppm), the only dose level tested.

The dermal LD_{50} for males was > 5,000 mg/kg bw (> 100,000 ppm)
for females was > 5,000 mg/kg bw (> 100,000 ppm)
combined was > 5,000 mg/kg bw (> 100,000 ppm)

B. CLINICAL OBSERVATIONS

Clinical signs included dark material on the facial area, and urine stains. Slight erythema was noted in two rats. All signs were resolved by the fourth day following exposure.

C. BODY WEIGHT

All animals gained weight during the study.

D. NECROPSY

No treatment related gross necropsy observations were noted.

E. DEFICIENCIES

Despite the concentration, homogeneity and stability of the dose not being determined and despite QA inspection not being carried out during the in-life phase, the study is nevertheless acceptable. The dose was prepared freshly prior to dosing. These deviations did not compromise the acceptability of the study.

III. CONCLUSIONS

The percutaneous LD_{50} of chemx was found to be in excess of 5,000 mg/kg bw (100,000 ppm). Chemx does not warrant classification as being harmful or toxic on the basis of its acute percutaneous toxicity.

(Jones KL 1993a)

IIA 5.2.3 **Acute inhalation toxicity**

Report: IIA 5.2.3/01 Smith CL 1994, Acute inhalation study of chemx herbicide, Report: CCC-13880

Guidelines

US EPA FIFRA Guideline § 81-3, which is equivalent to OECD 403

GLP: yes (certified laboratory), fully compliant

Executive Summary

In an acute inhalation toxicity study, groups of young adult Sprague-Dawley rats (5/sex), were exposed by the inhalation route to chemx (98.5 % purity) in air for 4 hours to nose only at a concentration of 3 mg/L. Animals observed for the following 14 days.

Inhalation LC_{50}	males	=	> 3 mg/L
	females	=	> 3 mg/L
	combined	=	> 3 mg/L

Chemx was found to be of a low order of acute toxicity following exposure of rats *via* the inhalation route. Clinical signs included dark material on the facial area, and urine stains. Slight erythema was noted in two rats. All signs were resolved by the fourth day following exposure.

During exposure, a red nasal discharge and a red ocular discharge were observed. Observations immediately following exposure included red/pink nasal discharge and red ocular discharge. From post-exposure days 1 to 14, all animals appeared normal. Three females lost weight in the two days following treatment. All animals gained weight by the seventh day following treatment and continued to gain weight until sacrificed. The only gross necropsy abnormality observed was the occurrence of enlarged livers in two males. On the basis of this study, chemx does not warrant classification as being harmful or toxic.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   Description: White powder
   Lot/Batch #: GHQ-9307-5385-T
   Purity: 98.5 % as [18]
   CAS #: 16335-17-2

Stability of test compound: Stable for at least 4 weeks stored in darkness at room temperature

2. **Vehicle and/or positive control:** chemx aerosol

3. **Test animals -**

Species:	Rat
Strain:	Crl:CD(SD)BR, albino
Age:	Young adult
Weight at dosing:	315 - 340 g males; 235 - 255 females
Source:	Charles River Laboratories, Portage, MI
Acclimation period:	8 days
Diet:	Chow (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages
Environmental conditions -	
Temperature:	Temperature was not specified
Humidity:	Relative humidity ranged from 35 to 84 %
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. **In life dates:** 13 February to 7 March 1994

2. **Animal assignment and treatment**

Animals were assigned to the test groups listed in Table IIA 5.2.3-1. Animals were observed approximately hourly during the 4-hour exposure period. Thereafter mortality and moribundity checks were conducted twice daily. Observations for signs of toxicity were conducted immediately following exposure and daily thereafter. Individual body weights were measured and recorded on days 2, 7 and 14. On day 14, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.3-1 Doses, mortality / animals treated

Dose (mg/L)	Males	Females	Combined
0	0/5	0/5	0/10
3	0/5	0/5	0/10

3. **Generation of the test atmosphere / chamber description**

An 80-L nose only exposure chamber was used. During exposure (4 hours), individual plastic tubes were positioned in two tiers around the outside of the chamber such that only the nose of test animals was exposed to the interior of the chamber. A JET-O-MIZER® jet mill was used to mill the test material

and generate the test aerosol. The test atmosphere was sampled at 20, 80, 140 and 223 minutes into the 240-minute exposure period for HPLC analysis, using a x column, 2 % acetonitrile mobile phase and UV (xx nm) detection. The limit of detection (LOD) for chemx was x µg as/L of air, while the limit of quantification (LOQ) was xx µg as/L of air. The test atmosphere concentration was 3.0 ± 0.16 mg as/L air.

Two samples were taken for particle size analysis using an Anderson cascade impactor. One of the samples was taken during the first part of the exposure period, the second during the second part of the exposure period:

Mass median aerodynamic diameter: 7.1 microns; Deomatric standard deviation: 2.6;
% particles < 10 microns: 65
% particles < 1 micron: 1.8

4. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. Mortality

Details are provided in Table IIA 5.2.3-1. No mortalities occurred at 3 mg/L, the only dose level tested.

The 4 hour inhalation LC_{50} for males was > 3 mg / L
for females was > 3 mg / L
combined was > 3 mg / L

B. CLINICAL OBSERVATIONS

During exposure, a red nasal discharge and a red ocular discharge were observed. Observations immediately following exposure included red/pink nasal discharge and red ocular discharge. From post-exposure days 1 to 14, all animals appeared normal.

C. BODY WEIGHT

Three females lost weight in the two days following treatment. All animals gained weight during by the seventh day following treatment and continued to gain weight until sacrificed.

D. NECROPSY

The only gross necropsy abnormality observed was the occurrence of enlarged livers in two males.

E. DEFICIENCIES

Although a mass median aerodynamic diameter of less than 4 microns was not achieved, the study is nevertheless acceptable. This deviation did not compromise the acceptability of the study.

III. CONCLUSIONS

The acute inhalation LC_{50} of chemx for the combined sexes was found to be in excess of 3 mg/L. The compound does not warrant classification as being toxic or harmful on the basis of its acute inhalation toxicity.

(Smith CL 1994)

IIA 5.2.4 **Skin irritation**

Report: in rats with Chemx Report: SB-92-480	IIA 5.2.4/01 Jones KL 1993, Primary dermal irritation study
Guidelines:	US EPA FIFRA § 81-5, which is equivalent to OECD 404
GLP:	yes (certified laboratory), fully compliant

Executive Summary

In a primary dermal irritation study, 6 young adult New Zealand rabbits, 2 males and 4 females, were exposed *via* the dermal route to 0.5 g of chemx (98.9% purity) per animal. The test material was applied as a powder for 4 hours to x % of the body surface area of test animals. Animals then were observed for 3 days. Irritation was scored using the Draize scheme.

Slight erythema was noted in one site at one hour post treatment. This had resolved by 24 hours. No other effect was seen. In this study, chemx was not a dermal irritant. On the basis of this study, chemx does not warrant classification as being irritating to the skin.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9209-4523-T
   - **Purity:** 98.9 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** not determined
2. **Vehicle and/or positive control:** test material dosed as received
3. **Test animals -**
   - **Species:** Rabbit
   - **Strain:** New Zealand
   - **Age:** Young adult
   - **Weight at dosing:** 2.1 – 2.3 kg males; 2.1 – 2.5 kg females
   - **Source:** Chalk Cliff Rabbitry, Whitesville, MI

Acclimation period: 5 days
Diet: Chow (#5322), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were individually housed in stainless steel suspended cages
Environmental conditions -
Temperature: Temperature was not specified
Humidity: Relative humidity ranged from 35 to 84 %
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 21 October to 1 November 1993

2. Animal assignment and treatment

On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal using a small animal clipper. The test material was applied, semi-occluded, as a single dermal administration to two male and four female New Zealand White rabbits. The application rate was 0.5 gm per animal. The substance was applied as a powder using moistened gauze. Two application sites were used. After an exposure period of 4 hours, the occlusion was removed and residual test material was removed with distilled water. The test sites were examined for signs of erythema and oedema at 1, 24, 48 and 72 hours following patch removal.

II. RESULTS AND DISCUSSION

A. FINDINGS

Slight erythema was noted in one site at one hour post treatment. This had resolved by 24 hours. No other effect was seen.

Table IIA 5.2.4-1: Individual and mean skin irritation scores according to the Draize scheme

	Erythema						Oedema					
Animal no	44529	44530	44531	44478	44448	44286	44529	44530	44531	44478	44448	44286
after 4 hr	0	1	0	0	0	0	0	0	0	0	0	0
after 24 hr	0	0	0	0	0	0	0	0	0	0	0	0
after 48 hr	0	0	0	0	0	0	0	0	0	0	0	0
after 72 hr	0	0	0	0	0	0	0	0	0	0	0	0
mean score 24-72 h	0.0						0.0					
Additional criteria specified in Directive 93/21/EEC Point 3.2.6.1 fulfilled: Yes/No												

III. CONCLUSIONS

Chemx was non-irritant to rabbit skin. On the basis of this study, chemx does not warrant classification as being irritating to the skin.

(Jones KL 1993b)

IIA 5.2.5 Eye irritation

Report:	IIA 5.2.5/01 Jones KL 1993, Primary eye irritation study in rabbits with chemx, Report: SB-92-480
Guidelines:	US EPA FIFRA Guideline § 81-4
GLP:	yes (certified laboratory), fully compliant

Executive Summary

In a primary eye irritation study, 0.084 g of chemx (98.9% purity) was instilled into the conjunctival sac of the right eye of 6 young adult New Zealand White rabbits (1 female and 5 males). Animals then observed for the following 7 days. Irritation was scored using the Draize scheme for unwashed eyes.

Moderate iritis was noted in 4/6 animals after one hour, an effect that had resolved by 24 hours. Slight to moderate conjunctival redness and slight to moderate swelling were observed in each of six rabbits at 1 hour after dosing, with ocular discharge in five of six rabbits. Conjunctival findings were resolved by 72 hours post-instillation in five rabbits and by day 7 in the sixth. In this study, chemx induced slight to moderate ocular irritation that was reversed during the study period. On the basis of this study, chemx does not warrant classification as being an eye irritant.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	Chemx
Description:	White powder
Lot/Batch #:	NPD-9209-4523-T
Purity:	98.9 % as [18]
CAS #:	16335-17-2
Stability of test compound:	not determined
2. Vehicle and/or positive control:	test material ground and passed through a number 40-mesh sieve used for dosing
3. Test animals -	
Species:	Rabbit
Strain:	New Zealand
Age:	Young adult
Weight at dosing:	2.5 to 2.9 kg
Source:	Chalk Cliff Rabbitry, Whitesville, MI
Acclimation period:	5 days
Diet:	Chow (#5322), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages
Environmental conditions -	
Temperature:	Temperature was not specified

Humidity: Relative humidity ranged from 35 to 84 %
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 11 November to 16 December 1993

2. Animal assignment and treatment

Test material, ground to pass through a number 40 sieve, was instilled into the conjunctival sac of the right eye of each of six (one female and 5 males) NZW rabbits (0.084 g test material per animal). The contralateral eyes served as controls for the animals used. This was followed by a 7-day observation period. Both eyes of each animal were examined for signs of irritation at 1, 24, 48 and 72 hours and 7 days after dosing. Flourescein dye retention was assessed at 24 hours and at each subsequent interval until a negative response was obtained.

II. RESULTS AND DISCUSSION

A. FINDINGS

Moderate iritis was noted in 4/6 animals after one hour, an effect which had resolved by 24 hours. Slight to moderate conjunctival redness and slight to moderate swelling were observed in each of six rabbits at 1 hour after dosing, with ocular discharge in five of six rabbits. Conjunctival findings were resolved by 72 hours post-instillation in five rabbits and by day 7 in the sixth.

Table IIA 5.2.5-1: Eye irritation scores according to the Draize scheme - unwashed eyes

	Cornea						Iris						Conjunctiva-redness						Conjunctiva-chemosis					
Time/ Rabbit	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 hour	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
24 hours	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
48 hours	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
72 hours	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
mean scores 24-72 h	0.0						0.0						0.0						0.0					
Additional criteria in Directive 93/21/EEC Point 3.2.6.2 fulfilled: Yes/No																								

* individual animal scores deleted for the purposes of this document

III. CONCLUSIONS

Slight to moderate ocular irritation was observed which was reversed within the study period. On the basis of this study, chemx does not warrant classification as being an eye irritant.

(Jones KL 1993c)

IIA 5.2.6 **Dermal sensitisation**

Report: IIA 5.2.6/01 Jones KL 1995, Guinea pig maximization test with chemx (Method of Magnusson and Kligman), Report: PL-95-047

Guidelines

US EPA FIFRA Guideline § 81-6, which is equivalent to OECD 406

GLP: yes (certified laboratory), fully compliant

Executive Summary

In a dermal sensitization study, chemx (98.8% purity) in Freund's Complete Adjuvant (FCA) Emulsion was tested using young adult Albino Dunkin Hartley Guinea pigs (10/sex). The treatment regime involved induction of sensitization by intradermal injection on day 1, induction of sensitization by topical administration on day 8 and challenge by topical administration on day 22.

One animal died of unknown causes on day 22. The death was not considered treatment-related. There was no dermal response to either induction or challenge applications. Appropriate historical control data using dinitrochlorobenzene demonstrated a positive response. On the basis of this study, chemx does not warrant classification as being a dermal sensitiser.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** GHQ-0307-5385-T
   - **Purity:** 98.8 % as ^{18}
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** not determined
2. **Vehicle and/or positive control:** polypropylene glycol, Freund's Complete Adjuvant (FCA) emulsion and saline 9 %
3. **Test animals -**
   - **Species:** Albino Guinea Pigs
   - **Strain:** Dunkin Hartley Haz:(DH)FBR
   - **Age:** 5 to 7 weeks at dosing
   - **Weight at dosing:** 345 to 420 g males; 270 to 435 g females
   - **Source:** GTP, Gainsville, Pa
   - **Acclimation period:** 14 days
   - **Diet:** Agway Prolab Purina Guinea Pig Diet, *ad libitum*
   - **Water:** Tap water, *ad libitum*
   - **Housing:** Animals were individually housed in stainless steel suspended cages with wire mesh bottoms

Environmental conditions -

Temperature:	18 to 24 °C
Humidity:	Relative humidity ranged from 30 to 60 %
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 11 November to 16 December 1993

2. Animal assignment and treatment

The treatment regime involved induction of sensitization by intradermal injection on day 1, induction of sensitization by topical administration on day 8 and challenge by topical administration on day 22. The test levels for dermal and intradermal inductions and challenge were selected following preliminary irritancy testing. The sites were pre-treated with 10 % sodium lauryl sulphate to elicit some dermal response, because of the known non-irritancy of the test substance. Propylene glycol was used alone for intradermal induction and mixed with chemx to produce a 5 % w/v mixture for intradermal induction. Freund's Complete Adjuvant (FCA) Emulsion was mixed 50 % v/v in distilled water for intradermal induction and mixed with chemx to produce a 5 % w/v mixture for intradermal induction. 0.9 % saline was used alone for topical induction and challenge and also used to moisten chemx for topical induction and challenge. The test material was administered at 5 % for the intradermal induction and at 100 % for the topical induction to 20 Dunkin Hartley guinea pigs (10 male and 10 female).

II. RESULTS AND DISCUSSION

A. FINDINGS

One animal died of unknown causes on day 22. The death was not considered treatment-related. There was no dermal response to either induction or challenge applications. Appropriate historical control data using dinitrochlorobenzene demonstrated a positive response.

III. CONCLUSIONS

Chemx did not exhibit dermal sensitisation potential under the test conditions. On the basis of this study, chemx does not warrant classification as being a skin sensitizer.

(Jones KL 1995)

IIA 5.2.8 **Summary of acute toxicity of chemx**

Table IIA 5.2.8-1: Summary of acute toxicity data for chemx

Type of study	Species	Results
Oral route	Rat	LD50 > 5000 mg/kg bw
Dermal route	Rat	LD50 > 5000 mg/kg bw
Inhalation	Rat	LC50 at 4 hours > 3.0 mg/L
Primary skin irritation	Rabbit	Non-irritating
Eye irritation	Rabbit	Slight to moderate eye irritation, but does not warrant classification as being an eye irritant
Skin sensitisation	Guinea pig	Not sensitising

IIA 5.3.1 **Oral 28-day studies**

IIA 5.3.1.1 **Mouse**

Report: IIA 5.3.1/01 White MW and Jones KL 1992, Four week range finding feeding study of chemx in CD-1 mice (screening study), Report No.: CCC-11959

Guidelines

None - the study was not intended to meet a regulatory requirement.

GLP

The study was in general performed in accordance with GLP (US EPA and OECD 1981, except for characterisation of test material and verification of its stability, homogeneity and concentration in the diet), but the data and report were not fully audited by the Quality Assurance Unit.

Executive Summary

In a mouse range-finding study, doses of 0, 10, 100, 1,000 and x,000 mg/kg diet (0, 10, 100, 1,000 and x,000 ppm) (mean achieved dose 2.0, 17, 186 and x01 mg/kg in males and 2.7, 22, 274 and xx7 mg/kg bw/day in females), were administered in the feed for four weeks to groups of CD-1 mice (5 animals of each sex per dose group).

The only findings were in the high dose males and consisted of slight reduction in mean weight gain, a single ocular opacity and slightly induced palmitoyl CoA activity. The NOAEL for males was xxx mg/kg bw/day and for females was xxx mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Chemx
Description: White powder
Lot/Batch #: NPD-9110-3378-T
Purity: 99 % as [18]
CAS #: 16335-17-2

Stability of test compound: The test material was stable for at least 7 days at room temperature and was distributed uniformly in the feed. Batches were prepared at weekly intervals.

2. Vehicle and/or positive control: None

3. Test animals -
Species: Mice
Strain: CD-1
Age: males 12 weeks; females 8 weeks
Weight at dosing: 34 to 37 g males; 27 to 29 g females
Source: Charles River Laboratory, Portage, Ml USA
Acclimation period: 13 days
Diet: Purina Mills rodent chow (#5002), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were individually housed in stainless steel suspended cages

Environmental conditions -
Temperature: 18 to 24 °C
Humidity: Relative humidity ranged from 40 to 60 %
Air changes: 15 – 20 changes/h
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: 11 October to 21 November 1993

2. Animal assignment and treatment

There were 5 animals of each sex per dose group. Animals were assigned using computer randomisation by weight. Chemx, was administered in the diet for 4 wk to CD-1 strain mice at the following doses - 0, 10, 100, 1,000 and x,000 mg/kg diet (0, 10, 100, 1,000 and x,000 ppm) (Table IIA 5.3.1.1-1). Animal housing and husbandry were in accordance with the provisions of the *Guide to the care and use of laboratory animals* (USPHS-NIH Publication No. 86-23). A negative control group received plain diet.

Table IIA 5.3.1.1-1 Study design

Test group	Concentration in diet (mg /kg feed)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	5	5
2	10	2.0	2.7	5	5
3	100	17.2	21.6	5	5
4	1,000	185.8	273.7	5	5
5	x,000	Xxx	Xxx	5	5

3. Diet preparation and analysis

The required amount of test material was weighed and mixed with the diet to produce the high-dose concentration. Further ground diet was added until the other required concentrations were achieved. Diets were prepared weekly and stored refrigerated or at room temperature before use. The concentration of the test substance in the feed was verified weekly.

4. Statistics

The following parameters were analysed statistically: body weight, body weight change, food consumption (2-tailed Dunnett's Multiple Comparison Test); incidence of histopathological lesions (1-tailed Fischer's Exact Test); palmitoyl CoA oxidase and cytochrome P-450 activity (Student's t-test); haematology, clinical chemistry, terminal body weights, absolute and relative organ weights (Bartlett's Test followed by either parametric [Dunnett's Test and linear regression] or non-parametric [Kruskall-Wallis, Jonckheere's &/or Mann-Whitney Tests] procedures); and outliers (Grubb's Test).

C. METHODS:

1. Observations

Animals were observed twice daily for mortality and morbidity and noteworthy signs of toxicity, and were also given a weekly detailed examination for clinical signs of toxicity.

2. Body weight

Body weights were measured weekly.

3. Food consumption and compound intake

Feed consumption was measured weekly.

4. Opthalmoscopic examination

Not reported

5. Haematology & clinical chemistry

At study termination, mice were asphyxiated with CO_2 and a fasted blood sample was obtained from the posterior *vena cava*. The following haematology and clinical chemistry parameters were measured: *haematology* - erythrocyte count (RBC), leukocyte count (WBC), neutrophil count, lymphocyte count, platelet count, haematocrit (HCT), haemoglobin (HGB), RBC indices (MCV, MCH, MCHC) and leukocyte differential; *clinical chemistry* - blood urea nitrogen (BUN), alanine aminotransferase activity (ALT), aspartate transaminase activity (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT). For control and high dose mice, hepatic palmitoyl CoA oxidase (leucodichlorofluorescein oxidation) and cytochrome P-450 (ethoxycoumarin deethylation) activities were determined in the peroxisomal and microsomal fractions, respectively.

6. Urinalysis

Urinalysis was not performed.

7. Sacrifice and pathology

Gross pathological examination was performed on all animals and the kidneys, liver, and spleen were weighed. Gross lesions, kidneys, liver, spleen and thyroid/parathyroid tissues were from control and high dose animals were examined histologically under light microscopy.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity

The only notable clinical sign was reported as “an eye opacity” in 1/5 males treated at x,000 mg/kg in the diet. It was unclear whether the opacity was uni- or bilateral. The finding is not attributed to treatment, due to the absence of a similar finding among mice treated at a higher dose in the 90-d dietary study, and a lack of treatment-related ocular opacity in the chronic mouse dietary study.

2. Mortality

There were no unscheduled deaths.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Although minor body weight loss occurred among females fed 10 mg/kg in the diet during wk 1 and among males fed x,000 mg/kg in the diet during weeks 1 and 2, statistical significance was not attained and the

trend reversed during the remainder of the study. By termination, there were no apparent effects on body weight or body weight gain.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Mean achieved chemx doses for the study were 2.0, 17, 186 and x01 mg/kg bw/day in males and 2.7, 22, 274 and xx7 mg/kg bw/day in females. Feed consumption was unaffected by treatment.

D. BLOOD ANALYSIS:

1. Haematological findings

Monocyte (MON) count was significantly ($p < 0.05$ *vs* control) elevated among females fed 100 mg/kg in the diet (Table IIA 5.3.1.1-2). Although MON counts were elevated in most treated groups, there was no dose-response relationship, the parameter displayed large within- and among-group variation, and was not affected at doses up to x,000 mg/kg in the diet in the 90-d mouse dietary study, so the result is not considered biologically significant. Haemoglobin (HGB) concentration displayed a statistical dose-relationship among treated female groups, and mean cellular haemoglobin (MCH) was significantly ($p < 0.05$ *vs* control) elevated in males fed 1,000 and x,000 mg/kg in the diet. Again, however, HGB and MCH were unaffected in animals fed at up to x,000 mg/kg in the diet in the 90-d study, and so these findings are not attributed to treatment.

2. Clinical chemistry findings

Plasma biochemistry parameters showed neither statistically nor biologically significant effects of treatment. There were also stated to be no effects on hepatic microsomal cytochrome P-450 activity in either sex, or on hepatic peroxisomal palmitoyl CoA oxidase activity in females. However, there was a small (1.4-fold *vs* controls) but statistically significant increase in palmitoyl CoA oxidase activity in males fed x,000 mg/kg in the diet.

E. SACRIFICE AND PATHOLOGY:

1. Organ weight

There were no treatment-related effects on absolute or relative organ weights.

2. Gross and histopathology

No gross lesions were reported from the treated or control groups. The only histopathological findings unique to treated mice were from females fed x,000 mg/kg in the diet, and comprised basophilic renal tubules and single examples of renal cortical cyst and epithelial vacuolation in the descending loop of Henle (Table IIA 5.3.1.1-2). The study authors did not regard these features as indicating a treatment-related effect, which is supported because these same renal lesions were not increased in incidence by treatment in the 90-day or chronic mouse studies.

Table IIA 5.3.1.1-2 Selected haematology and pathology findings among mice (at termination)

FEEDING LEVEL (mg/kg diet)	0	10	100	1000	x000	0	10	100	1000	x000
			MALES					FEMALES		
NUMBER IN GROUP	5	5	5	5	5	5	5	4	5	5
HAEMATOLOGY (mean values)										
MON (thousand / mm^3)	0.194	0.307	0.380	0.114	0.269	0.185	0.129	0.448~	0.303	0.243
HGB (g / dL)**	16.3	16.1	16.1	16.7	16.4	16.5	16.0	16.0	16.4	17.5
MCH (picograms)	15.3	15.9	16.0	16.2~	16.2~	15.8	15.8	15.7	16.0	16.3
HISTOPATHOLOGY (incidence)										
Kidney - basophilic tubules	2	-	-	-	0	0	-	-	-	2
- cortical cyst	0	-	-	-	0	0	-	-	-	1
- epithelial vacuolation	0	-	-	-	0	0	-	-	-	1
HEPATIC PEROXISOMAL PALMITOYL CoA ACTIVITY										
pmol/min/mg protein	1646	-	-	-	2280~	2028	-	-	-	2167
pmol/min/mg protein/g liver	1226	-	-	-	1640	1602	-	-	-	1640

~ Significant ($p < 0.05$) vs control

** Linearly related to dose ($p < 0.01$) with a positive slope (females only)

III. CONCLUSIONS

There were no treatment-related effects in female mice at doses up to and including the highest dose of x,000 mg/kg in the diet (NOAEL = xxx mg/kg bw/day) or in male mice at the x,000 mg/kg dose in the diet (NOAEL = xxx mg/kg bw/day) - ocular opacity occurred in males fed at x,000 mg/kg in the diet.

(White MW and KL Jones 1992a)

IIA 5.3.1.2 **Rat**

Report: IIA 5.3.1/02 White MW and Jones KL 1992, Four week range finding feeding study of chemx in CD rats (screening study) Report No.: CCC-11960

Guidelines

None - the study was not intended to meet a regulatory requirement.

GLP

The study was in general performed in accordance with GLP (US EPA and OECD 1981, except for characterisation of test material and verification of its stability, homogeneity and concentration in the diet), but the data and report were not fully audited by the Quality Assurance Unit.

Executive Summary

In a range-finding study, chemx was administered to rats for 28 days at levels of 20, 200, 2,000 and x,000 mg/kg diet (20, 200, 2,000 and x,000 ppm) (mean achieved dose 1.3, 13.7, 136.5 and xxx.x mg/kg be/day in males and 1.5, 15.6, 154.1 and xxx.x mg/kg bw/day in females) to groups of Sprague-Dawley (CD) rats (5 animals of each sex per dose group).

The only findings considered related to be treatment related occurred at the high dose (xxx mg/kg bw/day in males and xxx mg/kg bw/day in females). A slight decrease in activated partial thromboplastin time was noted in the high dose females. In addition slight kidney effects (protein accumulation in renal tubular epithelia) which may have been treatment related were seen in both sexes at the high dose. The NOEL for males was xxx mg/kg bw/day and for females was xxx mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9110-3378-T
   - **Purity:** 99 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** The test material was stable for at least 7 days at room temperature and was distributed uniformly in the feed. Batches were prepared at weekly intervals.

2. **Vehicle and/or positive control:** None

3. **Test animals -**
   - **Species:** Albino Rat
   - **Strain:** Sprague-Dawley (CD)
   - **Age:** males 12 weeks; females 8 weeks
   - **Weight at dosing:** 328.5 to 355.3 g males; 233.5 to 254.6 g females
   - **Source:** Charles River Laboratory, Portage, Ml USA
   - **Acclimation period:** 10 days
   - **Diet:** Purina Mills rodent chow (#5002), *ad libitum*
   - **Water:** Tap water, *ad libitum*
   - **Housing:** Animals were individually housed in stainless steel suspended cages
   - **Environmental conditions -**
     - **Temperature:** 18 to 24 °C
     - **Humidity:** Relative humidity ranged from 40 to 65 %
     - **Air changes:** 15 – 20 changes/h
     - **Photoperiod:** Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. **In life dates:** 15 October to 22 November 1993

2. Animal assignment and treatment

There were 5 animals of each sex per dose group. Animals were assigned using computer randomisation by weight. Chemx was administered in the diet for 4 weeks to Charles Rivers Sprague-Dawley rats - 0, 20, 200, 2,000, x,000 mg/kg feed (20, 200, 2,000 and x,000 ppm) (Table IIA 5.3.1.2-1). Husbandry conditions were in accordance with the USPHS-NIH publication *Guide to the Care and Use of Laboratory Animals*.

3. Diet preparation and analysis

The required amount of test material was weighed and mixed with the diet using a high-speed mixer. Diets were prepared approximately weekly. Further ground diet was added until the other required concentrations were achieved. Diets were prepared weekly and stored refrigerated or at room temperature before use. The concentration of the test substance in the feed was verified weekly.

Table IIA 5.3.1.2-1 Study design

Test group	Concentration in diet (mg /kg feed)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	5	5
2	20	1.3	1.5	5	5
3	200	13.7	15.6	5	5
4	2,000	136.5	154.1	5	5
5	x,000	Xxx	Xxx	5	5

4. Statistics

Depending on the results of tests for normality and homogeneity of variances (Bartlett's Test) statistical analyses used were either parametric (Dunnett's Test and Linear Regression) or non-parametric (Kruskal-Wallis, Jonckheere's and/or Mann-Whitney Tests). Fisher's Exact Test (one-tailed) was applied to the incidence of histopathological lesions, and Student's T-Test was applied to palmitoyl CoA oxidase and cytochrome P-450 activity. Grubb's test was used to help detect outliers for some data.

C. METHODS:

1. Observations

The animals were observed twice daily for mortality, morbidity and clinical signs of toxicity and examined weekly in detail.

2. Body weight

Body weights were recorded weekly.

3. Food consumption and compound intake

Food consumption was recorded weekly.

4. Opthalmoscopic examination

Not reported

5. Haematology & clinical chemistry

At study termination all animals were killed by CO_2 asphyxiation after an overnight fast. Blood was collected from the posterior *vena cava* for haematological and clinical chemistry determination. The following haematology and clinical chemistry parameters were measured: *haematology* - total erythrocyte count, total leukocyte count, platelet count, haematocrit, level of haemoglobin, and red blood cell indices (mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration), whole blood cell count, leukocyte differential, reticulocyte count and activated partial thromboplastin time; *clinical chemistry* - albumin, alkaline phosphatase, blood urea nitrogen, calcium, chloride, cholesterol, creatine phosphokinase, creatinine, direct bilirubin, gammaglutamyl transpeptidase, glucose, phosphorus, potassium, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, sodium, total bilirubin, total protein and globulin. For control and high dose mice, hepatic palmitoyl CoA oxidase (leucodichlorofluorescein oxidation) and cytochrome P-450 (ethoxycoumarin deethylation) activities were determined in the peroxisomal and microsomal fractions, respectively.

6. Urinalysis

Urinalysis was not performed.

7. Sacrifice and pathology

Gross necropsy was conducted on all animals. Spleen, liver, kidneys, thyroid and testes were removed, weighed, assessed histologically following fixation in 10 % buffered formalin and staining with haematoxylin and eosin [19].

[19] The recommendation in OECD Guideline 407 is that liver, kidneys, adrenals, heart and target organs be examined histologically

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity

There were no treatment-related clinical signs of toxicity observed in animals at any dose.

2. Mortality

No mortalities occurred in the test animals at any dose level.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Group mean cumulative weight gain in females at 2,000 and x,000 mg/kg feed, were significantly greater than controls ($p \leq 0.05$) for the first week (control = 5.44 ± 4.4 g; 20 mg/kg feed = 12.1± 6.4 g; 200 mg/kg feed = 11.98 ± 6.8 g; 2,000 mg/kg feed = 14.38 ± 4.7 g; x,000 mg/kg feed = 16.4 ± 3.7 g). This effect is not considered to be an adverse effect, since at study termination there were no effects on group mean body weights or cumulative weight gains in either sex for any other dose group.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Overall averages for consumption of test material at the 0, 20, 200, 2,000, x,000 mg/kg in feed corresponded to 1.3, 13.7, 136.5 nd xxx.xx mg kg bw/day in males and 1.5, 15.6, 154.1 and xxx.xx mg/kg bw/day in females. There were no statistically significant changes in food consumption in animals at any dose level.

D. BLOOD ANALYSIS:

1. Haematological findings

There were no statistically significant differences among treatment groups in any haematological parameter measured, except for a statistically significant ($p \leq 0.05$) decrease in activated partial thromboplastin time (APPT) in high-dose females (control = 16.3 ± 2.1 s; 20 mg/kg feed = 14.6 ± 0.6 s; 200 mg/kg = 17.4 ± 0.8 s; 2,000 mg/kg feed = 14.8 ± 1.0 s; x,000 mg/kg feed = 13.8 ± 1.3 s). A decrease in APPT is not considered to be toxicologically significant, however.

2. Clinical chemistry findings

There were sporadic statistically significant decreases in alkaline phosphatase in males and increased albumin and creatine phosphokinase in females. These changes were not dose-related and since they fell within the normal range of values are not considered treatment-related. There were no treatment-related effects on either the level of palmitoyl CoA oxidase or cytochrome P450 activity; therefore, the test material was not considered by the study author to be a peroxisome proliferator or cytochrome P-450 inducer (data not shown in Table IIA 5.3.1.2-2).

Table IIA 5.3.1.2-2 Clinical Chemistry - Selected Parameters (± SD)

Parameter	n = 5/sex	control	20 mg/kg feed	200 mg/kg feed	2,000 mg/kg feed	x,000 mg/kg feed
ALB (G/dL)	M	4.7 (0.1)	4.6 (0.2)	4.6 (0.1)	4.7 (0.2)	4.6 (0.4)
	F	4.7 (0.3)	5.3 *(0.2)	4.9 (0.3)	5.0 (0.3)	5.0 (0.3)
CPK (U/L)	M	111.0 (22.4)	98.4 (15.5)	115.3 (3.3)	100.4 (13.9)	110.8 (36.4)
	F	86.0 (18.2)	131.4 *(29.6)	97.8 (20.8)	81.4 (19.6)	101.4 (28.1)
ALP (g/dL)	M	362.0 (67.2)	267.6* (43.2)	364.6 (54.2)	285.2 (40.3)	268.6* (45.9)
	F	213.4 (38.0)	158.8 (49.0)	281.6 (76.4)	228.4 (80.2)	179.6 (56.6)

* $p \leq 0.05$; ** $p \leq 0.01$; CPK = creatine phosphokinase; ALB = albumin; ALP = alkaline phosphatase

E. SACRIFICE AND PATHOLOGY:

1. Organ weight

There were no dose-related or statistically significant changes to mean or absolute organ weights except for a 12 % decrease in absolute and relative testicular weight at 2,000 mg/kg feed.

2. Gross and histopathology

There were no dose-dependent or statistically significant changes in gross morphology noted. Hepatocellular necrosis noted in one high-dose female and the slightly increased incidence of protein accumulation in the kidney tubular epithelium in males and mineralization in the kidney tubular epithelium in females at the high dose were not considered treatment-related because they are seen frequently in this strain of rats (historical control data provided).

III. CONCLUSIONS

The NOAEL for the study is x,xxx mg/kg feed (the highest dose tested) since at that dose there were no toxicologically significant treatment-related effects in either male or female rats (NOAEL = xxx.x bw/day in males and xxx.x mg/kg bw/day in females).

(White MW and KL Jones 1992b)

IIA 5.3.1.3 **Dog**

Report: IIA 5.3.1/03 White MW and Jones KL 1996, Range finding study with chemx administered orally to beagle dogs, Report No.: CC-94-228

Guidelines

None - the study was not intended to meet a regulatory requirement.

GLP

The study was in general performed in accordance with GLP (US EPA and OECD 1981, except for characterisation of test material and verification of its stability, homogeneity and concentration in the diet), but the data and report were not fully audited by the Quality Assurance Unit.

Executive Summary

In a dog range-finding test using beagle dogs, doses of 30, 100, 300 and x,000 mg/kg bw/day were given neat in gelatine capsules daily, 5 days/week, for 4 weeks to 2 dogs/sex/dose.

The only findings considered possibly related to treatment were a slightly reduced weight gain in females at the two highest doses and elevated ALP and CPK in high dose males. The no-observed-adverse-effect level (NOAEL) was xxx mg/kg bw/day in females and xxx mg/kg bw/day in males.

I MATERIALS AND METHODS

A MATERIALS:

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IIA 5.3.2 **Oral 90-day studies (rodents)**

IIA 5.3.2.1 **Oral 90-day toxicity in the mouse**

Report: IIA 5.3.2/01 White MW and Jones KL 1995, 90-day feeding study with chemx in CD-1 mice, Report No.: CCC-14048

Guidelines

US EPA FIFRA Guideline § 82-1, equivalent to Directive 88/302/EEC (OJ No L133/8 of 30 May 1988)

GLP: Fully GLP compliant [17].

Executive Summary

Chemx was given for 90 days to groups of 20 CD-1 mice (10 of each sex) at concentrations of 0, 100, 1,000, 3,000 and x,000 mg/kg in the diet (0, 100, 1,000, 3,000 and x,000 ppm) (mean achieved dose 18, 163, 550 and x,xxx mg/kg bw/day in males and 32, 313, 887 and x,xxx mgt/kg bw/day in females).

The only possibly treatment-related effect was an equivocal decrease in ALP in high dose animals. The NOAEL for the study was x,000 ppm (xxx mg/kg bw/day for males and xxx bw/day for females).

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9209-4523-T
   - **Purity:** 99.1 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** Stability analyses of test material and of feed fortified with the test material were satisfactory. The concentration of test substance in the feed was verified each week. Batches were prepared at weekly intervals.

2. **Vehicle and/or positive control:** None

3. **Test animals -**
   - **Species:** Mice
   - **Strain:** CD-1
   - **Age:** 8 weeks
   - **Weight at dosing:** 29 to 35 g males; 24 to 28 g females
   - **Source:** Charles River Laboratory, Portage, Ml USA
   - **Acclimation period:** 29 days
   - **Diet:** Purina Mills rodent chow (#5002), *ad libitum*
   - **Water:** Tap water, *ad libitum*
   - **Housing:** Animals were individually housed in stainless steel suspended cages
   - **Environmental conditions -**
     - **Temperature:** 18 to 24 °C
     - **Humidity:** Relative humidity ranged from 40 to 65 %
     - **Air changes:** 15 – 20 changes/h
     - **Photoperiod:** Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. **In life dates:** 10 April to 7 August 1993

2. **Animal assignment and treatment**

   There were 10 animals of each sex per dose group. Animals were assigned using computer randomisation by weight. Chemx, was administered in the diet for approximately 90 days to CD-1 strain

mice at the following doses - 0, 100, 1,000, 3000 and x,000 mg/kg diet (0, 100, 1,000, 3,000 and x,000 ppm). Animal housing and husbandry were in accordance with the provisions of the *Guide to the care and use of laboratory animals* (USPHS-NIH Publication No. 86-23). A negative control group received plain diet.

3. Diet preparation and analysis

Each week, the test material was mixed into the diet at a concentration of x,000 mg/kg feed, and this mixture was used to prepare additional mixtures at 100, 1,000 and 3,000 mg/kg feed. Prepared diets were stored under refrigeration or kept in the animal room until use. The stability and homogeneity of chemx in the dietary mixtures was checked by analysis using HPLC. The test material was homogeneous throughout the feed and was stable for up to 35 days (Table IIA 5.3.2.1-2). The mean dietary concentrations throughout the study were 100, 1,040, 2,980 and x,xxx mg/kg feed.

Table IIA 5.3.2.1-1 Study design

Test group	Concentration in diet (mg /kg feed)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	10	10
2	100	17.9	32.8	10	10
3	1,000	162.8	313.1	10	10
4	3,000	549.5	887.3	10	10
5	x,000	Xxxx	Xxxx	10	10

Table IIA 5.3.2.1-2 Homogeneity and stability of test material

Nominal concentration	Concentration analysed[1] (mg/kg)	% of nominal
20 mg/kg	T=22 M=22 B=22 S=19.7	110 110 113 89
xx,000 mg/kg	T = xx,xxx M = xx,xxx B = xx,xxx S = xx,xxx	102 104 98 94

T = top; M = middle; B = bottom of mixing bowl; S=35 d stability, mean % of nominal

Homogeneity analyses revealed that the within-batch coefficient of variation in the test article concentration, was approximately 6 % at 100 and x,000 mg/kg. No data were presented for the intermediate concentrations.

4. Statistics

The following parameters were analysed statistically: body weight, body weight change, food consumption (2-tailed Dunnett's Multiple Comparison Test); incidence of histopathological lesions (1-tailed Fischer's Exact Test); haematology, clinical chemistry, absolute and relative organ weights (Bartlett's Test followed by either parametric [Dunnett's Test and linear regression] or non-parametric [Kruskall-Wallis, Jonckheere's &/or Mann-Whitney Tests] procedures); and outliers (Grubb's Test).

C. METHODS:

1. Observations

Animals were observed twice daily for mortality and morbidity and examined weekly for clinical signs of toxicity.

2. Body weight

Body weights were recorded weekly.

3. Food consumption and compound intake

Food consumption was recorded weekly.

4. Opthalmoscopic examination

Not reported

5. Haematology & clinical chemistry

At termination, fasted blood samples were obtained from the posterior vena cava. The following haematology and clinical chemistry parameters were measured: *haematology* - erythrocyte count (RBC), leukocyte count (WBC), neutrophil count, lymphocyte count, platelet count, haematocrit (HCT), haemoglobin (HGB), RBC indices (MCV, MCH, MCHC) and leukocyte differential; *clinical chemistry* - blood urea nitrogen (BUN), alanine aminotransferase activity (ALT), aspartate transaminase activity (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT).

6. Urinalysis

Urinalysis was not performed.

7. Sacrifice and pathology

At termination, mice were asphyxiated with CO_2. Gross pathological examination was performed on all animals and the kidneys, liver, spleen and testes were weighed. The following tissues examined histologically under light microscopy: gross lesions, kidneys, liver and lung from all animals and the spleen, testes and thyroids of control and high dose animals.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity

There were no treatment-related clinical signs at any dose.

2. Mortality

There were no unscheduled deaths.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no effects on body weight or body weight gain.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no effects on feed consumption. The mean achieved doses were [M/F] 18/32, 163/313, 550/887 and x,xxx/x,xxx mg/kg bw/day at the 100, 1,000, 3,000 and x,000 mg/kg feeding levels, respectively.

D. BLOOD ANALYSIS:

1. Haematological findings

Haematology determinations revealed a significant ($p < 0.05$) linear dose-related trend towards decreasing neutrophil (NEU) count in treated females, which also displayed a tendency towards depressed lymphocyte (LYM) and elevated eosinophil (EOS) counts, compared with controls. Treated mice of both sexes showed decreased basophil (BAS) counts relative to controls (Table IIA 5.3.2.1-3). However, group standard deviations were large and statistical significance was not attained with respect to LYM, EOS and BAS counts. The haematological findings are considered to be of equivocal biological significance, as they may have arisen from among-individual variation.

2. Clinical chemistry findings

Statistically significant increases in alkaline phosphatase (ALP) activity occurred in treated males (Table IIA 5.3.2.1-3) but were attributed by the study authors to a low control mean value (historical control data were presented). The response was less marked at x,000 mg/kg feed than at lower doses. By

contrast, ALP activity was significantly depressed in females given x,000 mg/kg feed. An elevated mean aspartate transaminase (AST) activity was detected in males given x,000 mg/kg feed, but was not statistically significant. A high reading in a single animal, which also displayed elevated alanine aminotransferase (ALT) activity, caused this.

Table IIA 5.3.2.1-3 Selected haematology, clinical chemistry and pathology findings among mice (at termination)

FEEDING LEVEL (mg/kg feed)	0	100	1000	3000	x000	0	100	1000	3000	x000
			MALES					FEMALES		
NUMBER IN GROUP	10	10	10	9	9	10	10	10	9	9
HAEMATOLOGY (mean values)										
NEU (thousand / mm^3)*	2.154	1.555	1.594	1.617	1.962	1.256	1.101	0.767	0.731	0.959
LYM (thousand / mm^3)	5.723	5.774	4.092	4.488	5.251	4.372	4.280	3.147	3.257	3.269
BAS (thousand / mm^3)	0.039	0.031	0.024	0.029	0.030	0.024	0.020	0.017	0.019	0.019
EOS (thousand / mm^3)	0.112	0.101	0.114	0.146	0.140	0.058	0.083	0.073	0.040	0.073
CLINICAL CHEMISTRY (mean values)										
ALP (IU/L)#	80	140~	152~~	148~~	104~	168	182	153	148	121~
AST (IU/L)	83	91	86	97	133	83	74	74	79	90
GROSS PATHOLOGY (incidence)										
Liver - discoloration	0	1	2	2	1	0	0	0	0	2
HISTOPATHOLOGY (incidence)										
Liver - haemorrhagic necrosis / fibrosis	0	0	0	0	0	0	0	0	0	1
- necrosis + inflammation	1	1	1	0	0	0	1	0	0	1
- mononuclear cell infiltrate	0	0	0	2	0	0	3	1	1	0
Spleen - excess haematopoiesis	0	-	-	-	2	0	-	-	-	0
Uterus - hydrometra	-	-	-	-	-	0	4~	0	0	0

* Linearly related to dose ($p < 0.05$) with a negative slope (females only).

~ ($p < 0.05$) and ~~($p < 0.01$) *vs* control

Linearly related to dose ($p < 0.05$) with a positive slope for males and a negative slope for females

E. SACRIFICE AND PATHOLOGY:

1. Organ weight

Absolute and relative organ weights, were unaffected by treatment.

2. Gross and histopathology

The only notable gross lesion was an increased incidence of hepatic discoloration among treated males and x,000 mg/kg feed females (Table IIA 5.3.2.1-3). However, histological findings in the liver were confined to sporadic, non-dose-related instances of necrosis with inflammation or mononuclear cell infiltration, and haemorrhagic necrosis/fibrosis in a single female given x,000 mg/kg feed. The only statistically significant microscopic finding was hydrometra, present in 4/10 females from the 100 mg/kg feed group. This is not considered to be treatment-related, in the absence of similar findings at higher doses. Excessive haematopoiesis was observed in the spleen of 2/10 males from the x,000 mg/kg feed group, but given that the finding was not replicated at the same dose in the 18-month dietary study in mice, it may be discounted.

III. CONCLUSIONS

The decreased ALP activity in female mice at the highest dose of x,000 mg/kg feed is not considered to be toxicologically significant, in the absence of evidence that depression of serum ALP activity is associated with tissue injury. As there were no treatment-related effects at up to and including the highest dose, the NOAEL can be set at x,000 mg/kg feed (x,xxx mg/kg bw/day in females).

(White MW and KL Jones 1995a)

IIA 5.3.2.2 **Oral 90-day toxicity in the rat**

Report: IIA 5.3.2/02 White MW and Jones KL 1995, 90-day feeding study with chemx administered in feed to Sprague-Dawley rats, Report No.: CCC-14049

Guidelines

US EPA FIFRA Guideline § 82-1, equivalent to Directive 88/302/EEC (OJ No L133/8 of 30 May 1988)

GLP: Fully GLP compliant [17]

Executive Summary

In a 90-day study, the test substance was administered to groups of 30 Sprague-Dawley rats (10 males and 20 females) at concentrations of 0, 20, 200, 2,000, 6,000 and xx,000 mg/kg in the diet (0, 20, 200, 2,000, 6,000 and xx,000 ppm) (mean achieved dose 1.22, 12.1, 123.2, 370 and x,xxx mg/kg bw/day in males and 1.47, 14.6, 144.3, 448 and x,xxx mg/kg bw/day in females). The study also included a preliminary reproductive toxicity test (10 females per group).

Dietary exposure to chemx resulted in slight body weight effects in males at the high dose and in pregnant females. Slight alterations to haematological parameters in high dose females may have been treatment-related. A number of lesions were identified in the kidneys, urethras and bladders of a number of high dose males and females. On the basis of the toxicological profile of chemx determined in other studies reported, it is concluded that the renal and bladder effects seen in this study at the high dose were treatment-related. The NOAEL was xxx mg/kg bw/day for males and xxx mg/kg bw/day for females) and was based on the renal and bladder effects observed.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Chemx

Description:	White powder
Lot/Batch #:	NPD-9209-4523-T
Purity:	99.3 % as [18]
CAS #:	16335-17-2
Stability of test compound:	The test material was stable for at least 7 days at room temperature and was distributed uniformly in the feed. The concentration of test substance in the feed was verified each week

2. Vehicle and/or positive control: None

3. Test animals -

Species:	Rat
Strain:	Crl:CD(SD)BR, albino
Age:	Approximately 7 weeks old
Weight at dosing:	215.4 - 286.7 g for males; 102.9 - 147.0 for females
Source:	Charles River Laboratories, Portage, MI
Acclimation period:	27 days
Diet:	Purina Mills rodent chow (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages
Husbandry:	Husbandry conditions were in accordance with the USPHS-NIH publication *Guide to the Care and Use of Laboratory Animals*.
Environmental conditions -	
Temperature:	22 ± 2° C
Humidity:	55 ± 10 %
Air changes:	16 - 20 changes/h
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: Start: 22 February 1993
End: 30 May 1993 for animals sacrificed immediately after the end of the treatment period
30 June for animals maintained for a 4-week recovery period following the treatment period

2. Animal assignment and treatment

There were 10 rats/sex/dose for the main study and an additional 10 females/group for the concurrent reproductive study (Table IIA 5.3.2.2-1). A satellite group consisting of 10 rats/sex for both the high dose and control treatments was maintained for 4 weeks post treatment to check recovery.

3. Diet preparation and analysis

The required amount of test material was weighed and mixed with the diet using a Hobart HCM-450 mixer to produce the high-dose concentration. Further ground diet was added until the other required concentrations were achieved. Diets were prepared weekly and stored refrigerated or at room temperature before use. The concentration of the test substance in the feed was verified weekly. Samples of the 20 mg/kg and xx,000 mg/kg formulations were taken for analysis to check stability and homogeneity at the beginning of the study and to check the concentration in the feed on a weekly basis. The test material was homogeneous throughout the feed and was stable for up to 35 days (Table IIA 5.3.2.2-2).

Table IIA 5.3.2.2-1 Study design

Test group	Concentration in diet (mg /kg feed)	Dose per animal		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	20	20
2	20	1.22	1.47	10	10
3	200	12.1	14.6	10	10
4	2,000	123.2	144.3	10	10
5	6,000	370	448	10	10
6	xx,000	x,xxx	x,xxx	20	20

Table IIA 5.3.2.2-2 Homogeneity and stability of test material

Nominal Concentration	Concentration determined by analysis	Actual concentration as % of the nominal concentration
20 mg/kg	T = 22	110
	M = 22	110
	B = 22	113
	S = 19.7	89
xx,000 mg kg	T = xx,x00	102
	M = xx,x00	104
	B = xx,x00	98
	S = xx,x00	94

T = top of mixing bowl; M = middle of mixing bowl; B = bottom of mixing bowl; S = 35 d stability, mean % of nominal

4. Statistics

Dunnett's Multiple Comparison Test (two-tailed) was applied to the body weight, cumulative body weight change, food consumption and APTT data. Fishers Exact Test (one tailed) was applied to data on the incidence of histopathological lesions. Barrette's Test, Dunnett's Test, Linear Regression and non-parametric tests were used where appropriate.

C. METHODS:

1. Observations

The animals were observed twice daily for mortality and weekly for clinical signs of toxicity. For the reproduction section of the study, females were paired for a maximum of 7 days with sexually mature males at a 1:1 ratio. The day on which spermatozoa were found in the vaginal smear or a vaginal plug was observed was designated day 0. At the end of the study period mating, fertility, gestation length, litter weights and survival were determined.

2. Body weight

Body weights of each animal were determined one-day pre-dosing and weekly thereafter. For the reproduction study, body weights were determined once weekly until mating, on days 0, 7, 14 and 21 of gestation and days 0 and 4 of lactation.

3. Food consumption and compound intake

Food consumption was recorded weekly for all animals in the main study and weekly until mating for the reproduction study.

4. Opthalmoscopic examination

Animals were examined opthalmoscopically once at pre-test and once prior to terminal sacrifice (all at pre-test, control and high-dose prior to terminal sacrifice).

5. Haematology & clinical chemistry

At study termination blood was collected from all main study animals (animals fasted overnight) for haematological and clinical chemistry measurements.

6. Urinalysis

Urinalysis was not performed.

7. Sacrifice and pathology

At study termination all animals were killed by CO_2 asphyxiation and exsangination. All appropriate tissues from the control and high-dose groups were removed, weighed, assessed for tumour incidence (grading system included in study attachment) embedded in paraffin, stained with haematoxylin and eosin and submitted for histopathological examination where applicable. Animals from the reproduction section of the study were killed by CO_2 asphyxiation and exsangination on or shortly after day 4 of lactation

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity

There were no treatment-related clinical signs of toxicity observed in animals at any dose for either the main or reproductive studies.

2. Mortality

No mortalities occurred in the test animals at any dose level for either the main or reproductive studies.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Cumulative weight gains in low and mid-dose males and all female dose-groups were not different from those of control animals (Table IIA 5.3.2.2-3). Cumulative weight gains in high-dose males were lower than those of control animals from days 31 - 92 (~ 15 % less) reaching statistical significance between days 3 - 31 and 31 - 79.

There was a dose-related lower terminal body weight in males (which was 10 % lower in the high-dose compared to control groups) (Table 5.3.2.2-4). For the reproduction study phase, there were no statistically significant differences among treatment groups in maternal group mean body weights or weight changes during gestation except for an 8 % lower body weights in high-dose females by the end of gestation.

Table IIA 5.3.2.2-3 Cumulative mean body weight gain, males (selected time periods)

Dose (mg/kg feed)	Day 3 n=10	Day 31 N=10	Day 79 N=10	Day 92 n=10
control	23.16 [1] (21.8) [2]	250.14 (22.9)	395.28 (60.5)	412.46 (64.6)
20	21.48 (17.8)	254.42 (18.6)	388.07 (37.8)	409.63 (42.7)
200	29.22 (7.1)	253.87 (27.3)	386.66 (61.1)	411.07 (73.4)
2,000	30.08 (7.7)	245.13 (18.7)	386.97 (49.8)	411.91 (62.4)
6,000	29.73 (6.7)	231.17 (25.7)	366.69 (53.9)	387.09 (58.4)
xx,000	28.20 (4.8)	219.79 *(16.3)	332.13* (39.9)	349.29 (47.1)

[1] weight in grams; [2] standard deviation

Table IIA 5.3.2.2-4 Terminal Body Weights

Parameter	n=10/ sex	control	20 mg/kg feed	200 mg/kg feed	2,000 mg/kg feed	6,000 mg/kg feed	xx,0000 mg/kg feed
Terminal Body Weights	Main Study M	630.0[1] (71.1)[2]	628.9 (52.5) (100)[3]	629.2 (82.4) (100)	626.5 (67.6) (99)	602.1 (67.0) (96)	575.8 (61.5) (90)
	F	280.5 (31.7)	298.5 (30.7) (106)	283.2 (34.8) (101)	299.9 (26.1) (107)	304.2 (37.5) (108)	283.2 (32.9) (101)
	Repro Study F	450.9 (41.6)	487.7 (38.8) (108)	465.8 (65.4) (103)	450.2 (62.5) (98)	441.4 (34.1) (98)	416.1 (52.2) (92)

[1] weight in grams; [2] standard deviation; [3] % of control group value

C. FOOD AND COMPOUND CONSUMPTION:

1. Food consumption and compound intake

There were no statistically significant changes in food consumption in animals at any dose level in either the main or the reproductive studies. There were no statistically significant differences in food consumption in pre-mated females used in the reproduction study. Food consumption was not measured during gestation or lactation. Overall averages for consumption of test material at the 0, 20, 200, 2,000, 6,000 or xx,000 mg/kg dose groups (0, 20, 200, 2,000, 6,000 and xx,000 ppm) corresponded to 1.2, 12.1, 123.2, 370 and x,xxx mg/kg bw/day in males and 1.5, 14.6, 144.3, 448 and x,xxx mg/kg bw/day in females respectively.

2. Food efficiency

Not considered relevant

D. REPRODUCTIVE EFFECTS

There were no statistically significant differences in measures of mating, fertility or gestation length at any dose level. There were no statistically significant differences in pup weights at birth or during the first four days of lactation at any dose group.

There were no statistically significant changes in litter weights or survival. There was an increase in numbers of pups found dead on lactation days 0 - 4 at 200 mg/kg feed (combined male/female survival as a % of control = 85.8); however, the reduction was not statistically significant or dose-related and was within historical control ranges for this strain of rat (historical control reference provided).

E. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ocular abnormalities.

F. BLOOD ANALYSIS:

1. Haematological findings

There were no statistically significant differences among treatment groups in any haematological parameter measured (total erythrocyte count, total leukocyte count, platelet count, haematocrit, haemoglobin concentration, red blood cell indices - mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular concentration).

2. Clinical chemistry findings

A statistically significant decrease in mean alanine amino transferase occurred in high-dose males however the level was within the normal range of values. Statistically significant increases in mean glucose, albumin, total protein and calcium levels occurred in females; however, these were not dose-related and since they fell within the normal range of values were not considered to be treatment-related. There was a dose-related increase in chloride levels in males, which reached significance at 6,000 mg/kg feed and xx,000 mg/kg feed; however, these levels were within the normal range of values and are not considered toxicologically significant. Chloride levels in the high dose satellite group were similar to those of the control group following the four-week recovery period.

Table IIA 5.3.2.2-5 Clinical chemistry - selected parameters

Parameter	n=10/ sex	control	20 mg/kg feed	200 mg/kg feed	2,000 mg/kg feed	6,000 mg/kg feed	xx,000 mg/kg feed
ALT/SGPT	M	40.0 (9.2)	36.3 (9.32)	33.4 (8.9)	34.6 (9.3)	50.0 (18.6)	31.9* (3.8)
(IU/L)	F	42.8 (20.9)	46.7 (27.4)	38.2 (17.8)	35.1 (10.1)	37.2 (11.0)	28.5 (6.7)
GLU	M	227.0 (20.1)	228.4 (25.2)	218.0 (27.3)	252.7 (39.4)	230.0 (44.3)	229.6 (26.7)
(mg/dL)	F	160.7 (28.1)	214.2* (38.3)	188.8 (46.8)	205.00 (35.5)	227.7** (43.7)	181.9 (41.7)
TP	M	6.58 (0.4)	6.82 (0.4)	6.87 (0.3)	6.77 (0/3)	6.78 (0.4)	6.55 (0.3)
(g/dL)	F	6.91 (0.4)	7.61 **(0.5)	7.06 (0.3)	7.34 (0.4)	7.26 (0.2)	7.01 (0.5)
ALP2	M	4.27 (0.2)	4.17 (0.3)	4.38 (0.3)	4.31 (0.2)	4.42 (0.2)	4.13 (0.1)
(g/dL)	F	4.72 (0.5)	5.47** (0.5)	5.00 (0.3)	4.99 (0.4)	4.90 (0.3)	4.61 (0.5)
Ca	M	11.46 (0.4)	11.68 (0.4)	11.60 (0.6)	11.71 (0.5)	11.61 (0.6)	11.51 (0.4)
(mg/dL)	F	11.22 (0.4)	11.90 *(0.5)	11.44 (0.7)	11.76 (0.6)	11.52 (0.5)	11.34 (0.5)
Cl	M	100.3 (1.9)	101.4 (1.7)	101.8 (1.1)	102.4 (2.5)	103.9** (1.9)	104.4** (2.0)
(meQ/L)	F	100.9 (1.8)	100.5 (1.8)	101.8 (1.8)	101.2 (1.6)	101.9 (1.4)	101.9 (2.3)

* $p \leq 0.05$ ** $p \leq 0.01$ ALT/SGPT alanine amino transferase/glutamic pyruvic transaminase;
GLU glucose; TP total protein; ALP alkaline phosphatase; Ca calcium; Cl chloride

G. SACRIFICE AND PATHOLOGY:

1. Organ weight

There were no dose-related or statistically significant changes in the mean or absolute organ weights (data not shown in Table 5.3.2.2-6).

2. Gross and histopathology

There were no dose-dependent or statistically significant increases in microscopic lesions noted. Lesions were noted in the mesenteric and submaxillary lymph nodes in males at 20 mg/kg feed, however, the incidence was not dose-related. At the high dose several lesion types (pyelonephritis, hydronephrosis and hyperplasia of the mucosal and/or pelvic epithelium in the kidneys) were seen which were in conjunction with kidney and/or bladder calculi. The incidence of lesions was not dose-related or statistically increased in comparison to the incidence levels in control animals. However, the incidence of calculi in animals of the age used in the study is unusual and may be a treatment-related effect.

Table 5.3.2.2-6 Incidence of Selected Pathologies

Parameter	n=10 /sex	control	20 mg/kg feed	200 mg/kg feed	2,000 mg/kg feed	6,000 mg/kg feed	xx,000 mg/kg feed
Kidney:	M	0	0	0	0	0	1
pylonephritis	F	0	0	0	0	0	2
Kidney:	M	2	0	2	0	0	0
hydro-nephrosis (bilateral)	F	0	0	0	0	0	2
Kidney:	M	0	0	0	0	0	1
hyperplasia, pelvic epithelium	F	0	0	0	0	0	1
Kidney:	M	1	0	4	2	4	4
mineralization or protein accumulation	F	3	0	4	3	2	2
Urinary and / or	M	0	0	0	0	0	1
Kidney calculi	F	0	0	0	0	0	2
Urinary bladder or	M	0	0	0	0	0	1
ureter hyperplasia	F	0	0	0	0	0	1
Urinary mineralization or	M	0	0	0	0	0	1
protein accumulation	F	-	-	-	-	-	-

III. CONCLUSIONS

At the high dose several lesion types were seen which were in conjunction with kidney and/or bladder calculi. The incidence of lesions was not dose-related or statistically increased in comparison to the incidence level in control animals. However, the incidence of calculi in animals of the age used in the study is unusual and therefore may be a treatment-related effect. There were no statistically significant differences among treatment groups in the reproduction part of the study: maternal group mean body weights or weight changes during gestation; measures of mating, fertility or gestation length; pup weights at birth or during the first four days of lactation; or litter weights or survival.

The NOAEL for the study was x,xxx mg/kg feed (xxx mg/kg bw/day) based on body weight reduction in males and the occurrence of calculi in both sexes at the next highest dose which may be treatment-related. The x,xxx mg/kg feed dose level is considered a NOAEL since there was a dose-related increase in chloride level in males which was within the normal range of values. The dose levels (0, x0, x00, x,000, and xx,000 mg/kg feed) were chosen for the long-term study.

(White MW and KL Jones 1995b)

IIA 5.3.3 **Oral 90-day toxicity (dog)**

Report: IIA 5.3.3/01 White MW and Jones KL 1995, 90-day feeding study with chemx administered by capsule to beagle dogs, Report No.: CC-94-324

Guidelines

US EPA FIFRA Guideline § 82-1, equivalent to Directive 88/302/EEC (OJ No L133/12 of 30 May 1988)

GLP: Fully GLP compliant [17].

Executive Summary

In a 90-day study, groups of 5 beagle dogs/sex/dose were administered chemx in gelatine capsules at doses of 0, 30, 100, 300 and x,000 mg/kg bw/day, 5 days/week, for 90 days.

The urinary tract was identified as the target organ. The pattern of response was somewhat unusual in that there was a low incidence of findings at ≥ 300 mg/kg bw/day but in one male animal the effect was very severe, necessitating premature termination. The entire urinary tract was targeted in this animal while only the bladder showed evidence of substance-related damage in the others. These findings are consistent with irritation caused by crystals in the urine and damage following the formation of calculi. Urinary crystals were identified previously in the ADME study (point IIA 5.1.1 to 5.1.3) and were identified in this study at the interim (males and females) and termination (females only) sampling. The NOEL was xxx mg/kg bw/day for females and xxx mg/kg bw/day for males.

I. MATERIALS AND METHODS

A. MATERIALS:

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IIA 5.3.7 **28-day percutaneous toxicity (rodents)**

Report: IIA 5.3.7/01 White MW and Jones KL 1997, Range finding and one month dermal study with chemx in CD rats, Report No.: CC-96-115

Guidelines

US EPA FIFRA Guideline § 82-2, broadly equivalent to Directive 88/302/EEC (OJ No L133/8 of 30 May 1988) and OECD Guideline 410

GLP: Fully GLP compliant [17].

Executive Summary

In a 28-day percutaneous toxicity study, the test substance was administered to groups of 16 Sprague-Dawley rats (8 males and 8 females) at concentrations of 0, 100, 300 and x,000 mg/kg bw per application. Duration and frequency of treatment was 6 hours per day, 5 days a week for a total of 20 applications in 28 days.

There was no evidence of treatment related toxicological effects in the rat following dermal exposure of up to x,000 mg/kg bw/day chemx.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9503-6466-T
   - **Purity:** 98.8 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** The test material was found to be stable over a one-year period.
2. **Vehicle and/or positive control:** Carboxmethylcellulose (CMC), lot no. 112H0386, from Sigma Chemical Company, MO (concentration = 1.96 %) was freshly diluted to a concentration of 0.5 %, for use as the vehicle twice during the first week of the study, weekly thereafter
3. **Test animals -**
   - **Species:** Rat
   - **Strain:** Sprague-Dawley (CD)

Age:	Approximately 8 weeks old
Weight at dosing:	276.9 – 320.8 g for males; 185.1 – 217.2 for females
Source:	Charles River Laboratories, Portage, MI
Acclimation period:	15 days
Diet:	Purina Mills rodent chow (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages
Husbandry:	Husbandry conditions were in accordance with the USPHS-NIH publication *Guide to the Care and Use of Laboratory Animals*.
Environmental conditions -	
Temperature:	22 ± 2° C
Humidity:	55 ± 15 %
Air changes:	16 - 20 changes/h
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: Start: 22 April 1996
End: 21 May 1996

2. Animal assignment and treatment

Animals were randomly assigned to the study groups (computer randomization by weight).

3. Dosing selection rationale

Dose levels were chosen on the basis of the results obtained in the range finding study (Appendix 6 of the study report, pages 186 to 260). In the dose-range finding study, chemx, purity 98.8 %, vehicle 0.5 % carboxymethylcellulose, was administered by dermal application to male and female Sprague-Dawley rats at dose levels of 0, 100, 300 and x,xxx mg/kg bw per application for 5 consecutive days, 6 hours per day, to 3 rats/sex/group. The NOEL was found to be x,xxx mg/kg bw/day since there were no treatment-related effects observed at any dose level tested. On the basis of these results, the dose levels chosen for the main study were 0, 100, 300 and x,xxx mg/kg bw per application to 8 rats/sex/dose group [20].

4. Preparation of dosing mixtures

Dosing mixtures were made twice during the first week of the study, weekly thereafter. The stability of the solutions (all dose levels) was checked by analysis on day 0 and after stored refrigerated for 7 days. Solutions prepared for week 1 were measured for homogeneity from duplicate samples taken from the top, middle and bottom of the container, for the 100 and x,xxx mg/kg bw dose levels. Actual test material concentration was determined for samples of dosing solutions, for all dose levels, prepared for each study week.

[20] OECD Guideline 410 specifies that at least 10 rats per sex should be used at each dose level

The actual concentration of chemx in the test solutions, expressed as percentage of the nominal concentrations, for the 100, 300 and x,xxx mg/kg bw dose levels, respectively, were as follows (stability):

a) immediately after preparation: 105.1 %, 117.0 % and 108.0 %.
b) after stored refrigerated for 7 days: 106.0 %, 119.0 % and 104.7 %.

The coefficient of variation for the 100 and x,xxx mg/kg bw dosing solutions (homogeneity) were 9.7 % and 7.3 %, respectively. The mean actual concentration of chemx in the test solutions, expressed as percentage of nominal concentrations, for the 100, 300 and x,xxx mg/kg bw dose levels, respectively, were 116.5 %, 115.0 % and 117.1 %. Based on these results, the stability, homogeneity and actual test material concentrations believed to be satisfactory for the purposes of the study.

5. Preparation and treatment of animal skin

Test material was directly applied to the skin on the back of each animal to cover an area of approximately 25-35 $cm^{2.}$. The report of the study does not indicate whether or not this area was clipped free of hair prior to application. Duration and frequency of treatment was 6 hours per day, 5 days per week for a total of 20 applications in 28 days. The test material was covered with a gauze wrap; a plastic collar was placed on each animal to prevent ingestion. After 6 hours, the gauze was removed and excess test material was wiped off.

6. Statistics

Dunnett's Multiple Comparison Test (two-tailed) was used to analyse in-life body weights, cumulative body weight changes, food consumption data and APTT. The Environmental Health Laboratory (EHL) decision-tree analysis (two-tailed) was used for haematology, blood chemistry, terminal body weights, and absolute and relative organ weights. Depending on the results of tests for normality and homogeneity (Bartlett-Box test) analyses used were either parametric (Dunnett's test and linear regression) or non-parametric (Kruskal-Wallis, Jonckheere's and/or Mann-Whitney tests). Fisher's Exact Test (one-tailed) was applied to the incidence of microscopic lesions. Grubb's test was used to help detect anomalies in the organ weight data.

C. METHODS:

1. Observations

Animals were observed twice daily for mortality, morbidity and overt clinical signs of toxicity. A more detailed physical examination was conducted on each animal on a weekly basis. Individual body weights and food consumption were measured weekly.

2. Body weight

Individual body weights were measured weekly.

3. Food consumption

Individual food consumption was measured weekly.

4. Haematology & clinical chemistry

At study termination blood was collected from the posterior *vena cava* of all study animals (animals fasted overnight) for haematological and clinical chemistry measurements. Haematological parameters examined were RBC count, Hgb, HCT, MCH, MCHC, MCV, WBC count and differential, platelet count, reticulocyte count and activated partial thromboplastin time. Clinical chemistry parameters measured were aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, sodium, chloride, phosphorus, blood urea nitrogen, creatinine, creatine phosphokinase, cholesterol, potassium, calcium, glucose, total bilirubin, direct bilirubin, total protein and albumin. Globulin was determined by subtraction of the albumin value from the total protein value.

5. Urinalysis

Urinalysis was not performed.

6. Sacrifice and pathology

At the end of the 28-day treatment period, animals were fasted and then anaesthetised by CO_2 inhalation, after which each animal was sacrificed and necropsied. At necropsy, the adrenals, kidneys, liver, spleen and testes were weighed. A complete tissue inventory was collected from each animal, preserved in 10 % neutral buffered formalin (eyes in 5 % neutral buffered formalin/0.5 % glutaraldehyde) and processed for routine histopathological examination (haematoxylin and eosin). The liver, kidneys, spleen, skin (treated and untreated) and urinary bladder from the control and high-dose group animals were examined microscopically.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity

There were no overt clinical signs of treatment-related toxicity. There were no signs of local skin irritation reported for any animal at any dose level tested.

2. Mortality

The only death occurring during the study period was one female in the 300 mg/kg bw group which died on day 9. Although the cause of death was not determined, it was not considered to be treatment-related since there were no mortalities in the x,xxx mg/kg bw group.

B. BODY WEGHT AND BODY WEIGHT GAIN

There were no treatment-related effects on mean body-weight gain throughout the study period.

C. FOOD CONSUMPTION

Mean food intake values were comparable amongst all groups throughout the study period.

D. BLOOD ANALYSIS:

1. Haematological findings

There were no effects on haematological that were considered to be related to treatment with chemx.

2. Clinical chemistry findings

There were no effects on clinical chemistry parameters that were considered to be related to treatment with chemx.

E. SACRIFICE AND PATHOLOGY:

1. Organ weight

Mean absolute and mean relative (to body weight) kidney weights for females in the x,xxx mg/kg bw group were slightly increased (Table IIA 5.3.7-2). However, all individual kidney weight values (absolute and relative) for females amongst all groups fell within the same range of values, and there was no dose-response relationship. This finding is therefore not considered to be treatment related.

Table IIA 5.3.7-2 Females - mean kidney weights, with standard deviations absolute weight (g) and weight relative to body weight (% bw)

Dose (mg/kg bw)	Absolute weight	Relative weight
0	2.05 ± 0.12	0.86 ± 0.03
100	2.07 ± 0.20	0.89 ± 0.06
300	2.01 ± 0.14	0.84 ± 0.06
x,xxx	2.22 ± 0.19	0.93 ± 0.03*

* statistically significantly different from control, $p \leq 0.05$

2. Gross and histopathology

There were no treatment-related findings observed on gross pathology or histopathology examination.

III. CONCLUSIONS

The NOEL was determined to be x,xxx mg/kg bw per application, since there were no treatment-related effects observed in male or female rats at any dose level tested. There were no signs of local dermal irritation observed in male or female rats exposed to chemx at any dose level tested.

(White MW and KL Jones 1997)

IIA 5.3.8 **90-day percutaneous toxicity (rodents)**

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IIA 5.3.9 **Summary of short-term toxicity**

Table IIA 5.3.9-1: Summary of short-term toxicity of chemx

Study	Dose levels mg/kg bw/day ($\bar{}/\alpha$)	Findings	NOAEL
28-day oral, mice	0, 2/2.6, 17.2 /21.6, 196.8/286, 700.9/987	ocular opacity	$\bar{}$ = xxx mg/kg bw/day α = xxx mg/kg bw/day
28-day oral, rats	0, 1.3/1.5, 13.7/15.6, 136.5/154.1, 669/768	non adverse cumulative weight gain in females	$\bar{}$ = xxx mg/kg bw/day α = xxx mg/kg bw/day
21-day oral, dog	0, 30, 100, 300, 1,000	clin. chem.	$\bar{}$ = xxx mg/kg bw/day α = xxx mg/kg bw/day
90-day feeding, mice	0, 18/33, 163/313, 549.5/887, 1,144/2,123	↓ALP	$\bar{}/\alpha$ = x,xxx mg/kg bw/day
90-day feeding, rat	0, 1.2/1.5, 12.1/14.6, 123.2/144.3, 370.3/447.5, 1,277.5/1,489.1	↓body weights in males calculi in $\bar{}/\alpha$	$\bar{}/\alpha$ = xxx/xxx mg/kg bw/day
90-day oral, dog	0, 30, 100, 300 and 1,000	urinary tract pathology	$\bar{}$ = xxx mg/kg bw/day α = xxx mg/kg bw/day
One-year oral, dog	0, 5, 20, 100 and 500	↑AST and ALT, bladder pathology	$\bar{}/\alpha$ = xxx mg/kg bw/day
28-day dermal, rat	0, 100, 300, 1,000	no treatment related effects	NOEL = x,xxx mg/kg bw/day

IIA 5.4 Genotoxicity testing

IIA 5.4.1 *In vitro* genotoxicity testing (bacterial assay for gene mutation)

Report: IIA 5.4.1/01 Smith A 1995, Ames / Salmonella mutagenicity assay of chemx, Report No.: CC-94002

Guidelines: EEC B 14, equivalent to OECD Guideline 471

GLP: Fully GLP compliant [18].

Executive Summary

In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA102, TA1535, and TA1537 of *Salmonella. typhimurium* were exposed to chemx (98.5% purity), using dimethylsulfoxide (DMSO) solvent at concentrations of 50, 150, 500, 1,500 and x,xxx μg/plate in the presence and absence of S9 activation. A single plate was used, per dose, per condition.

Chemx was tested up to cytotoxic concentrations or limit concentration, x,xxx μg/plate. Based on the qualitative data generated, cytotoxicity (as indicated by decreased revertants/plate) was evident at the highest dose tested (HDT) with and without S9 activation. Hence, x,xxx μg/plate ±S9 was chosen as the highest dose tested for mutagenicity testing using both the plate incorporation and pre-incubation procedures. An additional six doses (down to 5 μg/plate ±S9) were included for statistical purposes. Reproducible cytotoxic effects were seen under both plate incorporation and pre-incubation test conditions in the majority of strains at doses ≥ 1,500 μg/plate ±S9. The positive controls induced the appropriate responses in the corresponding strains

There were no significant ($p \leq 0.01$) elevations in revertants / plate or in dose responses in any of the tests. It was concluded that chemx was not mutagenic in the bacterial strains tested, either in the presence or absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9307-5385-T
   - **Purity:** 98.5 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** The test material was found to be stable over a one-year period. The test material was stored at room temperature. Dosing solutions were prepared on the day of use and samples of the stock solution were analysed for achieved concentrations.
   - **Solvent used:** dimethylsulfoxide (DMSO).

2. **Control Materials:**

   - **Negative:** culture medium

Solvent/final concentration: DMSO at 0.1 mL/plate

Positive: non-activation:

4-nitroquinoline-N-oxide	0.02, 0.1 & 0.2	µg/plate TA98, TA100
sodium nitrite	0.5, 2.5 and 5	mg/plate TA1535
9-aminoacridine	10, 50 and 100	µg/plate TA1537
cumene hydroperoxide	10, 50 and 100	Tg/plate TA102

activation:

2-acetylaminofluorene	3, 15 and 30	µg/plate TA98
benzo(a)pyrene	0.2, 1 and 2	µg/plate TA100
2-aminoanthracene	1, 5 and 10	µg/plate TA1535, TA1537
Dantron	5, 25 and 50	µg/plate TA102

3. Activation: S9 derived from male Sprague-Dawley rats (Aroclor 1254 induced rat liver)

The rat liver S9 (Lot No. MolTox 0339; protein content = 39.2 mg/mL) was purchased from Molecular Toxicology, Inc., College Park, MD. The metabolic activation ability of the S9 was characterized using varying S9 and positive control concentrations.

S9 mix composition:	**Component:**	**Concentration**
	sodium phosphate buffer (pH 7.4)	100 µmoles
	glucose 6-phosphate	5 µmoles
	NADP	4 µmoles
	KCl	33 µmoles
	$MgCl_2$	8 µmoles
	S9	10 % (v/v)

4. Test Organisms: *S. typhimurium* strains: TA98, TA100, TA102, TA1535, TA1537 - test organisms were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor)

5. Test Concentrations:

(a) Preliminary cytotoxicity assay: Two preliminary assays were performed:

Plate incorporation assay: 50, 150, 500, 1,500 and x,xxx µg/plate were evaluated with and without S9 activation in S. typhimurium strain TA100. A single plate was used, per dose, per condition.

Pre-incubation assay: 50, 150, 500, 1,500 and x,xxx µg/plate were evaluated with and without S9 activation in S. typhimurium strain TA100. A single plate was used, per dose, per condition.

(b) Mutation assays:

Plate incorporation assay: 5, 15, 50, 150, 500, 1,500 and x,xxx µg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Pre-incubation assay: As above for the plate incorporation assay.

Re-tests: Owing to contamination, poor performance of the positive controls or unacceptable analytical data, portions of the plate incorporation assay were repeated with strains TA1535, TA1537, TA98 and TA100. Doses and assay conditions were comparable to those used with the initial plate incorporation assay.

B. TEST PERFORMANCE: The study (Salmonella Assay - standard plate test, pre-incubation for 20 minutes) was conducted during the period January to October 1994 by the Chemco Research Laboratory, New York.

1. Preliminary cytotoxicity/plate incorporation mutation assay

In general, similar procedures were used for the preliminary cytotoxicity and the plate incorporation mutation assay.

A 0.1 mL aliquot of the appropriate test strain and 0.1 mL of the appropriate test material dose, positive controls (mutation test only) or solvent, were added to tubes containing 2.0-mL volumes of molten top agar. For the S9-activated tests, 0.5 mL of the S9-cofactor mix was also added. Test strains, and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E plates, and incubated at 37 ± 1°C for 48 hours. For the mutation assay, triplicate plates per strain, per dose, per condition were used for the test compound; nine replicate plates were prepared for the solvent controls, and single plates were used for the negative controls and each positive control concentration. Means and standard deviations were calculated for the mutation assay data.

2. Pre-incubation assay

The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1°C.

3. Statistics

Data were transformed using a $\log_{10}$ transformation and analysed using Bartlett's test for homogeneity of variance; test groups were compared with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine outliers and dose response was evaluated by regression analysis. Significance was established at $p \leq 0.01$.

4. Evaluation Criteria

The test material was considered positive for a particular strain and condition if it caused a statistically significant ($p \leq 0.01$) dose-related increase in revertants over the solvent controls at three treatment levels.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Results of the analytical determinations indicated that with the exception of a single sample (78 % of target), actual concentrations (high dose only) used in valid tests ranged from 87 to 119 % of the intended levels. The overall percent difference from target for the nine samples was -1.76%

B. PRELIMINARY CYTOTOXICITY ASSAY

Five doses of the test material ranging from 50 to x,xxx µg/plate ± S9 were evaluated in the plate incorporation and the pre-incubation cytotoxicity tests. No precipitation was observed up to the limit dose, x,xxx µg/plate. Based on the qualitative data generated, cytotoxicity (as indicated by decreased revertants/plate) was evident at the highest dose tested (HDT) with and without S9 activation (see study report, Appendix I, Table 1, p. 18). Hence, x,xxx µg/plate ± S9 was chosen as the HDT for mutagenicity testing using both the plate incorporation and pre-incubation procedures. An additional six doses (down to 5 µg/plate ± S9) were included for statistical purposes.

C. MUTATION ASSAYS

Reproducible cytotoxic effects were seen under both plate incorporation and pre-incubation test conditions in the majority of strains at doses ≥ 1,500 µg/plate ± S9. Reverent counts in chemx treated initial and repeat plate incorporation tests did not differ significantly from the DMSO solvent control or from the negative (culture medium) control data (see study report, Appendix I, Tables 2-9, pp. 19-26). Similar results were obtained in the initial and repeat pre-incubation tests (see study report, Appendix II, Tables 1-3, pp. 28-30). In contrast, positive controls responded appropriately with significant ($p \leq 0.01$) reverent values 5-fold to 50-fold over background (see study report, Appendix III, Tables 2-9).

III. CONCLUSIONS

It was concluded that the test article was not mutagenic in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested. Additionally, the sensitivity of both the plate incorporation and pre-incubation procedures to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls.

(Smith A 1995a)

IIA 5.4.2 *In vitro* genotoxicity testing (test for clastogenicity in mammalian cells)

Report: IIA 5.4.2/01 Smith A 1996, Chromosome aberration study in human whole blood lymphocytes with chemx and a confirmatory assay with multiple harvests, Report No.: CC-95-201

Guidelines: OECD 473 ≅ EPA FIFRA Guideline § 84-2 ≅ EEC B 10

GLP: Fully GLP compliant [18].

Executive Summary

In a mammalian cell gene mutation assay, human lymphocyte cells cultured *in vitro* were exposed to chemx (98.5 % purity) using dimethylsulfoxide (DMSO) solvent at concentrations of 100, 250, 500, 750, and x,xxx µg/mL with and without metabolic activation with 3 hour treatment and 22 hour harvests.

In a range finding trial, chemx was assayed at 500, 750 and x,xxx µg/mL. Positive controls induced the appropriate response. There was no evidence of induced mutant colonies.

In the confirmatory trial, replicate cultures of human lymphocytes cells were incubated with 100, 250, 500, 750, and x,xxx µg/mL with metabolic activation (3 hour treatment) and without metabolic activation (19.5 and 43.4 hour treatments). Cultures were harvested at 22.3 and 46 hours after initiation of treatment. Cultures treated with 500, 750 and x,xxx µg/mL from these assays were checked for chromosomal aberrations. Positive controls induced the appropriate response. No significant increase in cells with chromosomal aberrations or in polyploidy were observed at the concentrations tested.

Chemx was found to be negative in relation to induction of chromosomal aberrations or polyploidy in cultured whole blood human lymphocytes with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9503-6466-T
   - **Purity:** 98.5 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** The test material was shown to be stable in dimethylsulfoxide (DMSO) dosing solutions for 24 hours at room temperature.
   - **Solvent used:** dimethylsulfoxide (DMSO).

2. **Control Materials:**

   - **Negative:** Tissue culture medium
   - **Solvent:** DMSO, 1 %
   - **Positive:** **-S9**: mitomycin C (MMC) in water at 0.2, 2 and 10 µg/mL.
     +S9: cyclophosphamide (CP) in water at 30 and 40 µg/mL.

3. **Activation**: S9 derived from adult male Sprague-Dawley rats (Aroclor 1254 induced rat liver). The composition of the rat liver S9 reaction mix was: S9: 15 µL/mL; NADP 1.5 mg/mL; and isocitric acid: 2.7 mg/mL.

4. **Test Cells:** Human lymphocytes obtained from human venous blood from a single, normal, healthy donor.

5. Culture Medium: RPMI 1640, supplemented with 15 % foetal bovine serum, 1 % phytohaemaglutinin (to stimulate lymphocytes to divide), 1 % penicillin and streptomycin, and 1% L-glutamine.

6. Test compound concentrations used:

	Trial 1	Trial 2
	(µg/mL)	
Non-activated conditions	100, 250, 500, 750, x,xxx	100, 250, 500, 750, x,xxx
Activated conditions	100, 250, 500, 750, x,xxx	100, 250, 500, 750, x,xxx

B. TEST PERFORMANCE

The Chemco Research Laboratory, New York conducted the study during the period September to November 1995.

1. Preliminary Cytotoxicity Assay

Cultures were exposed to a range of doses from 33.3 to x,xxx µg/mL for 3 h ± S9, or to 3.33 to x,xxx µg/mL for 19.3 h without S9, and cytotoxicity determined as the mitotic index, MI (number of dividing cells/1,000 cells counted).

2. Cytogenetic Assay:

Cell Treatment: Cells were exposed to test compound, solvent or positive control for 3 h (initial trial) or 19.5 and 43.4 h (trial 2) (non-activated), 3 hours (activated, both trials).

Spindle inhibition: 0.1 µg/mL colcemid was administered 2 h before cell harvest

Cell harvest: Cells were exposed to test material, solvent or positive control were harvested 19 h (initial trial), or 2 h (trial 2) after termination of treatment (non-activated), 22 hours after termination of treatment (activated).

Slide preparation: Slides were prepared by dropping the harvested cultures on clean sides. The slides were stained with 5 % Giemsa solution. All slides were air-dried and cover slipped using Depex^{R} mounting medium.

Metaphase analysis: Slides were coded prior to analysis. 100 cells were examined per replicate culture (200 per dose) and were scored for structural aberrations and for numerical aberrations (polyploidy).

Evaluation criteria: The following factors were taken into account in the evaluation of the chromosomal aberrations data: the overall chromosomal aberration frequencies; the percentage of cells with any aberrations; the percentage of

cells with more than one aberration; evidence for increasing amounts of damage with increasing dose, *i.e.* a positive dose response.

Chromatid and isochromatid gaps, if observed, were noted in the raw data and were tabulated. They were not, however, considered in the evaluation of the ability of the test article to induce chromosomal aberrations since they may not represent true chromosomal breaks and may possible be induced by toxicity. Percent polyploidy was checked and results were tabulated. Historical control data were presented (see study report Table 8).

Definitions

TG = Chromatid gap SG = Chromosome gap
(Gaps are noted, but are not counted as chromosomal aberrations)

UC = uncoiled chromosome (failure of chromatin packing - probably not a true aberration. Not counted as a chromosomal aberration).

The following are considered to be chromosomal aberrations:

TB = Chromatid break SB = Chromosome break
ID = Interstitial deletion TR = Triradial
QR = Quadriradial D = Dicentric
DF = Dicentric with fragment
R = Ring chromosome
CI = Chromosome intrachange
GT = A cell which contains more than 10 aberrations.

3. Statistics

Data were evaluated for statistical significance at $p \leq 0.01$ using Fisher's Exact Test with adjustments for multiple comparisons. The Armitage procedure for testing linear trend was also performed.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY

Slight precipitation was noted in both activated and non-activated cultures treated at x,xxx µg/mL. In non-activated cultures treated for 3 h, there was considerable cytotoxicity (> 5 0% reduction in MI) at 100 and 333 µg/mL, but considerably less at x,xxx µg/mL (*ca.* 15 % reduction in MI). In non-activated cultures treated for 19.3 h, mitotic reductions of 39 and 61 % were observed at the two highest dose levels (333 and x,xxx µg/mL, respectively).

In the activated cultures, reductions of 14, 33 and 19 % were noted in the MI of cultures treated with 100, 333, and x,xxx µg/mL.

B. CYTOGENETIC ASSAYS

In 3h non-activated cultures of Trial 1 (Initial) chemx produced slight to moderate cytotoxicity (13 - 33 % reductions in MI) at 100 - x,xxx µg/mL, but no increase in structural or numerical chromosomal aberrations (Table IIA 5.4.2-1). In treated cultures with activation, the same dosage series produced greater cytotoxicity (up to 69 % reduction in MI), but again there was no significant amount of induction of aberrations (Table IIA 5.4.2-4). In the 22.3 and 46 h confirmatory trials, reductions of up to 54 % in the MI were observed, but there was not a significant increase in cells with chromosomal aberrations and/or polyploid chromosome number (Table IIA 5.4.2-2, Table IIA 5.4.2-3, Table IIA 5.4.2-5 and Table IIA5.4.2-6) at the dose levels evaluated (500, 750, and x,xxx µg/mL) either in the absence or presence of activation.

In contrast, treatment with both positive controls resulted in significant increases in the percentage of cells with aberration, but with little or no indications of polyploidy (characteristic of strictly clastogenic agents).

III. CONCLUSIONS

Chemx was not clastogenic and/or aneugenic in human lymphocyte cultures under the conditions of this study.

(Smith A 1996b)

Table IIA 5.4.2-1: Chromosome aberrations in human lymphocytes, Trial 1, without S9: Cells fixed 22.0 h after initiation of treatment (3 h treatment)

				Number and type of aberration														No of	% cells			
			Cells scored	Not computed			Simple		Complex								Other	aberrations	with aberrations	with >1 aberration	% Polyploidy	Mitotic Index
				TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT	per cell		tion		
CONTROLS																						
Negative RPMI 1640		A	100	9	2		1	2										0.03	3.0	0.0	0.0	3.6
		B	100	9	2			3							1			0.04	2.0	1.0	0.0	2.6
		A+B	200	18	4		1	5							1			0.04	2.5	0.5	0.0	3.1
Solvent: DMSO	10 µL/mL	A	100	5			1								1			0.02	2.0	0.0	1.0	3.2
		B	100	8	2		1											0.01	1.0	0.0	0.0	2.8
		A+B	200	13	2		2								1			0.02	1.5	0.0	0.5	3.0
Positive: MMC	2.0 µg/mL	A	100	23	8		20	10	2	7	17			1				0.57	38.0	13.0	0.0	0.7
		B	100	27	6	1	33	6	5	3	13	1			2			0.63	41.0	17.0	0.0	0.4
		A+B	200	50	14	1	53	16	7	10	30	1		1	2			0.60	39.5*	15.0*	0.0	0.6
TEST ARTICLE	100 µg/mL	A	**																			1.6
		B	**																			2.3
		A+B	**																			2.0
	250 µg/mL	A	**																			1.5
		B	**																			3.7
		A+B	**																			2.6
	500 µg/mL	A	100	4	1													0.00	0.0	0.0	0.0	3.2
		B	100	7														0.00	0.0	0.0	0.0	1.8
		A+B	200	11	1													0.00	0.0	0.0	0.0	2.5
	750 µg/ml	A	100	5	4		1											0.01	1.0	0.0	0.0	5.9
		B	100	8														0.00	0.0	0.0	0.0	2.5
		A+B	200	13	4		1											0.01	0.5	0.0	0.0	4.2
	x,xxx µg/ml	A	100	10			1	1										0.02	2.0	0.0	0.0	1.7
		B	100	11			2											0.02	2.0	0.0	0.0	2.4
		A+B	200	21			3	1										0.02	2.0	0.0	0.0	2.1

* Significantly greater than the solvent controls, $p \leq 0.01$

** Chromosome aberrations not checked since there were three higher dose levels checked

Table IIA 5.4.2-2: Chromosome aberrations in human lymphocytes, Trial 2, without S9: Cells fixed 22.3 h after initiation of treatment (19.5 h treatment)

				Number and type of aberration														No of	% cells			
			Cells scored	Not computed			Simple		Complex								Other	aberrations	with aberrations	with >1 aberration	% Polyploidy	Mitotic Index
				TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT	per cell		tion		
CONTROLS																						
Negative RPMI 1640		A	100	1				1					1					0.02	2.0	0.0	1.0	2.6
		B	100	2	2		1											0.01	1.0	0.0	0.0	2.0
		A+B	200	3	2		1	1					1					0.02	1.5	0.0	0.5	2.3
Solvent: DMSO	10 µL/mL	A	100	2														0.00	0.0	0.0	0.0	2.0
		B	100	3			1	1										0.02	2.0	0.0	0.0	2.2
		A+B	200	5			1	1										0.01	1.0	0.0	0.5	2.1
Positive: MMC	0.20 µg/mL	A	100	15	5		19	3	1	2	4							0.29	25.0	3.0	0.0	0.5
		B	100	8	2		8	9	2	1	4		2		1			0.27	24.0	1.0	0.0	0.9
		A+B	200	23	7		27	12	3	3	8		2		1			0.28	24.5*	2.0	0.0	0.7
TEST ARTICLE	100 µg/mL	A	**																			2.6
		B	**																			1.5
		A+B	**																			2.1
	250 µg/mL	A	**																			2.1
		B	**																			1.7
		A+B	**																			1.9
	500 µg/mL	A	100	3														0.00	0.0	0.0	0.0	1.0
		B	100	3														0.00	0.0	0.0	0.0	1.4
		A+B	200	6														0.00	0.0	0.0	0.0	1.2
	750 µg/ml	A	100	1				2										0.02	2.0	0.0	0.0	1.5
		B	100	6	1		2											0.02	2.0	0.0	0.0	1.0
		A+B	200	7	1		2	2										0.02	2.0	0.0	0.0	1.3
	x,xxx µg/ml	A	100	2	2													0.00	0.0	0.0	0.0	1.2
		B	100	3	1		1	1										0.02	1.0	1.0	0.0	0.9
		A+B	200	5	3		1	1										0.01	0.5	0.5	0.0	1.1

* Significantly greater than the solvent controls, $p \leq 0.01$

** Chromosome aberrations not checked since there were three higher dose levels checked

Table IIA 5.4.2-3: Chromosome aberrations in human lymphocytes, Trial 2, without S9: Cells fixed 46.0 h after initiation of treatment (19.5 h treatment)

				Number and type of aberration														No of	% cells			
			Cells scored	Not computed			Simple		Complex								Other	aberra tions	with aberra tions	with >1 aberra	% Poly ploidy	Mitotic Index
				TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT	per cell		tion		
CONTROLS																						
Negative RPMI 1640		A	100	6							1							0.01	1.0	0.0	0.0	3.4
		B	100	6				1										0.01	1.0	0.0	0.0	3.9
		A+B	200	12				1			1							0.01	1.0	0.0	0.5	3.7
Solvent: DMSO	10 µL/mL	A	100	4														0.00	0.0	0.0	0.0	3.5
		B	100	2		1												0.00	0.0	0.0	0.0	3.7
		A+B	200	6		1												0.00	0.0	0.0	0.0	3.6
TEST ARTICLE	100 µg/mL	A	**																			3.6
		B	**																			3.4
		A+B	**																			3.5
	250 µg/mL	A	**																			2.9
		B	**																			2.7
		A+B	**																			2.8
	500 µg/mL	A	100	4														0.00	0.0	0.0	0.0	2.3
		B	100	4														0.00	0.0	0.0	1.0	2.8
		A+B	200	8														0.00	0.0	0.0	0.5	2.6
	750 µg/ml	A	100	5	1													0.0	0.0	0.0	0.0	2.4
		B	100	5	1													0.0	0.0	0.0	0.0	2.8
		A+B	200	10	2													0.0	0.0	0.0	0.0	2.6
	x,xxx µg/ml	A	100	9	2		6				1							0.07	3.0	1.0	1.0	1.5
		B	100	10	3		1	1										0.02	2.0	0.0	0.0	1.9
		A+B	200	19	5		7	1			1							0.05	2.5	0.5	0.5	1.7

* Significantly greater than the solvent controls, $p \leq 0.01$

** Chromosome aberrations not checked since there were three higher dose levels checked

Table IIA 5.4.2-4: Chromosome aberrations in human lymphocytes, Trial 1, with S9: Cells Fixed 22 h after initiation of treatment (3 h treatment)

				Number and type of aberration														No of	% cells			
			Cells scored	Not computed			Simple		Complex								Other	aberrations	with aberrations	with >1 aberration	% Polyploidy	Mitotic Index
				TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT	per cell		tion		
CONTROLS																						
Negative RPMI 1640		A	100	4														0.00	0.00	0.0	0.00	2.3
		B	100	1	1													0.00	0.00	0.0	0.00	2.5
		A+B	200	5	1													0.00	0.00	0.0	0.00	2.4
Solvent: DMSO	10 µL/mL	A	100	3	1													0.00	0.00	0.0	0.00	2.7
		B	100	2														0.00	0.00	0.0	0.00	2.5
		A+B	200	5	1													0.00	0.00	0.0	0.00	2.6
Positive: CP	40 µg/mL	A	75	33	7		26	5	1	1	5		1					0.52	33.3	16.0	0.0	0.3
		B	75	17	5		21	3	1	2	2	1						0.40	30.7	9.3	0.0	0.3
		A+B	150	50	12		47	8	2	3	7	1	1					0.46	32.0*	12.7*	0.0	0.3
TEST ARTICLE	100 µg/mL	A	**																			1.4
		B	**																			2.1
		A+B	**																			1.8
	250 µg/mL	A	**																			1.3
		B	**																			1.8
		A+B	**																			1.6
	500 µg/mL	A	100	5	1	1	2											0.02	2.0	0.0	0.0	1.9
		B	100	2				1										0.01	1.0	0.0	0.0	2.1
		A+B	200	7	1	1	2	1										0.02	1.5	0.0	0.0	2.0
	750 µg/ml	A	100	3	1		1											0.01	1.0	0.0	0.0	2.4
		B	100	3	1													0.00	0.0	0.0	1.0	2.2
		A+B	200	6	2		1											0.01	0.5	0.0	0.5	2.3
	x,xxx µg/ml	A	100	7	1		1											0.01	1.0	0.0	0.0	0.7
		B	100	3														0.00	0.0	0.0	0.0	0.8
		A+B	200	10	1		1											0.01	0.5	0.0	0.0	0.8

* Significantly greater than the solvent controls, $p \leq 0.01$

** Chromosome aberrations not checked since there were three higher dose levels checked

Table IIA 5.4.2-5: Chromosome aberrations in human lymphocytes, Trial 2, with S9: Cells Fixed 22.3 h after initiation of treatment (3 h treatment)

				Number and type of aberration														No of	% cells			
			Cells scored	Not computed			Simple		Complex								Other	aberrations	with aberrations	with >1 aberra	% Poly ploidy	Mitotic Index
				TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT	per cell		tion		
CONTROLS																						
Negative RPMI 1640		A	100	3			2											0.02	2.0	0.0	0.0	3.1
		B	100	2			1	1							1			0.03	2.0	1.0	0.0	2.9
		A+B	200	5			3	1							1			0.03	2.0	0.5	0.0	3.0
Solvent: DMSO	10µL/mL	A	100	1			4											0.04	4.0	0.0	0.0	4.9
		B	100	2														0.00	0.0	0.0	0.0	3.2
		A+B	200	3			4											0.02	2.0	0.0	0.0	4.1
Positive: CP	30 µg/mL	A	75	8	4		26	4		3	3				1			0.49	38.7	6.7	1.0	0.7
		B	75	9	2		21	5	2	1	3	1						0.44	29.3	8.0	0.0	1.0
		A+B	150	17	6		47	9	2	4	6	1			1			0.47	34.0*	7.3*	0.5	0.9
TEST ARTICLE	100 µg/mL	A	**																			3.3
		B	**																			3.2
		A+B	**																			3.3
	250 µg/mL	A	**																			3.0
		B	**																			2.6
		A+B	**																			2.8
	500 µg/mL	A	100	5														0.00	0.0	0.0	0.0	3.0
		B	100	2			1											0.01	1.0	0.0	0.0	2.2
		A+B	200	7			1											0.01	0.5	0.0	0.0	2.6
	750 µg/ml	A	100	2	1		1	2										0.03	3.0	0.0	0.0	2.3
		B	100	4			1											0.01	1.0	0.0	0.0	1.5
		A+B	200	6	1		2	2										0.02	2.0	0.0	0.5	1.9
	x,xxx µg/ml	A	100	2				1										0.02	2.0	0.0	0.0	2.9
		B	100	6				2										0.02	2.0	0.0	0.0	1.8
		A+B	200	8				3										0.02	2.0	0.0	0.0	2.4

* Significantly greater than the solvent controls, $p \leq 0.01$

** Chromosome aberrations not checked since there were three higher dose levels checked

Table IIA 5.4.2-6: Chromosome aberrations in human lymphocytes, Trial 2, with S9: Cells Fixed 46.0 h after initiation of treatment (3 h treatment)

				Number and type of aberration														No of	% cells			
			Cells scored	Not computed			Simple		Complex								Other	aberrations	with aberrations	with >1 aberration	% Polyploidy	Mitotic Index
				TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT	per cell		tion		
CONTROLS																						
Negative RPMI 1640		A	100	2							1							0.01	1.0	0.0	1.0	7.4
		B	100	1	2								1					0.01	1.0	0.0	1.0	6.0
		A+B	200	3	2						1		1					0.01	1.0	0.0	1.0	6.7
Solvent: DMSO	10µL/mL	A	100	6	1		1	1										0.02	2.0	0.0	0.0	7.0
		B	100	4														0.00	0.0	0.0	0.0	7.1
		A+B	200	10	1		1	1										0.01	1.0	0.0	0.0	7.1
TEST ARTICLE	100 µg/mL	A	**																			6.3
		B	**																			4.1
		A+B	**																			5.2
	250 µg/mL	A	**																			6.2
		B	**																			5.3
		A+B	**																			5.8
	500 µg/mL	A	100	4														0.00	0.0	0.0	0.0	5.6
		B	100	5									1					0.01	1.0	0.0	0.0	6.6
		A+B	200	9									1					0.01	0.5	0.0	0.0	6.1
	750 µg/ml	A	100	4			2	1		1								0.04	4.0	0.0	0.0	4.1
		B	100	4	1													0.00	0.0	0.0	0.0	4.3
		A+B	200	8	1		2	1		1								0.02	2.0	0.0	0.0	4.2
	x,xxx µg/ml	A	100	6	2													0.00	0.0	0.0	0.0	5.2
		B	100	3	1			1										0.01	1.0	0.0	1.0	6.2
		A+B	200	9	3			1										0.01	0.5	0.0	0.5	5.7

* Significantly greater than the solvent controls, $p \leq 0.01$

** Chromosome aberrations not checked since there were three higher dose levels checked

IIA 5.4.3 *In vitro* genotoxicity testing (test for gene mutation in mammalian cells)

Report: IIA 5.4.3/02 Smith A 1996, CHO/HGPRT gene mutation assay of chemx, Report No.: CC-95-200

Guidelines

OECD 476 ≅ EPA FIFRA Guideline § 84-2 ≅ Directive 88/3023/EEC (OJ No L133/61 of 30 May 1988)

GLP: Fully GLP compliant [18]

Executive Summary

In a mammalian cell cytogenetics assay, Chinese hamster ovary (CHO/V79/5178Y) cell cultures were exposed to chemx (98.5 % purity), using dimethylsulfoxide (DMSO) as solvent. Mutation at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) gene locus was measured. Mutagenicity testing of chemx was performed initially using a range of Aroclor 1254-induced rat liver homogenate (S9) concentrations (0 – 10 %) followed by a confirmatory experiment with 0 and 5 % S-9 Mix.

In the initial mutagenicity experiment the concentration ranges tested were 624, 1,250, 2,500 and x,xxx µg/mL both without S-9 Mix and with 1 %, 5 % and 10 % S-9 Mix. In both the presence and absence of S-9 Mix, the top two concentrations, 2,500 and x,xxx µg/mL, were above the limit of solubility (precipitation of test material occurred). There was no significant cytotoxicity (≤ 50 % relative survival) observed for any treatment level tested in either the presence or absence of S-9 Mix. In the initial experiment, no significant increases in mutant frequency or dose responses were observed in either presence or absence of S-9 Mix.

In the confirmatory experiment, the chemx concentration range tested was from 312 to x,xxx µg/mL both without and with 5 % S-9 Mix. The test material was insoluble at the 2,500 and x,xxx µg/mL treatment level in both the presence and absence of 5 % S-9 Mix. There was no significant cytotoxicity observed for any treatment level tested in either the presence or absence or 5 % S-9 Mix. Positive control treatments induced the appropriate response. There were no evidence of the induction of chromosomal abberations by chemx.

In was concluded that in cultured Chinese hamster ovary (CHO) cells assays, both in the presence (1 %, 5 % or 10 %) and absence of S-9 Mix, chemx was not mutagenic when tested at concentrations up to the OECD recommended upper limit dose and beyond the limit of solubility.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx
   - **Description:** White powder
   - **Lot/Batch #:** NPD-9503-6466-T
   - **Purity:** 98.5 % as [18]
   - **CAS #:** 16335-17-2
   - **Stability of test compound:** The test material was stored at room temperature. Neat test material was stable over the duration of the study (≈ 7 weeks).

Solvent used: dimethylsulfoxide (DMSO).

2. Control Materials:

Negative: None
Culture medium: serum-free Ham's F12
Positive: **Non-activation:** (concentrations, solvent): ethyl methanesulfonate (EMS) was prepared in dimethylsulfoxide (DMSO) to yield a final concentration of 200 µg/mL.
Activation: (concentrations, solvent): benzo(a)pyrene (BaP) was prepared in DMSO to yield a final concentration of 2 µg/mL.

3. Activation: S9 derived from male Sprague Dawley (Aroclor 1254 induced rat liver)

Two lots of rat liver S9 fraction, purchased from Molecular Toxicology, Inc., Annapolis, MD, were used in the study. Lot No. MolTox 0339 (protein content = 39.2 mg/mL) was used in the range-finding assays and Lot No. MolTox 0340 (protein content = 41.4 mg/mL) was used in the mutation assays.

S9 mix composition:	**Component**	**Concentration**
	NADP	4 mM
	glucose-6-phosphate	5 mM
	potassium chloride	30 mM
	magnesium chloride	10 mM
	sodium phosphate buffer, pH 7.5	50 mM
	calcium chloride	10 mM
	S9 homogenate	1, 5 and 10 % were used in both the cytotoxicity and initial mutagenicity assays

4. Test Cells: Chinese hamster ovary (CHO) - the cell cultures were properly maintained, were periodically checked for mycoplasma contamination and were periodically "cleansed" against high spontaneous background. The medium used was Ham's F12 medium ($F12_0$); $F12_5$--$F12_0$ with 5 % foetal calf serum (FCS).

5. Locus Examined: hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) - the selection agent used was 10 µM 6-thioguanine (6-TG)

6. Test Concentrations:

(a) Preliminary cytotoxicity assay: Two trials were performed; doses were as follows:

Trial 1: Nine concentrations (5, 25, 50, 70, 250, 500, 700, 1,000 and x,xxx µg/mL) were evaluated in the absence and presence of 1, 5, and 10 % S9 in the S9 cofactor mix.

Trial 2: Four doses (500, 1,000, 2,500 and x,xxx µg/mL) were evaluated in the absence and presence of 1, 5, and 10 % S9 in the S9 cofactor mix.

(b) Mutation assay: Two non-activated and two S9-activated assays were performed; doses tested were as follows:

Non-activated conditions:	**Initial trial:**	624, 1,250, 2,500 and x,xxx µg/mL
	Confirmatory trial:	312, 624, 1,250, 2,500 and x,xxx µg/mL
S9-activated conditions:	**Initial trial:**	624, 1,250, 2,500 and x,xxx µg/mL + 1, 5 or 10 % S9
	Confirmatory trial:	312, 624, 1,250, 2,500 and x,xxx µg/mL with 5 % S9

Note: Duplicate cultures per group were processed in the initial trials and triplicate cultures per group were processed in the confirmatory trials.

Dosing solutions, which were prepared on the day of use at the desired concentration, were subjected to ultrasonic agitation for ≈ 1 h at 37 ± 1°C prior to addition to the treatment medium. Homogeneity and stability of the test material/solvent was determined on the initial stock suspension; samples of the highest dosing suspension used in the mutation assays were analysed for achieved concentrations and samples of the stock solution were analysed for achieved concentrations.

B. TEST PERFORMANCE

The Chemco Research Laboratory, New York conducted The study during the period September to November 1995.

1. Cell treatment

Cells were exposed to test compound, solvent or positive controls for 3 hours (non-activated and activated); after washing, cells cultured for 7 to 9 days prior to cell selection; following expression, 2 x 10^5 cells/plate (5 plates) were cultured for 6 to 10 days in selection medium to determine numbers of mutants and 200 cells/plate (3 plates) were cultured for 6 to 10 days without selection medium to determine cloning efficiency (CE).

2. Statistics

Data were evaluated according to the method of Snee and Irr [21] (*i.e.* mutation frequencies were transformed as follows: $Y = [X + 1]^{0.15}$ and analysed by Student's t-test at $p \leq 0.05$.

[21] Snee, R.D. and Irr J.D. (1981). Design of a statistical method for the analysis of mutagenesis at the hypoxanthine guanine phosphoribosyl transferase locus of cultured Chinese hamster ovary cells. Mutat. Res. 77+93.

3. Evaluation criteria

A test compound was considered positive in this test system if it induced a reproducible dose-related increase in the mean mutation frequency (MF) with a positive slope statistically different ($p \leq 0.05$) from zero. Additionally, at least one treatment level should have a mean MF that was ≥ 2-fold and statistically higher ($p \leq 0.05$) than the solvent control value.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The results of chemical analyses conducted indicated that in general, the suspensions of chemx prepared in culture medium were adequately stable, distributed in the culture medium and accurately prepared (the mean dosing suspension value was within 1 % of the target concentration). Although large variations were obtained in the individual dosing suspension values, the overall study results were not compromised since the compound precipitated at high levels and there was reproducible evidence that the highest assayed dose was marginally cytotoxic to the CHO cells in the absence of S9 activation.

B. PRELIMINARY CYTOTOXICITY ASSAY

Combined results from the two cytotoxicity tests indicated that compound precipitation occurred at doses ≥ 500 µg/mL. The highest non-activated dose (x,xxx µg/mL) caused an ≈ 54 % reduction in relative cell survival (RCS). However, no appreciable cytotoxicity was found at any S9-activated dose or at non-activated level ≤ 2,500 µg/mL. Based on these results, doses of 624, 1,250, 2,500 and x,xxx µg/mL without or with 1, 5 or 10 % S9 were selected for the initial mutation assay.

C. MUTATION ASSAYS - INITIAL ASSAY

Summarized results from the initial mutation assay with chemx are presented in the study report (Appendix I, Table 2, p 28). Compound insolubility was recorded at concentrations ≥ 2,500 µg/mL in both the absence and presence of varying concentrations of S9. RCS at x,xxx µg/mL without S9 activation was 61 %; the S9-activated test material and lower non-activated levels were not cytotoxic. There was no indication of a mutagenic effect under any of the assay conditions.]

D. CONFIRMATORY ASSAY

Consistent with the initial mutation assay data, there were no increases in 6TG-resistant cell colonies (presumed $HGPRT^-$ mutants) in either the presence or absence of S9 activation up to the limit dose of x,xxx µg/mL. Compound precipitation was again evident at ≥ 2,500 µg/mL (see study report Appendix I, Table 3, p 29). By contrast to the negative results for the test material, the non-activated (200 µg/mL EMS) and S9-activated (2 µg/mL BaP) positive controls were clearly mutagenic with MFs 9 to 16-fold higher than background levels in both trials (see study report, Appendix I, Table 4, p.30).

III. CONCLUSIONS

Chemx was assayed above the limit of solubility (to the limit dose, x,xxx µg/mL) but failed to induce a mutagenic response either in the absence or presence of graded concentrations of S9. The findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis. The study provides clear evidence that chemx is not a mutagen in this *in vitro* mammalian cell gene mutation assay.

(Smith A 1996a)

IIA 5.4.4 *In vivo* genotoxicity testing (somatic cells) (metaphase analysis in rodent bone marrow, or micronucleus test in rodents)

Report: IIA 5.4.4/01 Smith A 1995, Mouse bone marrow micronucleus assay of chemx, Report No.: CCC-14073

Guidelines: OECD 474 ≅ EPA FIFRA Guideline § 84-2 ≅ EEC B 12

GLP: Fully GLP compliant [18].

Executive Summary

In a bone marrow micronucleus assay using CD-1 mice, chemx was administered by oral gavage to groups of male and female animals at target doses of 1,000 (5 animals/sex) and x,xxx mg/kg bw (10 animals/sex) in a dose range-finding assay and at 1,250, 2,500 and x,xxx mg/kg bw (5-10 animals/sex/group) in the micronucleus assay.

Negative control groups were treated with vehicle only (tricaprylin, 10 mL/kg bw), and positive control groups were treated with cyclophosphamide (40 mg/kg bw). Mouse bone marrow was sampled at 24, 48 and 72 hours after dosing for the vehicle and chemx dosed groups. A single sampling time of 24 hours after dosing was used for the cyclophosphamide positive control group. Slides of bone marrow cells were prepared from five animals/sex/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (micronucleated PCEs) and PCE/total erythrocyte ratios.

No deaths were observed in the chemx-dosed groups, vehicle or positive control groups. Only two male mice, one in the 2,500 mg/kg body weight dose group and one in the high dose level group, displayed clinical signs of toxicity (hyperactivity). There were no statistically significant decreases in mean body weight change for any of the chemx treated groups or control groups (vehicle and positive control). There were no statistically significant decreases in mean PCE/total erythrocyte ratio observed for any of the chemx treated groups or control groups.

Analysis of the micronucleated PCE (MN PCE) data indicated a statistically significant ($p \leq 0.01$) increase in mean micronucleated PCE frequency compared to concurrent control values at the mid dose level (2,500 mg/kg bw) female treatment group sacrificed 24 hours after dosing. The increase observed was not considered to reflect a treatment-related response. No statistically significant increases in the micronucleated PCE frequencies were observed in any of the other chemx treated groups. The observed level of MN PCE for the group (2.7 MN PCE/1000 PCE) was within the laboratory historical corn oil vehicle control range (a combined female historical range of 0 - 7.4 MN PCE/1000 PCE). The fact that MN PCE's were not induced at the highest dose level is not consistent with the response at the mid dose level being treatment-related. The observed effect is considered a result of biological variation and is not a treatment-related effect.

Positive control treatment induced the appropriate response. The observations and findings of the study indicate that chemx does not exhibit *in vivo* mammalian genotoxicity in mouse bone marrow cells under the experimental conditions of the study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Chemx
- **Description:** White powder
- **Lot/Batch #:** NPD-9307-5385-T
- **Purity:** 98.5 % as [18]
- **CAS #:** 16335-17-2
- **Stability of test compound:** The test material was stable for at least 7 days at room temperature. Analysis of the top dosing solution used in the main mutagenicity experiment verified that the concentration of chemx was within 1 % of the nominal concentration (see Table IIA 5.4.4-1).
- **Solvent:** 1,2,3-trioctanoylglycerol

2. Control materials:
- **Negative:** None
- **Solvent:** 1,2,3-trioctanoylglycerol (also known as glycerol tricaprylate and as tricaprylin), 10 mL/kg bw (oral gavage)
- **Positive:** Cyclophosphamide monohydrate (40 mg/kg bw) in 1,2,3-trioctanoylglycerol (oral gavage).

3. Test animals -
- **Species:** Mouse
- **Strain:** Charles River CD-1
- **Age:** 8 to 10 week old
- **Weight at dosing:** weight range 29.6 - 38.3 g (males) and 18.8 - 29.0 g (females).
- **Source:** Charles River Laboratories, Portage, MI
- **Number of animals per dose:** range-finding test - 5-10 animals/sex/group
  micronucleus assay - 5 males and 5 females / group / sacrifice time)
- **Animal husbandry:** The animals were properly maintained.

4. Test compound concentrations:
- **Range-finding test:** 1,000 and x,xxx mg/kg bw administered by means of oral gavage
- **Micronucleus assay:** 0, 1,250, 2,500 and x,xxx mg/kg bw

B. TEST PERFORMANCE

The Chemco Research Laboratory, New York conducted the study during the period February to August 1994.

1. Treatment and sampling times

Sampling took place 24 h, 48 h after the single dose given was administered; the positive control was sampled only at 24 h.

2. Tissues and cells examined

Bone marrow; 2,000 polychromatic erythrocytes (PCEs) examined per animal (two scorers each evaluated 1,000 PCEs); the number of normochromatic erythrocytes (NCEs, more mature RBCs) per 1,000 PCEs was noted.

3. Details of slide preparation

At 24, 48 or 72 h after administration of the test material or vehicle control, or 24 h after administration of the positive control, the appropriate groups of animals were sacrificed. Bone marrow cells were collected from the femurs and cell smears were prepared and stained according to conventional cytological procedures.

Coded slides were scored for the presence of micronuclei in 2,000 PCEs (1,000 PCEs by each of two scorers) per animal. The ratio of PCEs to NCEs was also recorded for each animal, as an indication of cytotoxicity to the target tissue.

4. Evaluation Criteria

To determine whether a statistically significant response in MN PCE frequency was treatment related, the following criteria were applied:

(a) whether or not there were dose- and time-dependent effects that were consistent with a treatment-induced response and

(b) the degree of the response in relation to both concurrent and historical negative/vehicle and positive control data.

5. Statistical methods

Each individual test animal was the unit used for analysis of micronucleated PCE frequency, PCE/total erythrocyte ratio and body weight change. Micronucleated PCE frequencies observed for each animal were transformed to the square root prior to analysis - PCE / total erythrocyte ratios were not transformed. A Dunnett's test (one-sided) was used for comparison of treatment group and positive control values with vehicle control values - a critical value of $p \leq 0.05$ was used to determine statistical significance.

II. RESULTS AND DISCUSSION

A. RANGE-FINDING TEST

Five males and 5 females were dosed at 1,000 mg/kg bw, and 10 males and 10 females at x,xxx mg/kg bw. Animals were sacrificed at 72 hours. There were no mortalities from administration of chemx at these dose levels.

B. MICRONUCLEUS ASSAY:

1. Toxicity

No deaths occurred. Clinical signs of toxicity (hyperactivity) were observed in one male mouse at 2500 mg/kg bw on days 1-2 after dosing and in another animal from the x,xxx mg/kg bw group on day 1.

2. PCE ratio

There was no evidence of target cell cytotoxicity (a significant decrease in the PCE/total erythrocyte ratio) at any dose level or sacrifice time (see Table IIA 5.4.4-1).

3. Micronucleated polychromatic erythrocytes (MPCEs)

There was a significantly ($p \leq 0.01$) elevated incidence of MPCEs in mid-dose (2,500 mg/kg bw) females which were sacrificed at 24 hours (Table IIA 5.4.4-2). However, the incidence (2.7/1,000 PCEs) was within historical control data limits (0.0 - 7.4/1000 PCEs (Table IIA 5.4.4-3). The statistical significance of the effect may also have been a result of a relatively low concurrent (24 h) vehicle control value (1.0/1,000 PCEs; 48-hr control value: 1.3/1,000; 72-hr control value: 1.6/1,000). Furthermore, there was no evidence of an effect in females dosed at x,xxx mg/kg bw following sacrifice at 24 h (1.4/1,000 PCEs). It was therefore concluded that this was a sporadic event.

III. CONCLUSIONS

Chemx does not induce MPCEs in mice at doses up to the limit dose x,xxx mg/kg bw (oral gavage).

Table IIA 5.4.4-1: Summary of micronucleus results in male and female mice (PCE ratio data)

			Mean PCE/total erythrocyte ratio ± standard deviation				
Harvest time (h)	Sex	Number of animals	Vehicle Control #	Low dose 1,250 mg/kg bw	Mid dose 2,500 mg/kg bw	High dose x,xxx mg/kg bw	Positive Control #
24	male	5	0.26 ± 0.07	0.34 ± 0.10	0.30 ± 0.07	0.28 ± 0.06	0.37 ± 0.12
24	female	5	0.35 ± 0.06	0.40 ± 0.11	0.41 ± 0.09	0.43 ± 0.09	0.44 ± 0.03
48	male	5	0.28 ± 0.06	0.39 ± 0.08	0.28 ± 0.12	0.29 ± 0.09	
48	female	5	0.37 ± 0.05	0.46 ± 0.06	0.45 ± 0.09	0.43 ± 0.05	
72	male	5	0.36 ± 0.05	0.31 ± 0.08	0.37 ± 0.03	0.33 ± 0.11	
72	female	5	0.50 ± 0.08	0.44 ± 0.09	0.43 ± 0.06	0.51 ± 0.06	

* $p \leq 0.05$; ** $p \leq 0.01$ by one-sided Dunnett's test.
# Vehicle control, 1,2,3-trioctanoylglycerol (10 mL/kg body weight); positive control, cyclophosphamide (40 mg/kg bw)

Table IIA 5.4.4-2: Summary of micronucleus results in male and female mice (micronucleus data)

			Mean micronucleated PCE/1,000 PCE ± standard deviation				
Harvest time (h)	Sex	Number of animals	Vehicle Control #	Low dose 1,250 mg/kg bw	Mid dose 2,500 mg/kg bw	High dose x,xxx mg/kg bw	Positive Control #
24	male	5	0.6 ± 0.4	1.5 ± 1.9	1.5 ± 1.0	1.3 ± 1.2	13.1 ± 5.1**
24	female	5	1.0 ± 0.8	1.5 ± 0.6	2.7 ± 0.8**	1.4 ± 0.4	8.8 ± 3.0**
48	male	5	0.8 ± 0.4	0.7 ± 0.6	0.7 ± 0.3	1.5 ± 0.6	
48	female	5	1.3 ± 1.0	2.5 ± 1.4	1.8 ± 0.8	1.7 ± 1.7	
72	male	5	1.5 ± 1.1	1.7 ± 1.2	1.5 ± 0.4	1.6 ± 1.0	
72	female	5	1.6 ± 1.0	2.1 ± 0.8	1.4 ± 1.8	2.0 ± 1.5	

* $p \leq 0.05$; ** $p \leq 0.01$ by one-sided Dunnett's test.
# Vehicle control, 1,2,3-trioctanoylglycerol (10 mL/kg body weight); positive control, cyclophosphamide (40 mg/kg bw)

Table IIA 5.4.4-3: Historical corn oil historical control data (10 mL/kg) for CD-1 mice *

Males Mn (PCE) / 1,000 PCEs				
Time Point (h)	Number of animals	Mean ± standard deviation	Range of means	Standard deviation as a % of mean
Males Mn (PCE) / 1,000 PCEs				
48	70	1.200 ± 1.431	0.20 - 2.40	130.3% ± 55.5%
72	45	1.022 ± 1.469	0.20 - 2.20	143.8% ± 51.5%
Combined	180	0.954 ± 1.303	0.00 - 2.40	140.0% ± 55.4%
Females Mn (PCE) / 1,000 PCEs				
48	50	1.880 ± 4.074	0.20 - 7.40	132.5% ± 61.5%
72	45	1.356 ± 1.569	0.20 - 2.40	108.8% ± 53.7%
Combined	180	1.421 ± 2.676	0.00 - 7.40	123.1% ± 54.1%

Vehicle control data from 13 studies (male) and 10 studies (female).

(Smith A 1995b)

Report: IIA 5.4.4/02 Smith, A., 1996, Mouse bone marrow micronucleus assay of ^{14}C chemx, Report No.: CCC-14612

Guidelines: OECD 474 ≅ EPA FIFRA Guideline § 84-2 ≅ EEC B 12

GLP

GLP compliant [17] with the following exceptions - the test and control materials (solvent and positive control) were not fully characterized; the stability of control substances was not determined (mixtures of test substance with carrier were prepared on each day of use); the homogeneity of the test substance in the carrier was not confirmed

Executive Summary

In a bone marrow micronucleus assay using CD-1 mice, chemx (unlabelled 98.5% purity, chem2 ring labelled 98 % radiochemical purity) was administered by oral gavage to 4 male animals at x,xxx mg/kg bw. A further 2 male animals were administered vehicle (corn oil) only. Bone marrow cells were harvested at 2 and 8 hours post-treatment.

Bone marrow and blood plasma samples were processed by means of liquid scintillation counting (LSC), and radioactivity was measured as disintegrations per minute (DPM). The concentration of parent compound (in terms of 1g equivalents/g of tissue) was determined for blood plasma and for bone marrow at 2 and 8 hours after dosing. DPM values measured were corrected for background on the basis of the results for concurrent controls.

Exposure of bone marrow cells to chemx and / or its metabolites was confirmed by observation of radioactivity in bone marrow after administration of ^{14}C-radiolabeled chemx in corn oil at approximately x,xxx mg/kg.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Chemx - chem2 ring labelled: ^{14}C in the C-3 position (label 11 chemx); specific activity 11.23 mCi/mmol;

   Description: White powder
   Lot/Batch #: NPD-9307-5385-T
   Purity: radiochemical purity ≥ 98 %; unlabelled chemx 98.5 % [18]
   CAS #: 16335-17-2
   Stability of test compound: The test material was stable for at least 7 days at room temperature. Unlabelled chemx was stored at room temperature, ^{14}C chemx was stored in a frozen state.

2. **Vehicle:** corn oil

3. **Test animals -**
   Species: Mouse
   Strain: Charles River CD-1
   Age: 11 weeks old

Weight at dosing: weight range 31.0 – 45.2 g at study initiation.
Source: Charles River Laboratories, Portage, MI
Acclimation period: 10 days
Number of animals per dose: 4 male animals (x,xxx mg/kg bw group); 2 male animals (test vehicle)
Housing: Stainless steel, 1-2 cage (before treatment); polycarbonate cages with bedding material (after dosage)
Animal husbandry: The animals were properly maintained.

Environmental conditions -
Temperature: 17.5 - 21° C
Humidity: 40 - 70 %
Air changes: 16 - 20 changes/h
Photoperiod: Alternating 12-hour light and dark cycles

4. Test compound concentrations: Solutions/suspensions were prepared on the day of use, in corn oil, mixing radiolabelled chemx with non-radiolabelled test compound. The total amount of radioactivity administered per animal was approximately 1 0 µCi/mouse. The dose of chemx administered was x,xxx mg/kg bw administered (oral gavage). The stability of the test solutions was not reported.

B. TEST PERFORMANCE

The Chemco Research Laboratory, New York conducted the study during November 1995.

1. Treatment and sampling times

The test vehicle (corn oil - 10 mL/kg) was administered orally to two animals, one of which was sacrificed after 2 h, the second animal after 8 h. The test material (x,xxx mg/kg bw) was administered by the oral route to four animals, two of which was sacrificed after 2 h, the remaining two after 8 h.

2. Pharmacokinetic analyses

Bone marrow and blood plasma samples were processed by means of liquid scintillation counting (LSC), and radioactivity was measured as disintegrations per minute (DPM). The concentration of parent compound (in terms of µg equivalents/g of tissue) was determined for blood plasma and bone marrow at 2 and 8 hours after dosing. DPMs in test animals were corrected for background on the basis of the results for concurrent controls.

II. RESULTS AND DISCUSSION

Results obtained, which are summarized in Table IIA 5.4.4-4 are expressed in terms of chemx equivalents - calculated on the basis of the specific activity of the administered chemx. Bone marrow samples, extracted from femurs, were pooled for each animal prior to liquid scintillation counting and replicate samples of blood plasma were analysed.

Table IIA 5.4.4-4: Radioactive Distribution of chemx

Animal Number	Dose (mg/kg bw)	Time of Sacrifice (hr)	Chemx in Tissue (μg equivalents/g tissue)		
			Bone Marrow	Blood Plasma	
95106 M01 001	x,xxx	2	272	1213	1213
95106 M01 003	x,xxx	2	557	1384	1359
95106 M01 002	x,xxx	8	25	383	376
95106 M01 004	x,xxx	8	32	269	268

III. CONCLUSIONS

The presence of substantial radioactivity in bone marrow following oral administration of x,xxx mg/kg bw is evidence that the test material and/or its metabolites reach that organ. This is evidence then that the bone marrow cells in the mouse micronucleus assay were exposed to chemx and/or its metabolites. Since the mouse micronucleus assay was negative (there was no induction of cytogenetic damage, which would have been expressed as an increased incidence of micronucleated polychromatic erythrocytes), this is reasonably conclusive evidence that chemx is not a clastogen in this type of assay.

(Smith A 1996c