NRA Special Review of

Metham Sodium, Dazomet and Methylisothiocyanate (MITC)

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Preface

The NRA report on the Special Review of Metham Sodium, Dazomet and Methylsiothiocyanate (MITC) is published as a three volume set. The contents of each volume is as follows;

Volume one

- is a record of the special review, including a regulatory history of metham sodium and recommendations for use of metham sodium and dazomet containing products in Australia.

Volume two

- provides the summary reports of the assessment of toxicological data for metham, dazomet and MITC, including a summary of comparative toxicology of the three compounds. It also contains the occupational health and safety (OH&S) risk assessment of metham (soil fumigant use) dazomet and MITC and provides recommendations for use of dazomet and soil fumigant use of metham. This volume also includes an OH&S risk assessment of root inhibitor use of metham and recommendations for use of metham as a root inhibitor.

Volume three

- contains the full reports of the toxicological assessments for Metham-Sodium, Dazomet and MITC.

FOREWORD

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals. One of the NRA's regulatory responsibilities is to conduct reviews of registered agricultural and veterinary chemicals to ensure that they continue to do the job that they are supposed to do and that they do not pose unacceptable risks to people, the environment or trade.

The Special Review Program examines urgent or specific concerns about a currently registered agricultural or veterinary chemical, which may require a rapid resolution. It addresses one or more specific aspects of a given chemical, and can be triggered, for example, by the findings of new research, the availability of new scientific data or concerns raised about the use or safety of a chemical.

In undertaking reviews, the NRA works in close cooperation with advisory agencies including the Department of Health and Family Services (Chemicals and Non-Prescription Drug Branch), Environment Australia (Risk Assessment Branch), Worksafe Australia (Chemical Assessment Division) and State Departments of Agriculture.

The NRA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. When the NRA decides that a review is to be conducted, it consults parties affected by the review (such as applicants, commodity groups, State regulatory agencies) and gives them an opportunity to respond to concerns raised and participate in the review. All participants are notified of the Board's decision and outcomes of special reviews are published in the NRA's Agricultural and Veterinary Chemicals Gazette.

This review report provides an overview of the review that has been conducted by the NRA and advisory agencies. The review findings are based on information collected from a variety of sources, including data packages and information submitted by registrants, information submitted by members of the public, and government organisations and literature searches.

The NRA also makes these reports available to the regulatory agencies of other countries which are part of the OECD ad hoc exchange program. Under this program, it has been agreed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

The information and technical data required by the NRA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken. Details of required data are outlined in various NRA publications.

Other publications explaining the NRA's requirements for registration can also be purchased or obtained by contacting the NRA. Among these are: Ag Manual: The Requirements for Agricultural Chemicals; Vet Manual: The Requirements Manual for Veterinary Chemicals and Volume II of Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products.

The NRA welcomes comments on this review and its review program. They can be addressed to Manager, Chemical Review, National Registration Authority for Agricultural and Veterinary Chemicals, PO Box E240 Kingston ACT 2604 Australia.

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Volume 3

METHAM (AND ITS SODIUM SALT, METHAM-SODIUM)

SUMMARY

Introduction

Metham (and its sodium salt, metham-sodium) is a dithiocarbamate soil fumigant with fungicide, nematocide, herbicide and insecticide activity. Its activity is due to its rapid decomposition in soil to methylisothiocyanate (MITC). This decomposition is a chemical rather than a biological process. There are several end use products (EUPs) containing metham-sodium registered for use in Australia. They are used for a range of crops, including ornamentals, food and fibre crops and tobacco. Some of the EUPs are used as inhibitors of root growth in sewer lines. Metham is in Schedule 6 of the SUSDP. The Department agreed to clearance of metham sources in August, 1991. No ADI has been set.

Metham-sodium has been placed on the National Registration Authority's Ad Hoc Review Program following a report of adverse effects to an Victorian orchardist who experienced severe eye irritation and nausea when using metham-sodium to fumigate soil. This evaluation consolidates all available toxicological data which have been submitted by a range of companies between 1986 and 1991.

Metabolism and Toxicokinetics

MITC is likely to be the main metabolite of orally-administered metham, although no data were presented to support this. This supposition is based on the fact that both metham and dazomet are broken down in soil to MITC, and that after oral administration of [^{14}C] dazomet to rats, a volatile compound, presumably MITC, accounted for the majority of radioactivity in hepatic portal vein plasma.

Methyl and other alkyl isothiocyanates are excreted in the urine of rats as mercapturic acid derivatives following oral administration of metham-sodium.

Acute Toxicity

Metham-sodium has moderately high acute oral and moderate dermal toxicity, with lowest oral LD_{50} values of 50 mg/kg (mouse), 100 mg/kg (cat), and 450 mg/kg (rat), and lowest dermal LD_{50} values of 650 mg/kg (rat) and 800 mg/kg (rabbit). Acute inhalation toxicity was low in rats with an LC_{50} >4700 mg/m^3.

Metham-sodium (37% aqueous solution) produced severe skin irritation to rats and rabbits and the technical material was corrosive to rabbit skin. As a 3% aqueous solution, metham-sodium produced slight skin irritation in rats and rabbits. Metham-sodium (as a 3% or 37% aqueous solution) was a moderate eye irritant to rabbits. It was a moderate to strong skin sensitiser in guinea pigs.

Short Term Repeat Dose Studies

No short term, repeat dose studies with metham-sodium are available.

Subchronic Toxicity

In an inhalation study in rats, doses of up to 160 mg/m^3 metham-sodium for 6 h/day for 5 d/week for 13 weeks did not result in mortalities, but decreased food consumption, bodyweight, serum albumin concentration and increased relative liver weights were seen at the highest dose level. No effects were seen at doses up to 45 mg/m^3.

Chronic Toxicity

No chronic studies on metham-sodium are available. However, 2-year carcinogenicity studies with the metabolite, MITC, administered in drinking water, have been conducted. MITC was not carcinogenic in mice at doses up to 27 mg/kg/d or in rats at doses up to 1.6 mg/kg/d (males) or 2.7 mg/kg/d (females).

Reproductive Toxicity

The only available data from reproductive studies are from a published paper in which metham-sodium was given orally to rats at a dose of 1/20 LD_{50} prior to implantation and during pregnancy or for one month after parturition. No adverse effects were observed in the progeny of treated dams.

Developmental Toxicity

In a rat developmental toxicity study, maternotoxicity was noted at oral doses of 40 and 120 mg/kg/d, with decreased food consumption and weight gain. Foetotoxicity was noted at 40 and 120 mg/kg/d, with reduced foetal weights and reduced placental weights. There was a significant increase in skeletal variations at 120 mg/kg/d, possibly due to foetal immaturity, and foetal skeletal retardations at 40 and 120 mg/kg/d, corresponding to decreased foetal weights at these doses. There were 2 foetuses (out of 261 foetuses) from 1 litter at 120 mg/kg/d with meningocoele, a rare abnormality. In a range-finding study, this abnormality was seen in 12 foetuses from 7 litters at 240 mg/kg/d. Thus, meningocoele appears to occur in a dose-related manner in the rat, with an NOEL of 40 mg/kg/d. The overall NOEL was 10 mg/kg/d.

In a rabbit developmental toxicity study, maternotoxicity was seen after oral dosing at 100 mg/kg/d, and embryotoxicity was noted at 30 and 100 mg/kg/d po, and was

characterised by increased post-implantation loss at both doses. There was also a low incidence (2/48 foetuses (from 2 litters)) of rare abnormalities (spina bifida or meningocoele) at 100 mg/kg/d. These abnormalities were not observed at a dose of 200 mg/kg/d in a range-finding study. The NOEL was 10 mg/kg/d.

Genotoxicity

Metham-sodium was negative for genotoxicity in a range of *in vitro* and *in vivo* assays, both with and without metabolite activation, with the exception of a human lymphocyte *in vitro* assay, where chromosome damaging effects were noted both with and without metabolic activation. Chromosome aberrations were not induced in *in vivo* studies.

Human Studies

In a published paper, there was a report of 15 cases of contact dermatitis following the use of a 10% VAPAM formulation by an unspecified number of workers in an occupational setting. Skin irritation was moderate to severe. Subsequent exposure to lower concentrations of metham-sodium (down to 1.5% VAPAM) caused skin reactions suggesting sensitisation, although the non-irritating concentration of VAPAM was not determined for use in the challenge test.

The acute health effects of metham-sodium released in a railroad transport accident in northern California were described in a published paper. Reported health effects in residents of a nearby town included non-specific neurologic complaints (headache and dizziness) and irritation (eye, respiratory tract, gastrointestinal tract and skin), and were consistent with MITC exposure. Nausea was also commonly experienced. 14% of residents sought medical attention, but in nearly all cases, symptoms were not severe enough to warrant hospitalisation. Reliable air data for the first 2 days after the spill were not available, but modelling by these authors suggested that the highest air concentrations of MITC in the town were less than 160 ppb. Another published paper reported on the longer-term health effects following the spill. Persistent respiratory health complaints were identified in 48 patients (2.3% of the town's population). A futher paper described the risk assessment process used following the spill and the authors estimated that the range of MITC concentrations immediately after the spill was 140-1600 ppb.

DISCUSSION

The toxicology data package for metham-sodium is relatively limited. A proportion of the data submitted was from poorly conducted and/or documented studies, and detail of methodology was frequently lacking. Minimal toxicokinetic and metabolism data were submitted. Whilst it is known that metham breaks down in soil to MITC, there is no information on its conversion to MITC by metabolism in animal tissues, although this would appear to be likely. It is noteworthy that the related chemical, dazomet, which also breaks down in the soil to MITC, would appear to be largely absorbed (at least in rats) as MITC following oral administration.

No chronic or short term repeat dose studies were submitted and reproductive toxicology data were minimal. However, as human exposure to metham is associated

with systemic exposure to MITC, then these limitations in the data package for metham are overcome by the available data on the systemic toxicity of MITC and dazomet (see toxicological reports attached). For both of these latter compounds, data are available concerning short term repeat dose toxicity, subchronic toxicity, chronic toxicity, carcinogenicity and reproductive toxicity. Moreover, there was no evidence that these compounds had carcinogenic activity or affected reproductive performance. Target organs of metham toxicity were not clearly identified in the package of data on metham because of the lack of appropriate studies. Given the considerations discussed above, it is likely that appropriate studies would have identified the liver and red blood cells as targets of metham toxicity (given that these were identified as targets of dazomet and/or MITC toxicity). Indeed, the 13-week inhalation study of metham-sodium in rats suggested that the liver is a target organ (increased relative liver weights and decreased serum albumin concentration).

As noted above, metham-sodium is not stable when present in its EUP form and it rapidly breaks down to MITC, a highly volatile chemical. Because of this instability and because MITC is phytotoxic, especially to young seedlings, it has, in the past, been considered questionable whether exposure of the human population to metham (or MITC) via food residues is an issue. As is the case for MITC, no ADI has been set as PACSC recommendations were that an ADI is not relevant. Nevertheless, it is noted that the MRL Standard (ref. 26) does contain MRLs for metham.

In an occupational setting, systemic exposure to metham is possible via the dermal route. There are no data to indicate the extent of percutaneous absorption of the chemical or the extent of dermal metabolism. The most serious acute effects of metham appear to be severe to moderate skin and eye irritation and strong skin sensitisation both in humans and in laboratory animals. From the chemistry of metham-sodium, occupational exposure is most likely to the volatile breakdown product, MITC, rather than to metham itself. The effects of such exposure to MITC would be expected to be comparable to (or possibly more serious than) direct exposure to metham, because MITC is a severe skin and eye irritant and causes skin sensitisation. Additionally, it should be noted that MITC has a local irritant effect on the lung following inhalational exposure. Furthermore, inhalational or dermal absorption of volatile MITC may result in systemic exposure, although no data are available on the extent of absorption of MITC by these routes. By-stander exposure, as well as occupational exposure, to MITC may occur following the use of metham-sodium, because of the volatility of MITC. The risk associated with such exposure is difficult to quantify because of lack of relevant data, in particular ambient concentrations of MITC following the use of metham-sodium (which may vary considerably depending on the weather conditions at the time of use). This issue should be addressed by WSA using exposure modelling.

As noted above, such exposure could result in eye, skin and lung irritation (because of the high irritancy of MITC) and possible systemic exposure to MITC following dermal or inhalational absorption, depending on the a number of factors such as the amount applied, the method of application, weather conditions, proximity to the site of application. It should be noted that the orchardist who lodged the complaint that led to the review of metham-sodium noted that his mother, who was inside the house at the time that he was applying metham-sodium, detected the vapour. The distance that the house was from the site of application was not stated precisely ("at some distance away"). The woman apparently did not suffer any adverse effects.

Of note are the rare abnormalities detected in both rat and rabbit developmental studies. Meningocoele was observed in 2/261 (0.77%) rat foetuses at an oral dose of 120 mg/kg/d (main study) and in 12/291 (4.12%) foetuses at 240 mg/kg/d (range-finding study). In rabbits, at an oral dose of 100 mg/kg/d (main study), 1/48 foetuses (2.08%) had spina bifida and 1/48 foetuses had meningocoele, but no such abnormalities were detected at a dose of 200 mg/kg/d in a range-finding study conducted under identical conditions to the main study. It would seem reasonable to conclude that meningocoele appears to occur in a dose-related manner in rats, whilst there is less evidence for a clear effect of metham on abnormalities in rabbits. The doses at which these abnormalities occurred were around the threshold for maternotoxicity, and the effects would seem most likely to be related to the retardation of ossification of foetal skeletons (including those of the skull) associated with maternotoxicity. Such abnormalities were not observed in the developmental studies conducted with either MITC or dazomet, but the doses used in the studies for these two chemicals were lower than those used in the metham studies because of embryolethal effects. The highest doses of MITC used in the developmental studies were 25 mg/kg/d in rats and 10 mg/kg/d in rabbits, while the highest doses of dazomet were 30 mg/kg/d in rats and 75 mg/kg/d in rabbits (note that doses on a molar basis are still greater for metham; molecular weights for MITC, metham and dazomet are approximately 73, 107 and 162, respectively). A retardation of ossification of foetal skeletons was observed in the MITC studies, as well as the metham studies. It is unclear why metham appears to be less embryolethal than dazomet and MITC.

Metham-sodium was negative for genotoxicity, except in the human lymphocyte *in vitro* assay, where clastogenic effects were noted (both in the presence (20 - 40 µg/mL) and absence (20 µg/mL) of metabolic activation). It is noted that MITC also showed evidence of clastogenicity (in an *in vitro* chromosome aberration test in the Chinese hamster cell line V79).

DRAFT RECOMMENDATIONS

1. The lowest NOEL was 10 mg/kg/d po from the rat and rabbit developmental studies. The establishment of an ADI is not warranted given the lack of potential to form food residues.

2. The scheduling of metham sodium in schedule 6 would appear appropriate.

3. First Aid & Safety Directions**:**

   The current SDs for Metham are:

Metham-sodium	AC EC LD all strengths	120 130 131 132 133 161 162 163 164 180 210 211 220 222 279 280 285 290 292 294 299 300 303 330 332 340 342 350 360 361 364 365 366

   The avilable products are a soil fumigants (423 g/L), liquid soil fumigants (423 g/L), and foaming root fumigants (228 g/L). 423 g/L metham corrsponds to 510 g/L metham-sodium. On the basis that the active is the only ingredient which would contribute significantly to the toxicological profile of these products, and assuming a concentration of 510 g/L metham-sodium or less, the following SDs appear to be appropriate, based on hazard. It will be noted that there are no SDs related to PPE listed, as these will be supplied by Worksafe.

Amendments

   Metham-sodium AC EC LD all strengths - amend entry to read:

Metham-sodium	AE EC LD 510 g/L/kg or less	129 131 133 207 162 163 164 180 220 222 223 309 330 331 332 342 340 341 342 343 340 341 343 **...** **...** **...** PPE from Worksafe) **.....** **...** **...** **...** **...** **...** **...** **...** **...**

   Prescription of SDs for metham-sodium in fumigant form needs to be considered further (hence 309?). It is noted that a conservative approach to eye irritancy has been taken and thus SDs for a severe eye irritation on the basis that although the eye irritation is said to be moderate for a 37% solution, the skin irritancy is severe in the same concentration. It seems somewhat unusual for a product to be less damaging to the eyes than to the skin.

   No change to the existing First Aid Instructions for metham-sodium is recommended.

4.. WSA should consider by-stander exposure to gaseous MITC following the use of metham-sodium.

SUMMARY OF TOXICOLOGICAL HAZARD

(for TGAC, unless otherwise specified)

Date of Preparation:	May 1995
Chemical name:	Metham
Worst oral LD_{50} in rats:	450 mg/kg
Worst oral LD_{50} in other species:	50 mg/kg in mice
Worst dermal LD_{50}:	650 mg/kg in rats
Worst inhalation LC_{50}:	>4700 mg/m^3 in rats
Skin irritation:	severe in rats, rabbits (37% aqueous) slight in rats, rabbits (3% aqueous) technical material corrosive in rabbits
Eye irritation:	moderate in rabbits (3 and 37% aqueous)
Skin sensitisation:	moderate to strong in guinea pigs
Remarks:	Contact dermatitis observed in humans exposed to a 10% VAPAM (metham-sodium) solution
T-value:	20
NOEL:	10 mg/kg/d po in rat and rabbit developmental studies

METHAM

1. INTRODUCTION

1.1 Regulatory history of public health considerations

Metham (and its sodium salt, metham-sodium) is a dithiocarbamate soil fungicide, nematocide and herbicide with a fumigant action, and is in Schedule 6 of the SUSDP.

Metham-sodium has been considered on a number of occasions by PACC/PACSC. On the first two occasions, July, 1971 and February, 1974, issues of residues were considered. In 1971, metham-sodium was considered a substance which should not residues of any significance remaining in or upon food. In 1974 there was a reorganisation of the residues classification and it was then that dazomet and methylisothiocyanate were considered as substances which are exempted from the requirements of a maximum residue limit, whilst metham-sodium was given a maximum residue limit of 0.1 ppm (a value which is at or about the limit of determination) for berry fruits and vegetables.

In 1986, the committee received a TGAC submission from Stauffer Chemical Company. The committee noted that the toxicological data base was very limited as it consisted only of acute oral, dermal and inhalational toxicity data, ocular and dermal irritation data and data from a subchronic inhalation study in rats. However, the committee considered that, in view of the limited use of the compound and that residues would be below the limit of detection, further information was not required at that stage, but that any extension of use would require further data. In February, 1987, the committee noted an additional acute toxicity study that had been received from Stauffer Chemical Company, but also noted that additional studies were underway and that the reports of these studies should be requested. In February, 1988, the committee again noted that reports of recently completed studies (eg teratology and mutagenicity studies) should be requested. In August, 1991, the committee examined the toxicology data submitted and considered that agreement could be given to the clearance of the TGAC for metham-sodium from a number of sources (ICI, UCB, Stauffer, Hardie Trading Company, Rohm & Haas, Kemcon, Mineral and Chemical Traders and Micro Bros).

In June, 1991 DPSSC considered an evaluation of toxicological data supplied by various companies (see below). The committee decided that clearance of metham-sodium from a scheduling aspect could not be supported in the absence of chronic studies and a reproduction study. The committee also noted the observation of a rare developmental defect (meningocoele) in both species tested, and the severe eye and skin irritancy and sensitisation potential of metham-sodium. The companies were requested to provide any information relevant to a reconsideration. A number of companies indicated that they had studies in progress.

However, at the August 1991 meeting, the committee decided that there should be no objection to TGAC clearance from a scheduling aspect. It noted that chronic studies for MITC had been evaluated previously and were clear of carcinogenicity. Thus the major concern with metham-sodium remained the irritancy and sensitisation, and it was noted that these had been adequately dealt with by FASDs. The committee also noted that negotiations were underway with companies to remove inappropriate applications from the label. It decided that the existing scheduling was appropriate.

Metham-sodium was placed on the NRA's Ad Hoc Review Program after consideration at the 17th meeting of the Interagency Co-ordination Committee (ICC). This was following receipt of correspondence from an orchardist from Shepparton, Victoria, who experienced severe eye irritation and nausea when applying metham-sodium to fumigate a small nursery site. The FASD are to be reviewed by Worksafe, following review of toxicology data by Environmental Health and Safety Branch. It is noted that complaints following the use of metham-sodium have been made previously and in August, 1991, the DPSSC considered information provided by Victoria on the irrigation use of metham, the former Advisory Committee on Agricultural Chemicals (ACAC) consideration of its use and comments from the municipal council involved with the complaint. The committee considered that spraying, irrigation and flood irrigation were not appropriate application methods and ACAC agreed in principle with this.

The method of application of VAPAM by the Victorian fruitgrower whose correspondence initiated the review was by hand held boom from a motorised spray unit. The spray was then incorporated into the soil by a tractor mounted rotary hoe and the soil was sealed with black plastic.

This evaluation consolidates the information provided and evaluated in a number of previous submissions to the Department. Details of submissions are shown in **Appendix I.**

1.2 International Status

In 1994, the US EPA proposed to restrict use classification and labelling of metham-sodium as they consider it "a difficult product to use and requires special training" (Pesticide and Toxic Chemical News, April, 1994). The US EPA announced, on 21 September, 1994, its intent to cancel some registrations for Vaporooter (a Foaming Fumigant), Foam Coat Vaporooter and Sanafoam Vaporooter II (Chemical Regulation Reporter, February, 1995). The agency said that it had determined that a restricted use classification for metham-sodium to control the growth of roots in sewer lines was required "due to the elaborate and complicated methods of applying this chemical and the potential for harmful human exposure".

Objections to the EPA's intent to cancel the above registrations and a request for a hearing on the objections have been filed by Airrigation Engineering Co. Inc. A prehearing conference was set by the US EPA for 28 March 1995. Under a settlement between EPA and the company, it was determined that the use of metham-sodium to control tree roots in sewer lines will be restricted to certified applicators after 1 March , 1996 (Chemical Regulation Reporter, February 16, 1996).

1.3 Chemical Identity

Common name:	metham (present as the sodium salt)
Chemical name:	methyldithiocarbamic acid, sodium salt (IUPAC), sodium methyldithiocarbamate (C.A.)
CAS No:	144-5-54-7 (metham) 137-42-8 (metham-sodium anhydrous) 6734-80-1 (metham-sodium dihydrate)
Empirical formula:	$C_2H_5NS_2$ (metham) $C_2H_4NS_2Na$ (metham-sodium anhydrous) $C_2H_4NS_2Na.2H_2O$ (metham-sodium dihydrate)
Structural formula:	**S** **CH_3-NH-C-SH** (metham) **S** **CH_3-NH-C-SNa** (metham-sodium anhydrous) **S** **CH_3-NH-C-SNa.2H_2O** (metham-sodium dihydrate)
Molecular weight:	107.2 (metham) 129.18 (metham-sodium anhydrous) 165.21 (metham-sodium dihydrate)
Chemical class:	Dithiocarbamate

1.4 Chemical and Physical Properties

Metham-sodium forms a colourless crystalline dihydrate. The TGAC is generally a greenish-yellow liquid with a sulphide odour. Metham-sodium has appreciable solubility in water (722 g/L at 20^{o}C), is moderately soluble in ethanol and methanol, but is only practically insoluble in most other organic solvents.
Metham-sodium is stable in concentrated aqueous solution, but unstable when diluted. The following data on stability of metham-sodium were reported by Myers and Johnson (1985): solutions exposed to sunlight - DT_{50} (time to 50% loss) = 1.6 h (pH 7, 25^{o}C); on hydrolysis (25^{o}C), DT_{50} = 23.8 h (pH 5), 180 h (pH 7) and 45.6 h (pH 9).

Studies were conducted on the products formed upon hydrolysis and photolysis of metham-sodium in buffered aqueous solutions (Chang *et al.*, 1985). The major products of **hydrolysis** at pH 5 were carbon disulfide, MITC (about 43% on a molar basis) and methylamine. Minor hydrolytic degradation products included elemental sulfur and 1,3-dimethylthiourea. The major products of **photolysis** at pH 7 were identified as MITC (about 26% on a molar basis), N-methylthioformamide, methylamine and elemental sulfur. Minor photolytic degradation products included N-methylformamide, carbon disulfide, carbon oxide sulfide and hydrogen sulfide.

1.5 End Use Products

The following companies have TGAC approval for metham-sodium:

Nufarm Ltd (manufacturer - Nufarm Ltd, Western Australia)
ICI Crop Care (manufacturer - ICI Americas Inc, USA) and
UCB Chemicals (manufacturer - UCB S.A., Belgium).

2. METABOLISM AND TOXICOKINETICS

2.1 Rat

Methyl and other alkyl isothiocyanates are excreted in the urine of rats as mercapturic acid derivatives following oral administration of metham-sodium (Mennicke et al 1983). Nesterova (1970) reported that rats given 450 mg metham-sodium exhaled carbon disulfite (disulfide?) for the first 48 h after administration.

MITC is likely to be the main metabolite of orally-administered metham, although no data were presented to support this. This supposition is based on the fact that both metham and dazomet are broken down in soil to MITC, and that after oral administration of [^{14}C]dazomet to rats, a volatile compound, presumably MITC, accounted for the majority of radioactivity in hepatic portal vein plasma.

2.2 Soil

Methylisothiocyanate (MITC) is the major product formed from the hydrolysis and photolysis of metham. Metham-sodium is quickly broken down after application to soil. This is a chemical rather than a biological process. In soil, the time for 50% loss has been quoted in the Pesticides Manual to range from 23 min to 4 d. The rate of breakdown increases with the organic matter and clay content of the soil (Garban et al (undated)). These authors state that the time for decomposition can vary from 1 h in moist loam to 5 h in sand. Metham-sodium is non-volatile, but MITC is a volatile compound with a vapour pressure of 2.13 kPa (25^{o}C) (ref. 25). MITC is rapidly dissipated from soil, mainly due to volatilisation, but also degradation.

Smelt and Leistra (1974) examined the conversion of metham-sodium to MITC in soil using 120 mL flasks three quarters filled with loose, moist soil to which an aqueous solution of metham-sodium (20-50 mg) was added. Concentrations of MITC were measured in the vapour phase at intervals of a few hours. The conversion in soil of metham-sodium took only a few hours. The highest conversion rates were found in loamy soils. At 12^{o}C, conversion was nearly complete by 3 h in loamy soils and by nearly 6 h in humic sandy soil. With the latter soil at 21^{o}C, a period of 3 to 4 h was sufficient for complete conversion. The calculated amounts of MITC formed represented a large proportion of that which could theoretically be produced.

Turner and Corden (1963) reported that in a sandy loam soil, over 75% of the VAPAM applied was accounted for as MITC. High temperature and low moisture were reported to increase the rate of VAPAM decomposition. Increasing the soil temperature from 10^{o}C to 40^{o}C decreased the time required for maximum VAPAM decomposition from 7 to 2.5 h. Decreasing the moisture content from 20 to 6% decreased the time required from 7 to 2.5 h. This is because the production of MITC from VAPAM is primarily an oxidative process and conditions which favour aeration of VAPAM in the soil (such as low soil moisture) increase decomposition of VAPAM to MITC. Similarly, an increased liquid-air interface associated with smaller soil particles, increases decomposition of VAPAM to MITC. The disappearance of MITC from soil is also greatly accelerated at high temperature, but the rate of MITC loss was not greatly affected by soil moisture. The methodology used in the studies was chromatographic analysis of gas samples of the atmosphere above treated soil in sealed containers.

A field dissipation study was conducted in California (Kearny Agricultural Centre, 1986a). Metham-sodium, as VAPAM, was applied to a sandy loam soil via a sprinkler irrigation system at the maximum label rate (145 kg/acre). Soil samples were taken at various depths up to 6 feet at various intervals of time (0, 4, 8 and 12 h and up to 49 days after application). Half-life was 1.2 days at 0 - 0.5 ft and at 0 - 1 ft depths. A field dissipation study was also conducted in Washington in 1985 (Kearny Agricultural Centre(1986b)). Metham-sodium, as VAPAM, was applied to a sandy loam soil via a centre pivot sprinkler irrigation system at rates of 72 and 145 kg/acre. Half-life of metham-sodium in soil varied from 2.0 to 4.1 d, depending on the depth at which measurements were made.

Stauffer Chemical Company also submitted a study examining the metabolism of [^{14}C]metham-sodium in a sandy soil under both anaerobic (Burnett 1987a)and aerobic conditions (Burnett 1987b). Half-lives were determined to be 23 min at 28^{o}C under both aerobic and anaerobic conditions. Studies on degradates were conducted using a system developed to optimise the recovery of radiolabelled volatiles. About 65% (under anaerobic conditions) and about 80% (under aerobic conditions) of the radiolabel was recovered as a volatile degradate which was identified as MITC.

3. ACUTE TOXICITY

3.1 Lethal Dose Studies

A summary of acute toxicity studies with metham-sodium is shown in Table 1 below:

Table 1. Acute lethal dose findings for metham-sodium (technical)

Route	Species	Sex	LD_{50} (mg/kg)	Submission	Ref
PO	Mouse	M/F	46.5	89	Nesterova (1969)
PO	Mouse	M/F	285	196	Agchem Handbook(1986)*
PO	Mouse	M/F	50	650	not stated
PO	Rat	M/F	450	89	Nesterova (1969)
PO	Rat	M/F	820	196, 650, 869	Agchem Handbook(1986)* Ben-Dyke et al (1970)
PO	Rat	F	1700	320	not available
		M	1800	320	not available
PO	Rat	F	1428	1814	not available
		M	1294	1814	not available
PO	Cat	M/F	100	89	Nesterova (1969)
PO	Guinea pig	M/F	81	650	not stated
PO	Rabbit	M/F	320	650	not stated
Dermal	Rat	M/F	646.6	89	Nesterova (1969)
Dermal	Rabbit	M/F	800	196, 650, 869	Agchem Handbook(1986)* Ben-Dyke et al (1970)

Dermal	Rabbit	M/F	1300	320	not available
Dermal	Rabbit[a]	M/F	1012	1814	not available
Inhaln	Rat	M/F	> 4700[b]	320	not available

a. 24 h exp; b: LC_{50} (mg/m^3)

* The data presented in submission 196 were published data for metham-sodium from The Agrochemicals Handbook (1986), as it was stated in the submission that the original acute toxicity studies are unavailable. The data were from the 1986 edition of The Agrochemicals Handbook and differ from the data in the 1991 (third) edition.

Ben-Dyke *et al*. (1970) (submission 869) was a compilation of acute oral and dermal LD_{50} values from experimental results of the Toxicology Laboratory, Chesterfield Park Research Station (Fisons Limited Agrochemical Division), when available, or from published literature and manufacturers' bulletins. The data were from studies on standard commercial formulations where possible, otherwise on technical grade material. The source of the data for the LD_{50} values quoted for metham-sodium in submission 650 was not given.

No details of the LD_{50} studies reported in Nesterova (1969) (submission 89) were given. It was stated in Nesterova (1969) that symptoms after oral dosing in mice, rats and rabbits (no LD_{50} value given for rabbits) included reduced motor activity, tremor and muscular fibrillation and incoordination. Doses above the LD_{50} produced spasmodic twitching of the limbs. Profuse salivation was the dominant symptom in cats.

In submission no. 320, it was reported that the major toxic signs following **acute oral administration** in the rat were depression, salivation, lacrimation, reddened ears and feet, and involuntary shaking and convulsions. Necropsy findings were lung and GIT haemorrhage. Survivors had prominent adhesions of abdominal organs to the abdominal wall, particularly the stomach. Toxic signs following **acute dermal exposure** in the rabbit were depression and diarrhoea. Local toxic effects were marked, and included necrosis, severe oedema and infection. Necropsy findings included eroded mucosa in the aglandular area of the stomach with clots, pyloric sphincter thickening, bloated stomach and pale kidneys and liver. In the **acute inhalation** toxicity study, 10 rats/sex were exposed to 4.7 mg/L of technical material in fine aerosol for 4 h. One male died on day 2, but all survivors appeared normal by day 3. Dyspnoea was observed in male rats only, but depression and blood-like stains around the face were seen in both sexes. 4/10 males had small foci on their lungs after the 14-day observation period. Female rats had significant depression of bodyweight at days 3, 8 and 14.

In the studies presented in submission 1814, the major toxic signs following **acute oral administration** in the rat were depression, salivation, ptosis and lacrimation. Toxic signs following **acute dermal exposure** in the rabbit were depression and ataxia. Local toxic effects were severe erythema and oedema and darkened dose sites.

3.2 Dermal and ocular irritancy

A summary of studies on the dermal and ocular irritancy of metham-sodium is shown in Table 2 below:

Table 2. Dermal and ocular irritancy of metham-sodium

Route	Species	Test material	Findings	Submission	Ref.
Skin irritation					
	Rat	37% aqueous	Severe irritation	89	Nesterova (1969)
	Rat	3% aqueous	Slight irritation	89	Nesterova (1969)
	Rabbit	37% aqueous	Severe irritation	89	Nesterova (1969)
	Rabbit	3% aqueous	Slight irritation	89	Nesterova (1969)
	Rabbit?	Technical	Irritant	196	Agrochem (1986)
	Rabbit	Technical	Corrosive	320	
	Rabbit	50% solution	Moderate-severe irritation	1814	
Eye irritation					
	Rabbit	37% aqueous	Moderate irritation	89	Nesterova (1969)
	Rabbit	3% aqueous	Moderate irritation	89	Nesterova (1969)
	Rabbit?	Technical	Irritant	196	Agrochem (1986
	Rabbit	0.1 mL EUP	Slight irritant	320	
	Rabbit	50% solution	Mild-moderate irritation	1814	

Nesterova (1969) (submission 89) reported that concentrated (37%) metham-sodium solutions applied to the skin of rats and rabbits caused hyperaemia, haemorrhagic crusts, cutaneous plication, erosion and ulceration. The inflammatory process persisted for 2-3 weeks in rats and 4-6 weeks in rabbits. Washing the site within one hour of application prevented the inflammation. Application of a 3% solution resulted in hyperaemia and skin dryness. The instillation of the above solutions into the conjunctival sac of rabbits produced lacrimation, conjunctival hyperaemia and slight miosis.

In a submission no. 196 it was stated that the original acute toxicity studies are unavailable published data for metham-sodium were provided from The Agrochemicals Handbook(1986).

In submission 320, it was stated that technical material was corrosive to skin at both intact and abraded sites of application. Skin was necrotic with surrounding oedema and skin colour was greenish black. Ocular administration of 0.1 mL of 'product' caused mild conjunctival reddening in 2/6 unwashed eyes at 24 h.

The **dermal irritation** study presented in submission 1814 involved a 4 h exposure under occlusive dressing. Moderate-severe oedema in both intact and abraded skin persisted for 72 h. By day 7, darkened dose sites, eschar formation and sloughing were present in all rabbits. In the **ocular irritation** study presented in submission 1814, mild-moderate conjunctival irritation was observed, but disappeared by day 2 in unwashed eyes, and by day 1 in eyes washed 20-30 s after application.

3.3 Acute inhalational toxicity of metham and MITC

No data were provided on the acute inhalational toxicity of metham-sodium, although submission 196 provided the following statement for metham-sodium from the Agrochemicals Handbook (1986): Inhalational toxicity - causes irritation of the mucous membranes. The following data on the acute inhalational toxicity of MITC are presented because exposure to MITC by the inhalational route following use of metham-sodium is possible. This is because metham can be rapidly broken down (photolysis and hydrolysis) to MITC which is a volatile chemical. The data are from the published paper by Nesterova (1969). The study was conducted in a chamber for dynamic inhalation poisoning and in vacuum desiccators. The metham-sodium solution was placed in a flask with wetted soil and the necessary MITC concentrations were produced by varying the amount of metham-sodium added to the soil and the rate of air supply into the chamber, and by heating the flask containing the metham-sodium. MITC concentrations in air were determined colourimetrically. Single inhalation exposure to MITC (4 h) produced the following results:

Concentration (mg/m³)	**Species**	**Observed Effects**
75-79	mice	Death of 80-100% of animals
11.3-27.9	mice	Irritation of upper respiratory tract mucosae
30-79.1	rats	Irritation of upper respiratory tract mucosae
8.3-9.3	rats	Decrease of WBC; increase in neutrophils
0.3-0.5	rats	Salivation and lacrimation
0.1-0.3	cats	Irritation of ocular mucosae

Acute inhalation toxicity data submitted with an application for clearance of MITC TGAC (submission no. 256; August, 1990) were from a study by Huntingdon Research Centre, UK (SHG 132/77372, July, 1977) and were as follows:

Inhalation LC_{50} in rats (1 h exposure) 1900 mg/m^3.

3.4 Dermal Sensitisation

3.4.1 Guinea Pig Maximisation Test

(Til & Keizer (1980) CIVV/TNO, The Netherlands. Report no R 658. September 1980. Submission nos. 649 and 869). GLP/QA: no.

The test material was a 510 g/L aqueous solution of metham-sodium. Male albino SPF guinea pigs (10 in the test group and 5 in the control group) received six intradermal injections (each 0.05 mL) as follows: 2 injections of Freund's adjuvant, 2 injections of a 10% dilution of the test material in water and 2 injections of a 10% dilution of the test material in water and Freund's adjuvant (1:1), one of each pair being given in each shoulder. A concentration of 10% was chosen for induction because this concentration caused moderate skin reactions. One week after the induction injections, topical induction was conducted on the same shaved areas as follows: patches of 2 x 4 cm filter paper spread with a 1.5% dilution of the test material in vaseline were placed on the shaved skin for 48 h and covered by an adhesive bandage. Controls were treated in the same manner for both intradermal and topical induction except that water replaced the test material.

All the animals (both test and control groups) were challenged 14 days after the topical induction using a non-irritating concentration (0.5% (w/w) in vaseline), a "small amount" of which was applied to a shaved area (2 x 2 cm) of the flank under an occlusive dressing. Skin reactions were measured 24 h after challenge. The challenge dose provoked well-defined to severe erythema in all (10) test animals, whereas only 1 of 5 control animals showed very slight erythema. It was concluded that metham-sodium exhibited severe sensitisation properties.

3.3.2 Modified Buehler Test

(Smutter (1987) tauffer Chemical Company, Richmond Toxicology Laboratory, Richmond, CA. Study no. T-12378. Final report - April, 1987 Submission no. 320). GLP/QA: yes.

VAPAM (containing 32.95% metham-sodium) was tested using a modified Buehler test in male Hartley strain guinea pigs, which included two range-finding primary irritation studies (one with metham-sodium and one with MITC) and a sensitisation study with metham-sodium.

In the first primary irritation study, a single application of a 30% solution of VAPAM caused delayed irritation (characterised by slight erythema (5/5 animals) and slight oedema (4/5 animals) at 72 h). A 10% solution of VAPAM required two applications (made at 3 day intervals) to clearly produce irritation (erythema and/or oedema responses were observed in 3/5 animals at 48 h). Single applications of 3% or 1% solutions did not cause irritation although a second application of the 3% solution produced irritation (erythema in 2/5 animals). A second application of the 1% solution did not produce erythema/oedema. The 1% solution was therefore chosen as the non irritating concentration for subsequent induction and challenge.

In the second primary irritation study, a single application of 0.5% MITC produced slight erythema in 4/10 animals. A 0.1% solution of MITC did not produce irritation and was therefore the concentration chosen for challenge in the sensitisation study.

The experimental design of the sensitisation study is shown below:

Group	Induction	First challenge	Second challenge	Third challenge
Test material	1% VAPAM	1% VAPAM; vehicle	1% VAPAM 0.1% VAPAM vehicle	1% VAPAM 0.1% MITC; vehicle
Negative control	Vehicle	1% VAPAM vehicle	1% VAPAM 0.1% VAPAM vehicle	1% VAPAM 0.1% MITC; vehicle
Positive control	0.1% DNCB*;	0.1% DNCB vehicle	0.1% DNCB vehicle	Not tested

* dinitrochlorobenzene

In the sensitisation study all solutions were applied topically for 6 hours per day under occlusion. Induction applications were made on alternate days for a total of 10 applications. Challenge applications were made on days 35-38, 42-45 and 49-52 after the first induction application. For challenges, each animal was concurrently tested with test solution and vehicle (water).

At the end of the three week induction phase some slight erythema was observed in 7/10 animals that received repeated 1% VAPAM application which may have been due to cumulative irritation and/or sensitisation.

After the first challenge, sensitisation reactions (persistent erythema and oedema of mild intensity) was observed in 5/10 test animals, 0/10 negative controls and 10/10 positive controls. Eschar responses were also noted in two test animals. There were no positive findings for vehicle controls from any of the test groups at any of the challenges. After the second challenge, sensitisation reactions (persistent erythema and/or oedema of mild intensity) were observed in 6/10 test animals challenged with 1% VAPAM, but in 0/10 animals challenged with 0.1% VAPAM (some transient reactions observed), suggesting that the sensitisation reactions are concentration dependant. 5/10 negative control animals showed sensitisation reactions at the 1% concentration and 2/10 at the 0.1% concentration. 9/10 positive controls showed sensitisation reactions. After the third challenge, 6/10 animals showed sensitisation reactions to 1% VAPAM (4 also having eschar responses), while 6/10 animals showed sensitisation reactions to 0.1% MITC. Thus, exposure to metham-sodium sensitised animals to both metham and MITC. 9/10 negative controls showed sensitisation reactions to 1% VAPAM and 3/10 to 0.1% MITC.

From the results of the first challenge, it can be concluded that metham-sodium is a sensitiser. It is interesting that a single exposure to metham-sodium of the negative control animals at the first challenge sensitised the animals.

The mechanism by which metham caused sensitisation to MITC is unknown. Metham-sodium may be metabolised by the skin and therefore act as the sensitising agent or alternatively, it could be an example of cross-reactivity (ie. the sensitisation responses represent cross reactions of the sensitised lymphocytes).

4. SHORT TERM REPEAT DOSE TOXICITY

No data submitted.

5. SUBCHRONIC TOXICITY

5.1 Rat

5.1.1 3-Month Inhalation Study

(Stauffer Chemical Company. Report T-11006. 31 August, 1983. Submission no. 320)

Rats were exposed to 0, 6.5, 45 or 160 mg/m^3 for 6 h/d for 5 d/week for 13 weeks. No mortalities were attributed to treatment, but a minimal decrease in body weights and food consumption occurred in both sexes at 160 mg/m^3. These rats also had depressed serum albumin concentrations and increased relative liver weights, but no overt morphological changes.

Mild nasal epithelial hyperplasia and lymphocytic rhinitis, and evidence of gastric erosion were found at the top exposure level.

5.1.2 4-Month inhalation study

(Nesterova, 1969)

The following data on the subchronic inhalational toxicity of MITC are presented because exposure to MITC by the inhalational route following use metham-sodium is possible (see above).

Repeated exposure of rats (strain, number and sex not specified) to MITC for 4 months (4 h/day) produced the following effects:

Concentration mg/m^3	**Species**	**Observed Effects**
	1.09-1.29	rats No visible signs of poisoning; Increase in SH groups in serum; Decrease in relative weight of lungs; Morphological changes to internal organs*
0.34-0.58	rats	Morphological changes (vascular disturbances in the lungs)

* vascular disturbances, emphysematous areas in the lungs, and infiltrates of plasma cells and lymphoid cells. Numerous bi- and trinucleate cells were found in hepatic parenchyma, as well as in the kidneys and myocardium.

No further details were provided.

5.1.3 13-Week Inhalation Study on MITC

(Schering Report 374/77. August, 1990. Submission no. 256)

Rats (10/sex/group) were exposed nose only to MITC for 4 h/d for 12-13 weeks at MITC atmospheric concentrations of 0, 1, 10 and 45 ppm (0, 3.16, 30.67 and 137 mg/m^3). There were no compound-related effects on the eyes and only the high-dose group exhibited clinical signs (apathy, increased salivation and nasal discharge and vocalisation). This group also had decreased food consumption and body weight gain. There were no changes in haematology, urinalysis, blood chemistry, necropsy or histology (performed on control and high-dose groups only). Lungs, trachea and bronchi were examined, but the nasal turbinates were not. The NOEL was 30.67 mg/m^3 based on clinical signs and decreased body weight.

5.2 Cat

5.1.1 4-Month inhalation study *(Nesterova, 1969)*

Repeated exposure of cats (number and sex not specified) to MITC for 4 months (4 h/day) produced the following effects:

Concentration mg/m³	**Species**	**Observed Effects**
0.06-0.14	cats	No visible signs of poisoning and no change in biochemical indices (considered to be a NOEL)

6. CHRONIC TOXICITY

No data submitted.

7. REPRODUCTIVE TOXICITY

7.1 Rat *(Nesterova, 1969).*

Metham-sodium was administered to rats (strain and number not stated) by gavage at a dose of 1/20 LD_{50} prior to impregnation (number of doses not stated) and throughout pregnancy or for one month after parturition. MITC was administered by inhalation at 1.1 mg/m^3/d for 50 days. It is unclear whether the animals received MITC concurrently with metham-sodium or were a separate group, presumably the former. The following observations were made on the progeny: number, vitality, mean weight and length (presumably at birth). Further observations were made (time unspecified) of height, weight, behaviour and appearance of coat, and age at eye opening was measured. The progeny of treated rats were stated not to differ significantly from the progeny of control animals. No actual data were presented.

8. DEVELOPMENTAL TOXICITY

8.1 Rat

8.1.1 Oral study

(Hellwig & Hidebrand (1987) BASF Corporation, Chemicals Division, USA . 87/0128. Final report - March, 1987. Study completed February, 1986.). GLP/QA: yes.

Metham-sodium was administered by gavage to female Wistar rats (25/group) as an aqueous solution (42.2%) at doses of 0 (vehicle - distilled water), 10, 40 and 120 mg/kg/d from days 6 through 15 post coitum. Dams were killed on day 20 post coitum, and fetuses removed by caesarean section. Fetus were examined macroscopically for external and internal abnormalities. Visceral microscopic examination was conducted on 1/3rd of fetuses and skeletal examination by staining and stereomicroscope was conducted on 2/3rds of fetuses.

A preliminary dose-range-finding study was conducted at doses of 0, 60, 120 and 240 mg/kg/d under study conditions identical to those of the main study. There were dose-dependent reductions in body weights in the treatment and post-treatment periods. Food consumption over the treatment period was also reduced in a dose-dependent manner. Fetal weights were reduced in a dose-dependent manner. At the high dose, 12 fetuses (out of a total of 291 fetuses (4.1% of foetuses)) from 7 litters (out of 24 litters (29.2% of litters)) showed meningocoele.

In the main study, there were no mortalities and no treatment-related clinical signs. Reduced food consumption, bodyweight and bodyweight gain during treatment and post-treatment periods were noted in animals dosed at 120 mg/kg. Reductions in food consumption and bodyweight gain were also seen in animals at 40 mg/kg during the treatment period. "Corrected" body weight gain (body weight on day 20 post coitus minus body weight on day 0 minus uterus weight) was also reduced at the high dose. Necropsy findings in dams were unremarkable and uterus weights were comparable for all groups. The mean numbers of live fetuses were comparable for all groups but postimplantation losses (resorptions plus dead fetuses divided by the number of implantations) were increased at the 10 mg/kg and 120 mg/kg doses, but there was no clear dose-relationship for this parameter. Decreased fetal and placental weights were seen at 120 mg/kg. At 40 mg/kg, decreased placental weights, and slightly (not significantly) decreased fetal weights were observed.

Two fetuses out of a total of 261 foetuses (0.77% of fetuses) in one litter (out of a total of 22 litters) from the 120 mg/kg group showed meningocoele. This a rare abnormality in the strain of rats used in this study; thus, historical control data from BASF Laboratories revealed that this malformation was not observed in 3646 untreated fetuses. Though the 2 fetuses with this abnormality in the current (main) study were from one litter, it would seem reasonable to conclude (after consideration of data from both the range-finding and main studies) that meningocoele appears to occur in a dose-related manner in rats. The NOEL for these malformations was 40 mg/kg/d.

There was a significant increase in skeletal variations in the 120 mg/kg group, mainly caused by a higher incidence of dumbbell-shaped thoracic vertebrae. This variation can be considered as a retardation and its increase was attributed to the immaturity of the foetuses from this group. There was an increase in the incidence of retardations (incomplete or missing ossification) in the 40 mg/kg and 120 mg/kg groups (about 91% cf. about 76% in the control), which correlated with the decreased fetalweights in these groups.

There was a significant increase in skeletal variations at 120 mg/kg, mainly caused by an increased incidence of dumbbell-shaped thoracic vertebrae, and this may be an indication of fetal immaturity. A significant increase in skeletal retardations was observed at 40 and 120 mg/kg. This correlates with the decrease in fetal weight at these doses, and may be due to treatment-related fetal immaturity.

The NOEL for maternal and embryotoxicity in this study was considered to be 10 mg/kg/d.

8.2 Rabbit

8.2.1 Oral teratology study

(Hellwig (1987) BASF Corporation, Chemicals Division, USA . 87/0255. Final report - July, 1987. Study completed - May, 1986). GLP/QA: yes.

Metham-sodium (42.2% aqueous solution) was administered to female Himalayan rabbits (15/group) by gavage at dose levels of 0 (vehicle - distilled water), 10, 30 and 100 mg/kg/d on day 6 through day 18 post insemination. The dams were killed on day 29 post insemination. Fetuses were examined macroscopically and organs were examined *in situ*. Sections were examined for the heart, kidneys and head. Skeletal examination was largely done by X-rays.

A preliminary dose-range-finding study was conducted at doses of 0, 50, 100 and 200 mg/kg/d under study conditions identical to those of the main study. Food consumption and body weight gains of the dams receiving 100 and 200 mg/kg were reduced. Uterus weights (including fetuses) were also reduced in these groups. Postimplantation loss was increased and the number of live fetuses was reduced, both in a dose-dependent manner, and reaching statistical significance at 100 and 200 mg/kg. There were no differences between the four groups with respect to incidences of fetal abnormalities or anomalies.

In the main study, there were no substance-related mortalities and no substance-related clinical signs. In dams of the 100 mg/kg group, slightly reduced food consumption was noted during the treatment period, and diminished bodyweight gain was noted during treatment and post-treatment periods. Animals displayed reduced uterus weights (including fetuses) and increased placental weights at this dose level. There were no dose-related differences between the groups with respect to "corrected" body weight gain (ie. body weight on day 29 post implantation minus body weight on day 0 minus weight of uterus). There was a marked increase in postimplantation loss, and consequently a reduced number of live fetuses, at 100 mg/kg. Necropsy findings for the dams were unremarkable. Mean fetal weights were comparable for all groups, although slightly higher at 100 mg/kg.

At 30 mg/kg, there was a slight (not significant) increase in postimplantation loss, and a slight decrease in the number of live fetuses. Macroscopic, visceral and skeletal examination of foetuses did not reveal any statistically-significant treatment-related increases in fetal anomalies, variations or retardations at any of the dose levels tested, but at 100 mg/kg, one fetus exhibited meningocoele and one exhibited spina bifida (which are rare abnormalities in the strain of rabbits used in this study) out of a total of 48 fetuses. This a rare abnormality in the strain of rabbits used in this study; thus, historical control data from BASF Laboratories revealed that these malformations were not observed in 1638 untreated foetuses. The fetuses affected in the current study were from two different litters (out of a total of 14 litters). The incidence was very low and no such abnormalities were seen at the higher dose of 200 mg/kg in the range-finding study.

Thus, a relationship between the abnormalities and treatment with metham-sodium appears to be unlikely in the rabbit, but cannot be ruled out. The incidence of skeletal retardations was reduced in the 30 mg/kg group, and particularly in the 100 mg/kg group, possibly due to reduction in number of live fetuses in these groups, resulting in more mature fetuses.

Under the conditions of this study, maternotoxicity was displayed at 100 mg/kg, and embryo/fetotoxicity was noted at dose levels of 30 and 100 mg/kg. The NOEL was 10 mg/kg/d.

9. GENOTOXICITY

9.1 Gene Mutation Assays

9.1.1 Bacterial Mutation (Ames) test

(Willems (1980) CIVV/TNO, The Netherlands. Report no R 6619. October 1980. Submission nos. 649, 869). GLP/QA: no.

Metham-sodium was tested in the Ames test (standard plate test) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 at five concentrations (plus the negative control) over the range 0-3000 g/plate. The concentrations refer to g of test liquid (metham-sodium 510 g/L) per plate. The concentration range used was based on a preliminary test (no details given). The following positive control compounds were used: sodium azide (strains TA 1535 and TA 100, without S9), hycanthone methanesulphonate (strains TA 1537, TA 1538 and TA 98, without S9) and 2-aminoanthracene (all strains with S9). Positive controls produced the expected results, except for strain TA 100 without S9, for which the positive control did not result in a doubling of the background frequency of revertants. Each concentration was assayed in triplicate. There was no increase in his^+ revertants at concentrations of up to 330 g/plate in any of the tester strains, either in the presence or absence of metabolic activation (S9 mix), and at higher concentrations, the test material was toxic to the bacteria, with the exception of strain TA 100 in the absence of activation. In the latter case, at 1000 g/plate, the number of revertants was increased, but to less than twice the control value, and the standard deviation was relatively high. For some strains, cytotoxicity was observed at 330 g/plate, as revealed by a less dense growth of the bacterial lawn.

A second experiment was conducted using the same experimental conditions as the first experiment, except that only three concentrations (plus negative and positive controls) were tested, with the highest concentration being 330 g/plate. Again, for some strains, there was evidence of cytotoxicity at the 330 g/plate concentration, but there was no increase in his^+ revertants at concentrations of up to 330 g/plate in any of the tester strains. There were several instances in which the positive control compounds did not double the frequency of revertants seen in the negative control (strain TA 100 without S9 and strains TA 1535, TA 1537 and TA 100 with S9).

It was concluded that under the conditions of this study, metham-sodium did not reveal any genotoxic activity.

9.1.2 Bacterial Mutation (Ames) test *(Moriya et al., 1983, submission no. 869).*

These workers conducted an Ames test using *Salmonella typhimurium* strains TA 100, TA 98, TA 1535, TA 1537 and TA 1538 and *Escherichia coli* strain WP2 *hcr,* on 228 pesticides. The sodium salt of metham was not tested, but the ammonium salt of metham was found to be negative in the assays. It is of interest, however, that some other dithiocarbamates tested positive. Thus, 7 out of 13 of the dithiocarbamate compounds tested were found to be direct-acting mutagens for the base-change-type strains, especially TA 100. However, all of these pesticides have a common moiety: $(CH_3)_2NCSS$-, whereas metham only has one CH_3 group, the other being simply an H. It seems that the dimethyl moiety is essential for the mutagenicity because other dithiocarbamates lacking this moiety, such as the ethylenebis-type dithiocarbamates, were also non-mutagenic.

9.1.3 Bacterial Mutation (Ames) test

(Engelhardt (1987a) BASF Aktiengeselleschaft, Department of Toxicology, Germany. Report 87/0208. Study completed, February, 1987. Final report - March, 1987. Submission no. 838). GLP/QA: yes

Metham-sodium was tested for genotoxicity in the Ames test (standard plate test and preincubation test) both in the presence and absence of S9 mix. The test compound was a 42.2% aqueous solution which was diluted in distilled water. *Salmonella typhimurium* strains TA1535, TA100, TA92, TA1537, TA1538 and TA98 were used. Positive control compounds were 2-aminoanthracene in the presence of S9 mix (for all strains except TA92), and in the absence of S9 mix, N-methyl-N'-nitro-N-nitrosoguanidine for strains TA100 and TA1535, 4-nitro-o-phenylendiamine for strains TA98 and TA1538, 9-aminoacridine for strain TA1537 and mitomycin for strain TA92. Assays were done using 3 plates/test concentration. Two experiments were conducted using the standard plate test over concentration ranges of 0 (solvent control) - 5000 µg/plate and 0-2500 µg/plate. Two experiments were conducted using the preincubation test over concentration ranges of 0-2500 µg/plate (exp. 1) and, in exp. 2, 0-2500 µg/plate in the presence of S9 mix, or 0-300 or 0-1000 µg/plate in the absence of S9 mix, depending on the strain.

A bacteriotoxic effect (reduced his- background growth, decreased number of his^+ mutants) was observed, but depended on the strain and test conditions. In the standard plate test, bacteriotoxicity was observed from about 500 µg/plate upward for TA92, from about 1000 µg/plate for TA1535, from about 1500 µg/plate upward for TA1538 and TA100, and at 2000 µg/plate for TA1537 and TA98. In the preincubation test, bacteriotoxicity was observed from about 100 - 500 µg/plate upward (for all tester strains in the absence of S9 mix, and for TA1535 and TA100 with S9 mix) or at concentrations of 2500 µg/plate (remaining strains in the presence of S9 mix).

Positive control compounds showed the expected responses. The test compound did not result in any increase in numbers of revertants for any strain in any of the experiments and it was concluded that metham-sodium was not genotoxic under the conditions of this study.

9.1.4 Chinese hamster ovary cells - HGPRT locus

(Engelhardt (1987b) BASF Aktiengeselleschaft, Department of Toxicology, Germany. Report 87/0280. Study completed - May, 1987. Final report - August, 1987 Submission no. 838). GLP/QA: yes.

The genotoxic potential of metham-sodium was investigated on the HGPRT locus of CHO cells over the concentration range 0-0.01 mg/mL, both in the presence and absence of S9 mix. Preliminary range-finding cytotoxicity tests were performed to determine the effect of the test compound on cell survival (as measured by relative cloning efficiencies). Decreased cloning efficiencies (measured at 24 h post exposure) were observed in the main experiments. These were particularly marked at the highest concentration, but some decrease in cloning efficiency also occurred at 0.001 mg/mL.

Ethylmethanesulfonate (absence of S9) and 3-methylcholanthrene (MCA, presence of S9) were used as positive controls. The experimental procedure was as follows: pretreatment of cells in HAT medium for 7 d, seed out 10^5 cells/flask (3-5 flasks per concentration) allowing 24 h for attachment, expose the cells to the test agent for 4 h, allow 8 d for expression (with subculturing), and select mutants with medium containing 6-thioguanine. The assay was carried out three times at the same eight concentrations, as the cloning efficiency in the first experiment was low (51.5% in the negative control in the absence of activation and 43.75% in the presence of activation, for measurements at transition into selection medium) and in the second experiment, the positive control, MCA, resulted in a lower mutant yield than was normally achieved in this particular laboratory. Some concentrations gave an increased number of mutants in these experiments, but these generally appeared to be singular events and no clear dose-response relationship was observed in any of the three experiments, either in the presence or absence of metabolic activation. However, at the highest concentration (0.01 mg/mL) a slight mutagenic response was noted in the presence of activation, mainly in the first and third experiments. Under the conditions of this study, metham-sodium was observed to be genotoxic, although only at cytotoxic concentrations.

9.1.5 Published data on metham-sodium

(Gentile et al., 1982, submission no. 89)

In this paper, commercial and technical grades of 10 insecticides were evaluated for genotoxicity by three different tests: (i) the Ames test (*Salmonella typhimurium* histidine reversion assay; standard plate test), (ii) *Saccharomyces cerevisiae* forward mutation assay, and (iii) gene mutation assay at the *waxy* (*wx*) locus in *Zea mays in situ*.

The assays were conducted both in the presence and absence of mammalian metabolic activation (S9 mix) and a plant metabolic activation system (IS fraction, a 1000x*g* supernatant of *Z. mays* homogenate (roots, stems and leaves of three-leaf stage plants).

Strains used in the Ames test were TA1535, TA1537, TA1538 and TA100. Positive controls were 2-nitrofluorene (TA1538 and TA98), sodium azide (TA1535 and TA100), 9-aminoacridine (TA1537) and aflatoxin B_1 (TA100 in the presence of S9) and results were discarded unless expected results were obtained. The assays were conducted in triplicate and a minimum of 3 plates was used for each strain in each experiment. Metham-sodium (both commercial and technical grade; exact concentrations used were not stated) either in the absence of activation or in the presence of mammalian or plant metabolic activation systems, gave negative results (any increase in number of revertants per plate was less than twice negative control values, and non significant test (Katz, 1979) and no dose-response) in this assay.

In the yeast assay, *S. cerevisiae* strain D4 was used and gene conversion at the *ade 2-1/ade 2-2* and *trp 5-12/trp 5-27* loci was monitored using N-methyl-nitro-N-nitrosoguanidine as positive control in the absence of S9 mix and aflatoxin B_1 in the presence of S9 mix. Log-phase yeast cells were incubated for 30 min at 30^oC in the presence of test compound, with or without S9 mix or IS fraction. After washing, the cells were plated on minimal agar supplemented with either 0.1% adenine sulphate or 0.1% L-tryptophan. Colonies were scored after 4 d incubation (5-6 d for colonies on minimal media only). Gene conversion frequencies were calculated by dividing the number of convertants by the number of viable cells on minimal plates. Metham-sodium (both commercial and technical grade; exact concentrations used were not stated) either in the absence of activation or in the presence of mammalian or plant metabolic activation systems, gave negative results (any increase in number of convertants per 10^5 survivors was less than twice negative control values, non significant test (Katz, 1978) and no dose-response) in this assay.

The plants used for the maize assay were inbred W22 homozygous for the *wx*-C allele. The *waxy (wx*) locus controls the synthesis of amylase. Pollen grains containing the recessive allele (*wx*) stain tan with an iodine stain. When a reverse mutation has occurred, pollen grains contain the dominant allele starchy (*Wx*) and stain black. Commercial-grade metham-sodium (technical grade chemical was not tested in this assay) was applied to test plots (which consisted of 5 seedlings) at the rate of 2.24 kg/ha prior to the emergence of the seedlings. Control plots did not receive the metham application. At early anthesis, pollen was collected, stained and the frequency of revertant grains was calculated by dividing the total number of *Wx* (black) grains by the estimated number of viable grains on the microscope slide (the estimated number of pollen grains analysed was 1.6×10^6). A test was considered positive if there was a doubling of the mutation rate, a significant *t* test and a significant test. Metham-sodium was negative in this test (by all three parameters).

9.2 Chromosomal Effects Assays

9.2.1 In vitro cytogenetic assay using human lymphocytes

(Gelbke & Englehardt (1987a) BASF Aktiengeselleschaft, Department of Toxicology, Germany. Report 87/0116. Final report - March, 1987. Submission no. 838). GLP/QA: yes.

Metham-sodium was tested for the ability to induce chromosomal aberrations in human lymphocytes following *in vitro* exposure in the presence and absence of S9 mix. Concentrations tested were 1, 5, 10 and 20 g/mL (without S9) and 10, 20 and 40 g/mL (with S9). These concentrations were based on a preliminary range-finding study, but few details were given. Selection of test concentrations was based on quality of the metaphases and not mitotic index, because at test substance concentrations causing a reduction in mitotic index, the chromosomes were so severely affected that their evaluation was not possible. Negative controls (untreated and solvent - distilled water) and positive controls (cyclophosphamide, in the presence of activation, and mitomycin, in the absence of activation) were also tested. Duplicate cultures were used for each experimental point. After incubation at 37^{o}C for 48 h, cultures were treated with test compound for 24 h in the absence of S9, or for 2 h in the presence of S9, followed by 22 h in the absence of test substance. Cells were arrested in metaphase 2-3 h prior to harvesting by the addition of colcemid. For each culture, 100 metaphases (50 for positive control) were analysed (except the 20 g/mL cultures in the absence of S9 for which only 50 metaphases were examined because of cytotoxicity).

The positive controls gave the expected results. Mitotic index was not decreased relative to negative controls at any concentration of metham-sodium tested. Dose-related increases in the number of aberrant metaphases, including and excluding gaps, were noted in the presence of the test compound, both with and without metabolic activation. In the absence of S9, metaphase aberrations were significantly increased at 20 g/mL. In the presence of S9, significant increases in metaphase aberrations were noted at 20 and 40 g/mL. There were no increases in the frequency of exchanges following treatment with the test substance, but significant increases in the frequency of exchanges were induced by the positive control compounds.

Under the conditions of this *in vitro* study using human lymphocytes, metham-sodium had a chromosome damaging (clastogenic) effect at 20 g/mL and above.

9.2.2 In vivo cytogenetic assay in Chinese hamsters: bone marrow chromosome analysis

(Gelbke & Engelhardt (1987b) BASF Aktiengeselleschaft, Department of Toxicology, Germany. Report 87/0238. Final report - June, 1987. Submission no. 838). GLP/QA: yes.

Chinese hamsters (5/sex/group) were given a single oral dose of 150, 300 or 600 mg/kg metham-sodium. These doses led to irregular respiration for about 1 - 2 h at about 30 min after administration of the test substance. The doses were selected on the basis of preliminary acute oral toxicity testing. Higher doses (1200 and 900 mg/kg) were initially tested, but resulted in deaths and difficulty in evaluation due to poor quality of the chromosomes and/or few cells found for analysis. Control animals received the solvent (aqua dest) only and cyclophosphamide (40 mg/kg PO) was administered to positive control animals. Animals were sacrificed and bone marrow preparations made from the two femora at 24 h after administration of the test substance. There were 2 additional groups at the 600 mg/kg dose, for sacrifice at 6 and 48 h time points. 100 Metaphases per animal were analysed.

There were no significant differences in the types or frequency of aberrations between the dose groups and solvent control group. No exchanges, multiple aberrant metaphases or disintegration of chromosome structure (pulverisations) were observed following treatment with the test compound, but these were observed for the positive control. A higher frequency (not analysed statistically) of polyploid cells was observed in smears from animals receiving 300 mg/kg and 600 mg/kg (6 and 24 h sacrifice) compared with the frequency observed for the solvent control (1.7 - 2.3% cf. 0.3%). Expected results were observed for the positive control animals.

Under the conditions of this study, metham-sodium did not have chromosome-damaging effects.

9.3 DNA Damage and Other Genotoxic Effects Assays

9.3.1 *Bacillus subtilis* rec-assay

(Hoorn (1987) Hazleton Biotechnologies, Holland. BASF Report 87/0163. Final report - March, 1987. Study completed - February, 1987. Submission no. 838). GLP/QA: yes.

Metham-sodium (42.2% aqueous solution) was examined for genotoxic activity in the recombination assay using *Bacillus subtilis* strains H17 (rec^+) and M45 (rec–) at concentrations of 0.1-150 L/plate, with and without metabolic activation (S9 mix). Solvent control (water) and positive control compounds (sterigmatocystin, in the presence of activation, and methylmethanesulfonate, in the absence of activation) were assayed concurrently with the test material. Three plates per concentration (for each strain) were tested. Any test in which a differential of 4 mm or more occurred was considered to be positive.

In the first test, both in the presence and absence of metabolic activation, concentrations of 25 µL/plate or greater, produced zones which were equal to the diameter of the plates, while 0.1 µL/plate did not produce any inhibition. Therefore only concentrations of 1, 5 and 10 µL/plate were useful for detecting DNA damage. In this test, neither positive control gave a positive result, although methylmethanesulfonate produced a 3 mm difference in inhibition zones. The test compound showed some activity, both in the presence and absence of metabolic activation, but it was below that required for defining a positive result and was not observed in a dose-dependent manner. The test was repeated, with similar results, although in this test, methylmethanesulfonate produced a positive result and the results of the test compound were weaker than in the first test. Because of the large inhibition seen at concentrations of »25 µL/plate, a third test was conducted using concentrations over the range 0.1 - 15 µL/plate. In this test, methylmethanesulfonate approached producing a positive result and sterigmatocystin produced a positive result. The test compound showed no activity in the presence of activation, and in the absence of activation, its activity was below that required for defining a positive result and did not occur in a dose-related manner.

Under the conditions of this study, metham-sodium did not display genotoxic activity to *Bacillus subtilis* organisms.

9.3.2 Unscheduled DNA synthesis in rat primary hepatocytes

(Cifone (1987) Hazleton Laboratories, USA. BASF Report 87/0240. Study completed April, 1987. Final report - July, 1987. Submission no. 838). GLP/QA: yes.

Primary rat hepatocytes were exposed to metham-sodium (42.2% aqueous solution) for 18 h at eight concentrations over the range 0.5 nL/mL to 250 nL/mL. In a preliminary concentration selection test conducted over the concentration range of 0.025 nL/mL to 1000 nL/mL, the test compound was found to be lethal at concentrations of 500 and 1000 nL/mL. At 100 nL/mL, survival was 17.3% of the negative control value and at 50 nL/mL, survival was 55.3%. The test material was soluble in the culture medium with 1% serum at all concentrations tested. 2-Acetyl aminofluorene was the positive control and gave the expected response. The viability of the hepatocytes prepared by perfusion and of the monolayer cultures was satisfactory. The data were obtained by autoradiography and the grain counts were expressed as the average net nuclear grain counts (nuclear counts minus cytoplasmic counts for a nuclear-sized area) for 150 cells. In the negative control, there were 1.71 grains/nucleus (6% of nuclei having 6 grains and no nuclei having 20 grains). No effects which indicated unscheduled DNA synthesis were observed at the concentrations of metham-sodium tested and no dose-related effects were seen. Under the conditions of this study, metham-sodium did not induce unscheduled DNA synthesis in primary rat hepatocytes.

10. HUMAN STUDIES

10.1 Occupational Exposure

(Wolff and Jung, 1970)

In a published paper from Western Germany, 15 cases of contact dermatitis were reported in an unspecified number of workers in potato production occupations following the use of a 10% VAPAM solution. Symptoms ranged from erythema, infiltration and itching of the skin, to severe irritation with burning redness, acute swelling and blistering of the skin. Two of the cases displayed punctiform cutaneous haemorrhages of a purpuric nature. The areas of the skin affected were exclusively those which had been in contact with the VAPAM solution or the vapours of the solution.

Once their contact dermatitis had subsided, the patients were tested for skin sensitisation effects. The metham-sodium solution (presumably VAPAM) was applied to the patients' skin at dilutions of 10, 5, 2.5 and 1.5% as a patch test. Acute positive cutaneous and epicutaneous reactions were seen for all strengths of the test material, and even exposure to a mist of the solution caused itching and burning to previously exposed individuals.

These results indicate that humans occupationally exposed to a 10% VAPAM solution suffered from moderate or severe skin irritation. They also appeared to display strong skin sensitisation reactions to the formulation at subsequent exposure, but the non-irritating concentration of VAPAM was not determined for use in the challenge test.

10.2 Accidental exposure

10.2.1 (Kreutzer et al., 1994)

Kreutzer *et al.* (1994) described the acute health effects of metham-sodium released in a railroad transport accident in northern California. Residents of the town of Dunsmuir (population 2129) were exposed to metham's volatile decomposition product, MITC, and 14% of residents sought medical attention (of course, others may have been affected, but did not seek medical attention). Reported health effects, including non-specific neurologic complaints (headache and dizziness) and irritation (eye, respiratory tract, gastrointestinal tract and skin), were consistent with MITC exposure. Nausea was also commonly experienced. In nearly all cases, symptoms were not severe enough to warrant hospitalisation. An unexpected finding was the continued reporting of health complaints one week after the spill or later, and the reasons for this are unclear. Symptoms experienced at this time were very similar to those experienced earlier. There were no adverse outcomes from 6 pregnancies. The environmental fate and transport model used by these authors suggested that the highest air concentrations of MITC in Dunsmuir were less than 160 ppb and may have occurred over 12 hours after the spill as a result of a rapid photochemical conversion of metham-sodium to MITC in sunlight. However, the model predictions were not able to be validated because reliable air data for the first 2 days after the spill were not available. In fact, it is not known with certainty which compounds were responsible for the observed health effects, although the toxicological data suggest that MITC is the most likely candidate.

10.2.2 (*Cone et al., 1994)*

Cone *et al.* (1994) reported on the longer-term health effects following the metham-sodium spill in Northern California. They reported that persistent respiratory health complaints were identified in 48 patients who had suffered initial symptoms of irritation over the few days following the spill. Persistent irritant-induced asthma was confirmed in 20 and persistent exacerbation of asthma in 10 of these 48 patients. The respiratory symptoms persisted for at least 3-14 months of follow-up.

10.2.3 *(Alexeeff et al., 1994)*

Alexeeff *et al*. (1994) described the risk assessment process used following the spill. Reference exposure levels (RELs) were developed and compared to exposure concentrations. The following RELs of MITC in air were determined by dividing the relevant NOELs by an uncertainty factor of 100: 0.5 ppb to prevent discomfort, 40 ppb to prevent permanent disability and 150 ppb to prevent life-threatening injury.

An estimated range of MITC concentrations immediately after the spill was 140-1600 ppb. Air monitoring data available beginning 3 days after the incident gave average levels of MITC of about 4-5 ppb and a range of 0.2 - 37 ppb.

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APPENDIX I:

Departmental Submissions: Metham sodium

submission no. 320	Stauffer Chemical Company (note that Stauffer Chemical Company was later acquired by ICI Pty Ltd) for TGAC clearance of metham-sodium (September, 1986) and supplementary data (February, 1988);
submission no. 1814	(company not stated) - supplementary data (February, 1987);
submission no.196	John James O'Brien (manufacturer, Kemcon Pty Ltd) for TGAC clearance (October, 1987 and February, 1991) - note that Kemcon was later acquired by Nufarm Ltd;
submission nos. 649	Hardie Trading Limited for TGAC clearance (January, 1991) and 869and data submitted in response to a request for toxicological data on metham-sodium from PACC (submission no. 649, July, 1988);
submission no. 650	Mirco Bros Pty Ltd for clearance of the TGAC manufactured by UCB Chemicals Pty Ltd (refer to submission no. 89, see below) (January, 1991) and data submitted in response to a request for toxicological data on metham-sodium from PACC (July, 1988);
submission no. 891	Rohm and Haas Australia Pty Ltd for clearance of the TGAC and the EUP, VAPAM (January, 1991) (application withdrawn);
submission no. 89	UCB Chemicals Pty Ltd for TGAC clearance (January, 1991);
submission no. 838	ICI Australia - data submitted in response to a request for toxicological data on metham-sodium from PACC (January, 1991);
submission no. 592	Mineral and Chemical Traders, also for clearance of the TGAC manufactured by UCB Chemicals (refer to submission no. 89, see above) (February, 1991); and
submission no. 9620	Chemark, Chemical and Marketing Services Pty Ltd; Foret SA; (contained only physico-chemical data).

Some of the original data were not available to the evaluator conducting the Ad Hoc Review. These included the LD_{50} values and skin and eye irritation data from submission nos. 320 and 1814 and the 3-month inhalation study in rats (submission no. 320).

List of Abbreviations/Acronyms

mg	Milligram
kg	Kilogram
mL	Millilitre
L	Litre
ng	Nanogram
µg	Microgram
m	Metre
d	Day
h	Hour
s	Second
iv	Intravenous
po	Oral
sc	Subcutaneous
id	Intradermal
ip	Intraperitoneal
im	Intramuscular
NOEL	No Observed Effect Level
ADI	Acceptable Daily Intake
MRL	Maximum Residue Limit
TGAC	Technical Grade Active Constituent
EUP	End Use Product
FASD	First Aid and Safety Directions
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
GLP	Good Laboratory Practice
QA	Quality Assurance
DPSSC	The former Drugs & Poisons Schedule Standing Committee of the NHMRC (National Health & Medical research Council)
NDPSC	Formerly the DPSSC, now the National Drugs & Poisons Schedule Committee of AHMAC (Australian Health Ministers' Advisory Council)
PACC	Pesticides and Agricultural Chemicals Committee of the NHMRC
PACSC	Pesticides and Agricultural Chemicals Standing Committee of the NHMRC

DAZOMET

SUMMARY

Introduction

Dazomet is a soil fumigant effective for the control of nematodes, insects, germinating weeds and soil fungi. Dazomet is strongly phytotoxic, acting by virtue of the chemical release of methylisothiocyanate (MITC). Dazomet is in schedule 6 of the SUSDP. The previous evaluator calculated an ADI of 0.005 mg/kg/d is based on a NOEL of 0.5 mg/kg (established in a 1-year dietary dog study and a 2-year dietary rat reproductive study) and a safety factor of 100. In the past, it has been considered questionable whether residues in food are an issue because dazomet breaks down in soil to MITC, which is highly volatile chemical. Also, MITC is phytotoxic, especially to young seedlings, and as the fumigant must be dissipated from the soil before a crop can be planted, no residues should appear in any crops. In the Australian MRL Standard, dazomet appears in Table 5 (Uses of substances where maximum residue limits are not necessary. Situations where residues do not or should not occur in foods or animal feeds;...). However, residue data submitted on dazomet for a variety of crops (submission number 7872), indicated that MITC may be a residue in crops following the use of dazomet.

Toxicokinetics and Metabolism

Results were provided on the absorption, distribution, metabolism and elimination in the rat following single oral dosing of [^{14}C]dazomet at levels of 10 and 100 mg/kg. One study was presented in full and the second study (which included the distribution data and involved dosing at the 10 mg/kg level) was presented in summary form only. [^{14}C] Dazomet was extensively absorbed from the GIT (>60% and possibly approaching 100%). Excretion was largely urinary, but a substantial amount of administered radiolabel was excreted in expired air. Analysis of portal vein plasma revealed very low concentrations of [^{14}C] dazomet, suggesting decomposition of dazomet during its passage across the gastrointestinal tract. Most of the radioactivity was detected as volatile compound, which was presumed (but not shown) to be MITC. Biliary excretion accounted for approximately 7% of radiolabel, while faecal excretion accounted for 2-3%, suggesting that enterohepatic recycling may occur. Following TLC analysis of radioactivity in urine, bile, liver and kidneys, a metabolic pathway was proposed. Repeated dosing did not alter the excretion or distribution of radioactivity. Tissue distribution studies revealed high concentrations of radioactivity in lungs, kidneys and liver, followed by ovaries, adrenals, thyroid and blood.

Acute Toxicity

Dazomet is of moderate acute oral toxicity. The oral LD_{50} values for dazomet from two different studies in rats were about 600 - 900 mg/kg for males and 400 - 550 mg/kg for females. The LD_{50} of dazomet, given subcutaneously to mice, was 248 mg/kg. The LD_{50} of dazomet, given subcutaneously to rats, was 470 and 550 mg/kg in males and females, respectively. The dermal LD_{50} of dazomet in rats was greater than 2000 mg/kg. Symptoms associated with acute dazomet toxicity were shaking, salivation, tonic convulsions, trembling, dyspnoea and lassitude.
In two studies, the introduction of 39 or 50 mg dazomet into the eye of rabbits caused slight irritation (moderate conjunctival erythema and slight oedema).

Results of two acute dermal irritation studies employing 50% aqueous preparations of dazomet in rabbits were reported. No irritation was observed in the study employing a 4 h exposure period. After a 20 h exposure period, moderate erythema and oedema were observed. Application of the EUP, Basamid Granular (2 g coated on a cottonwool carrier), to the rabbit ear for 20 h caused slight inflammation.

Skin sensitisation was not observed in two studies following the application of dazomet or Basamid Granular to the guinea pig. No justification was given for the doses/concentrations used in one of these studies and positive control compounds were not tested in these studies.

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Short-Term Repeat-Dose Studies

In a range-finding study, rats were given dazomet in the diet at 0, 200, 800 and 3200 ppm for 3 weeks. Pronounced signs of toxicity (deteriorated general state, piloerection, squatting posture, paralysis of forelegs, pareses of hindlegs, strutting gait etc.) were seen only at the two highest doses. At 800 and 3200 ppm, food consumption was dramatically reduced, with reduced bodyweight gain or loss of bodyweight.

In a preliminary study, rats were given dazomet in the diet at 0, 20, 60, 180 and 540 ppm for 4 weeks. Clinical signs of toxicity (motor disturbances including pareses of hindlegs, strutting gait, abnormal foreleg position) were seen in high-dose females. There was reduced food consumption and bodyweight gain in high-dose females (also a trend in males) and also in 180 ppm females over the first 2 weeks. Several clinical chemistry changes were noted at 60 ppm and above. Liver weights were increased, accompanied by intermediary- and centro-acinary fatty degeneration in the form of large fat droplets (180 and 540 ppm males, 540 ppm females). There were no compound-related effects at 20 ppm.

A 21-day (6 h/d) inhalation study was carried out with rats. The inhalation of dazomet (33 g/m^3 of air) dust by rats did not result in any observable signs of toxicity.

Two 21-day dermal studies were conducted in rabbits. In the first study, the dermal application of dazomet in carmellose at doses of 10 and 100 mg/kg/d (6 h/d) to abraded skin resulted in well-defined erythema and oedema. Cutaneous hardening and discolouration resembling chemical burns were seen in 8/10 and 10/10 animals receiving, respectively, 0.5% (10 mg/kg/d) and 5.0% (100 mg/kg/d) dazomet to a 10 cm^2 area. In the second study, the dermal application of dazomet (5 days/week, 6 h/d to unabraded skin) at doses of 10, 100 and 1000 mg/kg/d did not result in any dermal irritation. There were no significant effects on clinical chemistry parameters, body weight, organ weights or macroscopic or microscopic findings. The NOEL in this study was 1000 mg/kg/d.

Subchronic Toxicity

In a mouse 10-week dietary range-finding study (summary only, provided), 800 and 1200 ppm dazomet in the diet for 71 days led to a slight reduction in bodyweight in high-dose males and, at both doses, reduced haemoglobin (Hb), red blood cell (RBC) counts, haematocrit (Hct) and (in females) reduced mean corpuscular haemoglobin concentration (MCHC), and increased MCH (mean corpuscular haemoglobin) and MCV (mean corpuscular volume).
In a 3-month study, mice were given 20, 60, 180, 360 and 540 ppm dazomet in the diet (summary only, provided). There were no clinical signs for any of the dose levels tested. At 360 and 540 ppm, haematological changes, similar to those seen in the 10-week mouse study, were observed (reduced Hb, RBC counts and MCHC, and in males, Hct, and increased MCV, reticulocytes, polychromasia and anisocytosis). There was also a slight increase in splenic haemosiderin deposition. An increase in absolute and relative liver weight was noted in males at 180 ppm and above and in high-dose females. On the basis of this finding, the NOEL was considered to be 60 ppm (estimated compound intake of 9 mg/kg/d).

In a 3-month study, rats were given dazomet in the diet at 0, 20, 60, 180 and 360 ppm. At the high dose, there was a slightly reduced bodyweight gain in both sexes. Some changes in serum chemistry were observed at 180 and 360 ppm and at the high dose there was a decrease in Hb levels. Increased liver weights were seen at 60 ppm and above, with fatty degeneration of liver cells (males only at 60 ppm). On the basis of this finding, the NOEL was 60 ppm (about 4.6 mg/kg/d) in females and 20 ppm (about 1.8 mg/kg/d) in males.

In a 3-month study, beagle dogs were given dazomet in the diet at 0, 25, 100 and 400/200 ppm. The high dose was reduced to 200 ppm on day 23 because of vomiting, inappetence and large losses in bodyweight at 400 ppm. At 200 ppm there was still reduced bodyweight gain. Hb, RBC counts and Hct values were reduced at the high dose and there were some changes in clinical chemistry parameters. Relative liver weights were increased in the 200 ppm animals and the 100 ppm males, without any histopathological correlates. There was a slight increase in splenic haemosiderin deposition at the high dose. The NOEL was 25 ppm (0.8-1.0 mg/kg/d) for males and 100 ppm (3.1-4.0 mg/kg/d) for females, on the basis of liver weight changes.

Chronic Studies

In a 78 week study, mice were given dazomet in the diet at 0, 20, 80 and 320 ppm. Compound intakes were estimated as follows: males - 0, 4, 16 and 68 mg/kg/d; females - 0, 6, 22 and 93 mg/kg/d. Survival was not affected and there were no noteworthy clinical signs, or bodyweight or food consumption changes. There was a significant elevation of liver weight at the high dose and an increased number of mid-dose and high-dose animals with liver discolouration, liver masses and centrilobular lipid deposition. At the high dose, females showed a slightly increased incidence of hepatocellular adenomas (3, 0, 1 and 7 females, out of 50, in the control, low dose, mid dose and high dose groups, respectively) and a significantly increased incidence of basophilic foci. Increased splenic haemosiderin deposition and extramedullary haematopoiesis were noted at the mid dose (males) and high dose. Three/60 females from each dose group had malignant lymphoma at one or more sites; because of the low incidence, lack of a dose-response, and lack of any effect in males, it was not considered to be directly compound-related. The NOEL was 20 ppm (about 4 mg/kg/d in males, 6 mg/kg/d in females).

Three 2-year rat studies were submitted. In the first, rats were given dazomet in the diet at 0, 5, 20, 80 and 320 ppm. Mean compound intakes were: males - 0, 0.3, 1, 4 and 18 mg/kg/d; females - 0, 0.3, 1, 6 and 23 mg/kg/d. Survival was not affected. At the high dose there was a reduction in bodyweight gain. Target organs were the liver and red blood cells (at the high dose in males and mid and high doses in females). Liver effects included increased relative weights, hepatocellular fat deposition, vacuolation, reduced plasma proteins and triglycerides, while red cell effects included reduced cell counts, Hb and Hct values. There was no evidence of any oncogenic effect of dazomet. The NOEL was 20 ppm (about 1 mg/kg/d) for females and 80 ppm (about 4 mg/kg/d) for males.

In the second 2-year study, rats were given dazomet in the diet at 0, 5, 20 and 80 pp. Mean compound intakes were: males - 0, 0.3, 1 and 4 mg/kg/d; females - 0, 0.3, 1 and 6 mg/kg/d. Survival was not affected and there were no clinical signs or effects on bodyweight gain and food intake. At the high dose, there was a slightly increased incidence of diffuse hepatocellular fat deposition and vacuolation, and in females, a slightly increased incidence of mixed cell and basophilic cell foci. There was no evidence of an oncogenic effect of dazomet. The NOEL was 20 ppm (about 1 mg/kg/d).

The third 2-year rat study was an old (1960) study. Rats were given dazomet in the diet at 0, 10, 40, 160 and 640 ppm. Mean compound intakes were: males - 0, 0.4, 1.7, 6.4 and 28 mg/kg/d; females - 0, 0.5, 2.0, 7.4 and 31.8 mg/kg/d. Survival was not affected. At the highest two doses there was a reduction in food consumption. Bodyweight was reduced at the 640 ppm dose and in females, also at the 160 ppm dose. Target organs were the liver and kidney. Liver and kidney weights were increased at the highest dose. The main lesions were glomerular nephritis in the kidney and focal necrosis in the liver. There was no evidence of an oncogenic effect of dazomet. There was no clear NOEL in this study, with effects being observed at all doses.

Dazomet was administered in the diet (0, 15, 50 and 150 ppm) to beagle dogs for 12 months. The high dose of 150 ppm (about 4.8 mg/kg) was hepatotoxic, resulting in increased liver weight, decreased albumin, chronic hepatitis, cirrhosis, hepatocellular hypertrophy, hepatocellular fatty change and increased pigment deposition in Kupffer cells. The latter was also observed in females at the mid dose. The NOEL was 50 ppm (about 1.6 mg/kg/d) for males, 15 ppm (about 0.5 mg/kg/d) for females.

Reproduction Studies

Dazomet was fed to rats at 0, 5, 30 and 180 ppm for at least 70 days prior to mating, throughout mating and lactation, during production of F_1a and F_1b litters. Selected F_1a pups were maintained on compound-containing diets post-weaning to produce F_2 litters. Hepatotoxicity was observed in both generations, mainly at the high dose, but to some extent at the mid dose. Liver weights were increased and there was an increased severity of liver fatty change. Some serum enzyme and serum protein changes also indicated effects on the liver. There was no impairment of mating or reproductive performance and no adverse effect on reproductive organs or pup development. The NOEL with respect to reproductive function in rats was 180 ppm (about 18 mg/kg/d), while that for systemic toxicity was 5 ppm (about 0.5 mg/kg/d).

Developmental Toxicity

An oral (gavage) developmental study was conducted in rats at dazomet doses of 0, 3, 10 and 30 mg/kg/d. Food intake and body weight and also uterine weights were reduced at the high dose and to a lesser extent at the mid dose. There was a higher incidence of runts at 10 mg/kg and above, however, without a clear dose-response relationship. There was no evidence of teratogenic effects. The NOEL for maternal and foetal effects was 3 mg/kg/d.

Two oral (gavage) developmental studies were carried out in rabbits. In the first study (0, 25, 50 or 75 mg/kg/d), clinical signs such as severe diarrhoea, apathy and unsteady gait, as well as depressed food consumption and body weight, were seen in does at 50 and 75 mg/kg/d. The number of live foetuses was greatly reduced (by 80%) at 50 and 75 mg/kg/d. This effect corresponded to a high number of dead implantations. There was no evidence of treatment-related foetal abnormalities, but low numbers of foetuses at 50 and 75 mg/kg/d made this difficult to assess. The NOEL for both maternal and embryo toxicity was 25 mg/kg/d.

In the second study (0, 6.25, 12.50 or 25.00 mg/kg/d), embryotoxicity, expressed as increased dead implantations, in particular, increased early resorptions, resulting in reduced numbers of live foetuses, was seen at 25 mg/kg/d. There was no evidence of a teratogenic effect of dazomet. Maternal toxicity was not observed in this study (NOEL > 25 mg/kg/d). The NOEL for embryotoxicity was 12.5 mg/kg/d.

Genotoxicity Studies

A wide range of genotoxicity studies were conducted on dazomet including both *in vitro* and *in vivo* tests and covering gene mutation, chromosome effects and DNA damage assays. Some of these studies gave weakly positive results and some gave negative results.

Results from Ames tests were reported by five different groups. Tests were done using *S. typhimurium*, and in one case, also *E. coli WP2 hcr*; four of the studies were done both in the presence and absence of metabolic activation. None of these studies was positive, nor was a test using *Saccharomyces cerevisiae.* Negative results were also obtained in a host-mediated assay using mice and *S. typhimurium* G46. In contrast, in a gene mutation assay at the HGPRT locus in Chinese hamster ovary cells, dazomet induced small increases in mutation rate, both in the presence and absence of metabolic activation, although this increase did not appear to be concentration dependent in the presence of metabolic activation. A forward mutation assay at the TK locus in L5178Y mouse lymphoma cells produced equivocal results. In this assay, dazomet did not increase the mutation frequency when tested in the presence of metabolic activation, but in the absence of metabolic activation, an increase in mutation frequency (in the order of 2- to 3-fold) was observed in 2 out of 3 tests, but these increases were not concentration dependent.

Six chromosome effects assays were conducted, three *in vitro* and three *in vivo.* Dazomet was negative in four of these assays: a mouse micronucleus assay and chromosome aberration assays in human lymphocytes *in vitro,* and in rat bone marrow cells and Chinese hamster spermatogonia after dosing *in vivo.* In contrast, dazomet was positive in two assays, both being chromosome aberration assays in mouse lymphoma L5178Y cells *in vitro*, but these assays were conducted by two different laboratories. In one study (Stauffer Chemical Company), positive results were only observed in the absence of metabolic activation. However, reproducible, concentration-dependent increases in both structural and numerical aberrations were observed in two separate experiments by this laboratory. Endoreduplication, a rare numerical aberration, was observed at most concentrations of dazomet and translocations, triradials and quadriradial, which are rare structural aberrations, were observed at some concentrations. In the other study (Litton Bionetics, Inc.), significant increases in the numbers of cells with aberrations were observed both in the presence and absence of metabolic activation, but they were not clearly concentration dependent. However, again, some rare structural aberrations were observed (mainly in the absence of metabolic activation).

Dazomet was not found to be genotoxic in two *B. subtilis* rec assays which were both conducted in the presence and absence of metabolic activation.

Two laboratories conducted studies of the potential of dazomet for inducing SCEs. One study produced negative results (Stauffer Chemical Company). In the other study (Litton Bionetics, Inc.), in the absence of metabolic activation, there was a small, but significant, increase in the number of SCEs/chromosome at the highest concentration tested.

Two laboratories conducted studies of the potential of dazomet to induce unscheduled DNA synthesis in rat primary hepatocytes. One study (Hazleton Biotechnologies Company) was conducted with dosing *in vivo*, whereas the other study (Litton Bionetics, Inc.) was conducted *in vitro.* The *in vivo* study was negative, but positive results (weak, but reproducible increases in nuclear labelling) were obtained in the *in vitro* study.

Two laboratories conducted studies of the potential of dazomet to induce cell transformation in BALB/c-3T3 cells. In neither study did dazomet induce transformation.

Human Studies

A published article described case reports of contact dermatitis arising from exposure to dazomet.

DISCUSSION

The data package for dazomet is reasonably extensive, covering information on the impurities in the TGAC and data on metabolism (summary form only) and subchronic, chronic, reproductive and developmental toxicity.

Dazomet is degraded in the soil to MITC (a highly volatile chemical), which appears to be a chemical rather than a biological process. Because of this instability, and also the phytotoxicity of MITC, it is questionable whether exposure of the human population to dazomet (or MITC) via food residues is an issue. It would appear that following oral ingestion (at least in the rat), dazomet is rapidly degraded to MITC during its passage across the intestinal tract. It is not known to what extent dazomet is metabolised to MITC by the skin or lung epithelium.

High dietary doses of dazomet appeared to cause nervous system effects. Thus, doses of 540 to 800 ppm and above (in 3- and 4-week studies) in rats caused strutting gait, foreleg paralysis, and paresis of hindlegs in rats. Doses of 80 and 320 mg/kg in dogs caused high-stepping gait and forelimb weakness.

Dazomet has moderate to low acute oral, dermal and inhalational toxicity. It appears that the toxicity of dazomet is somewhat greater by the oral route than by the dermal and inhalational routes. Dazomet is only a slight dermal and ocular irritant. This is in contrast to MITC which is a severe local irritant. Unlike MITC, dazomet did not cause stomach lesions, which is probably a reflection of its lower local irritancy than that of MITC (dazomet at least must pass across the gut wall before it is metabolised to MITC, therefore following oral dosing with dazomet, all organs/tissues are exposed to MITC except the stomach mucosa). Nevertheless, dazomet might exert eye, skin and lung irritancy following conversion to MITC and volatilisation of the latter. Furthermore, systemic exposure to MITC might possibly occur, via the inhalational or dermal routes, following the use of dazomet.

Skin sensitisation tests in guinea pigs were negative, but it is not clear to what extent this may reflect inadequacy of the doses/concentrations tested/experimental conditions.

Haematological effects (predominately on red blood cells) were major toxicological effects of dazomet. Red blood cells appeared to be a target organ. Thus, in mice (10-week and 3-month dietary studies) and dogs (3-month dietary study) there was a reduction in red cell parameters (Hb, RBC count and Hct), with polychromasia and anisocytosis observed in the 10-week mouse study. These findings were accompanied by increases in splenic haemosiderin deposition. Also, extramedullary haematopoiesis was noted in a 78-week dietary mouse study, while deposition of iron-positive pigment was noted in the liver of dogs in a 1-year dietary study. It is unclear why the red blood cells appear to be a target of dazomet but not of MITC, although it is possible that this observation can be explained by the higher doses used in the dazomet studies than the MITC studies (which were possible to achieve because of the lower local irritancy of dazomet than MITC).

Another target organ for toxicity was the liver (which was also a target organ of MITC). Increases in absolute and relative liver weights were consistently seen in repeat-dose studies in mice, rats and dogs. For these three species the three-month studies gave the lowest doses (dietary) at which increased liver weights were observed and these doses were 180 and 540 ppm in male and female mice, respectively, 60 and 180 ppm for male and female rats and 100 and 200 ppm for male and female dogs. Fatty degeneration of hepatocytes, vacuolation and liver discolouration (rats), centrilobular lipid deposition (rats and mice), reduced total protein and albumin (rats and dogs), moderate-severe chronic hepatitis and cirrhosis (dogs) were also observed after long-term administration of dazomet. Hepatotoxicity was more marked in the dazomet studies than in the MITC studies, which is probably due to the higher doses used in the dazomet studies than the MITC studies.

In one of the two-year rat studies, there was also evidence for the kidney as the target organ, with the main lesion resembling glomerular nephritis.

Rat studies showed no clear evidence of any carcinogenic effect of dazomet. In mice, there was a slight increase in hepatocellular adenomas (not carcinomas) following 78 weeks of treatment at the high dose (320 ppm). There was also an increase in malignant lymphoma in females, but because of the low incidence, the lack of effect in males and the lack of any dose-response, it was not considered to be directly compound-related. The lack of a carcinogenic effect of dazomet is consistent with the data for MITC.

The lowest NOEL of about 0.5 mg/kg was established in a 1-year dietary dog study and in a 2-generation dietary rat reproductive toxicity study. This NOEL was based on deposition of iron-positive pigment in the livers of female dogs at the next highest dose (50 ppm or approx. 1.6 mg/kg/d). In rats, the lowest NOEL determined was in the 2-generation reproductive toxicity study, and was based upon reduced bodyweight gains and increased incidence and severity of liver fatty changes at the next highest dose (30 ppm, or about 3 mg/kg/d). However, in one of the 2-year chronic toxicity studies, an NOEL was not determined, with increases being observed in the incidence of histological lesions in the liver and the kidney at the lowest dose tested (40 ppm, ie. 0.4 - 0.5 mg/kg/d). The LOEL in the Mellon 2-year rat study (0.5 mg/kg/d) was the same as the NOEL from the dog study on which the current ADI is based.

Dazomet was not teratogenic at oral doses of up to 30 mg/kg in the rat and at oral doses of up to 25, 50 or 75 mg/kg in the rabbit (although at the latter two doses, there were not a large number of live foetuses for examination). This lack of evidence of a teratogenic effect of dazomet is consistent with the data for MITC.

An acceptable package of mutagenicity tests has been conducted covering all three end points. The results are the genotoxicity tests are not clear cut. While the majority of tests gave negative results, there were sufficient positive results to indicate some genotoxic potential of dazomet. In summary, there were positive results in one gene mutation assay (HGPRT locus in Chinese hamster ovary cells), equivocal results in another gene mutation assay (TK locus in mouse lymphoma L5178Y cells), and positive results in two chromosome aberration assays (both *in vitro* assays in mouse lymphoma L5178Y cells), in one *in vitro* assay for of unscheduled DNA synthesis in primary rat hepatocytes and in one *in vitro* assay of sister chromatid exchange. In all cases, the positive findings were relatively weak. There were no positive *in vivo* studies and there was a trend for results to only be positive (or to be stronger) in the absence of metabolic activation than in its presence. This suggests that unchanged dazomet has greater genotoxic potential than the metabolites of dazomet. The unscheduled DNA synthesis assay was the only assay which gave results suggesting that the metabolites of dazomet may have some genotoxic potential, even if only weak.

Although exposure of the human population to dazomet via food residues appears to be unlikely, an ADI has been previously set at 0.005 mg/kg/d based on the NOEL of 0.5 mg/kg/d from the dog study and using a safety factor of 100. Given the lack of determination of an NOEL in the Mellon 2-year rat study, and the evidence that dazomet (at least in the unchanged form) has some genotoxic potential, it would be appropriate to increase the safety factor from 100 to 1000, and correspondingly revise the NOEL down by a factor of 10.

DRAFT RECOMMENDATIONS

1. The scheduling of dazomet in the SUSDP at schedule 6 is considered appropriate on toxicological grounds.

2. ADI

   The ADI for dazomet should be reviewed in light of the data from the 2-year chronic study in Carworth-Wistar rats (Mellon Institute of Industrial Research, University of Pittsburgh). No NOEL was determined in this study (ie. the NOEL was lower than the lowest dose tested). It is recommended that the ADI is 0.005 based on the LOEL in the above study of 0.5 mg/kg/d and a safety factor of 1000.

   The National Registration Authority should review the residue data in light of the information suggesting that MITC may be a residue in crops following the use of dazomet.

3. First Aid and Safety Directions

   Dazomet EUP is Basamid Granular which is a 97% formulation of dazomet, comprising granules. The only excipient listed is 0.1% silica gel. Use pattern is as a soil fumigant which is applied directly to the soil in granular form.

   Current Safety Directions are:

Dazomet	GR 980 g/kg or less	120 130 133 161 162 163 164 210 211 220 221 279 283 290 294 297 320 340 342 350 360 361 363 366

   Given the acute toxicological profile of dazomet, there is no evidence to suggest that a change to the existing safety directions, based on hazard, is required. Worksafe Australia may wish to review the safety directions relating to PPE.

   No change to the existing First Aid Instructions for dazomet is recommended.

4. WSA may wish to consider by-stander exposure to gaseous MITC following the use of dazomet.

SUMMARY OF TOXICOLOGICAL HAZARD*

Date of Preparation:	July 1996
Chemical name:	Dazomet
Worst oral LD_{50} in rats:	415 mg/kg (females)
Worst oral LD_{50} in other species:	Not available
Worst dermal LD_{50}:	> 2000 mg/kg in rats
Worst inhalation LD_{50}:	8400 mg/m^3 in rats
Skin irritation:	Slight in rabbits
Eye irritation:	Slight in rabbits
Skin sensitisation:	Nil in guinea pigs at the doses tested
Remarks:	Contact dermatitis reported in humans
T-value:	40

NOEL: An NOEL was not determined in a chronic (2-year) dietary study in rats (Mellon Institute of Industrial Research, University of Pittsburgh). At the lowest dose tested (10 ppm, corresponding to about 0.5 mg/kg/d), there was an increase in the incidence of kidney and liver lesions. The lowest established NOEL was 0.5 mg/kg/d, in a 1-year dietary dog study and a 2-generation dietary rat reproductive toxicity study.

* This summary largely refers to the TGAC, but it would be expected that there would be little difference between the toxicity of the TGAC and EUP, given that the difference between the two forms is only one of particle size (larger for Basamid Granular than for the TGAC).

DAZOMET

1. INTRODUCTION

1.1 Historical Background

Dazomet, by virtue of the chemical release of methyl isothiocyanate, is a soil fumigant effective for the control of nematodes, germinating weeds and soil fungi. Dazomet is currently scheduled in schedule 6 of the SUSDP. No MRLs have been set due to its lack for potential residues in food. Dazomet is strongly phytotoxic and treated soils should not be planted until shown to be free of the compound and its decomposition products (generally within 8-24 days).

Metham-sodium has been placed on the NRA's Ad Hoc review program following a report of adverse effects to an Victorian orchardist who experienced severe eye irritation and nausea when using metham-sodium to fumigate soil. Dazomet and methylisothiocyanate (MITC) have been evaluated concurrently with metham-sodium under the Ad Hoc review program because both dazomet and metham-sodium break down to the active component, MITC, in the soil. This evaluation consolidates available toxicological data which have been submitted to the Department on dazomet over a period of time from the original submission by BASF Australia Ltd in May, 1986. It covers submission numbers 265, 7872, 10065, 10236 and 10580.

Dazomet was considered by the PACC in February, 1969 at which it was accepted that "as the fumigant must be dissipated from the soil before a crop can be planted no residues will appear in any crops." It was also considered in July, 1971 and in February, 1974 in relation to residues in food and was considered to be a substance which is exempted from the requirements of a maximum residue limit. Dazomet was also considered in December, 1988, at which time it was recommended that the company should provide ongoing toxicological studies as they become available. Previous evaluations were conducted by the Department in December, 1990 and January, 1994 covering submissions 265 (May, 1986), 7872 (August, 1991), 10065 (July, 1993; did not include toxicity data), 10236 (October, 1993; did not include toxicity data) and 10580 (June, 1994). Submission 10580 comprised summary data only. Many of the studies for which summaries were provided had been evaluated previously. The metabolism studies in the rat (refs. 10 and 11), however, has not been evaluated previously (submission number 10580). The summaries provided in submission 10580 revealed that some studies have been conducted that have not been submitted for evaluation. In December, 1994, the Department requested the company to submit these additional data. Following their submission, these data were evaluated in May 1996.

BASF Australia Ltd was the original applicant and all submissions have been under the auspices of BASF Australia Ltd, although the data for submissions 7872 and 10065 were from Hoechst Australia Ltd and submissions 10236 and 10580 were made through Lanercost Pty Ltd.

The company was informed in January, 1994 that the TGAC for dazomet manufactured by BASF Aktiengesellschaft, Germany, was cleared.

The original data for a number of studies were not available to the evaluator of this Ad Hoc Review. These studies are: Gelibeke (1986) -acute inhalational toxicity study in rats, ref. Kirsch & Kieczka (1985a)- eye irritation study in rabbits, Kirsch & Kieczka (1985b) -skin irritation study in rabbits, Kirsch & Kieczka (1986)- skin sensitisation study in the guinea pig), Zeller & Merkle (1980a & 1980b)- developmental toxicity studies in the rabbit, and Hazleton (1987)- *B. subtilis* rec assay.

Dazomet has been reviewed by DPSC on two occasions (1/69 and 6/69).

1.2 Chemical Identity

Common name: Dazomet

Chemical name: 3,5-dimethyl-1,3,5-thiadiazinone-2-thione (IUPAC)
tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-thione (CA)

CAS number: 533-74-4

Empirical formula: $C_5H_{10}N_2S_2$

Structural formula:

Molecular weight: 162.3

1.3 Chemical and physical properties (for 99% pure dazomet)

Physical state: solid

Colour: white to off-white

Odour: weak intrinsic odour

Melting point: 104 - 105^{o}C

Vapour pressure: 3.73 x 10^{-6} mbar at 20^{o}C

Solubility: water 0.3 g/100 g at 20^{o}C

Solubility:

organic solvents -	ethylether	0.60 g/100 g
	cyclohexane	0.04 g/100 g
	benzene	5.1 g/100 g
	acetone	17.3 g/100 g
	ethyl alcohol	1.5 g/100 g
	chloroform	39.1 g/100 g

Hydrolysis: half life at 22^{o}C and pH: 5 8.60 h
pH 7: 2.60 h
pH 9: 1.46 h

The structures of dazomet and the impurities are given in **Appendix I**.

1.4 End Use Products

Basamid Granular (97% dazomet), which is formulated as granules, is currently marketed in Australia. The difference between the TGAC and the EUP is only one of particle size (larger for Basamid Granular than for the TGAC).
Note that Basamid Granular EUP is produced by an integrated process, meaning that there is no isolated TGAC during the production process. The nominal concentration of dazomet in Basamid Granular is 97%. Silica gel (0.1%) is added to prevent electrostatic charging of the EUP.

2. METABOLISM AND TOXICOKINETICS

2.1 Rat

2.1.1 Oral study

(Hawkins et al (1985) Huntingdon Research Centre. HRC/BSF 423/85945. Reg. Doc. No. BASF 85/0455. December, 1985). GLP/QA: yes.

Sprague-Dawley (COBS (SD) CD) rats received a single dose of [^{14}C]dazomet by gavage (vehicle - 1% aqueous carmellose) at 100 mg/kg. The position of the radiolabel was at the carbon of the C=S bond

Excretion study

Six rats (3/sex) were used in an excretion study. Urine and faeces were collected and expired air was trapped for intervals up to 120 h after dosing. Livers, kidneys and gastrointestinal tract were taken for analysis at sacrifice (120 h).

Absorption and excretion results are shown in the following table.

Percentage of administered radioactivity

	Males	Females
Urine (0-120 h)*	62.40	59.68
Faeces (0-120 h)	2.78	2.21
Expired air (0-120 h)	29.44	32.28
Kidney	0.1	0.14
Liver	0.52	0.17
GIT	0.07	0.08
Remaining tissues	1.76	2.61
Total in tissues	2.44	3.00
Recovery	97.07	97.17

* including cage washings

It can be seen that dazomet was rapidly and extensively absorbed. Administered radioactivity was largely excreted via the urine, but a substantial amount was excreted in expired air. Faecal excretion was low. The large proportion of radioactivity in expired air indicates that the administered dazomet was extensively metabolised. Most of the urinary radioactivity was excreted in the 24 h following dosing (56.8% of the dose in males and 46.3% in females) and in the following 24 h period (3.7% in males and 11.1% in females). Most of the radioactivity in expired air was excreted in the 24 h following dosing (27.6% of the dose in males and 29.0% in females).

Analysis of urine (0-6, 6-24 and 24-48 h samples) by TLC revealed that none of the metabolites detected corresponded to dazomet, a finding that also indicatives extensive metabolism. Patterns of metabolites in urine were unchanged following incubation with -gucuronidase/aryl sulphatase, indicating that conjugates of dazomet or its metabolites were not present in urine. There were 5 peaks of radioactivity in the urine chromatograms. GCMS of the major metabolite gave a mass spectrum indicating methylisothiocyanate as a possible fit. N-acetyl-S-(N-methyl thiocarbamoyl)-L-cysteine was synthesised and was shown by TLC to correspond to the major metabolite. Thus, the main metabolic degradative product in urine of rats was identified as the mercapturic acid conjugate of methylisothiocyanate.

Plasma kinetics

Six rats (3/sex) were used in a study of plasma kinetics, with blood samples being taken at intervals from 0.5 h to 168 h after dosing. In males, a mean peak concentration of radioactivity (15.0 µgeq./mL) was reached at 0.5 h after dosing, with a second peak at 12 h. In females, the mean peak concentration was observed at 12 h (11.0 µgeq./mL), but concentrations were high over the period 0.5 to 12 h after dosing. In males, mean concentrations declined to 0.33 µgeq./mL at 168 h, with a mean half-life of about 55 h. In females, mean concentrations declined to 0.47 µgeq./mL at 168 h, with a mean half-life of 52 h. The occurrence of two peaks suggests enterohepatic recycling. The extended terminal half-life of radioactivity suggests binding of radioactivity to plasma or tissues.

Liver and plasma metabolites

Eight rats (4/sex) were used in this study, with pairs (1/sex) being sacrificed at 0.5, 2, 12 and 24 h after dosing. Samples of portal blood and liver were taken for analysis of radioactivity, and of plasma dazomet concentrations by HPLC.

At most time points, concentrations of radioactivity in liver were higher (about 3.5-fold) than those in plasma. Elimination of radioactivity from the liver occurred at a slower rate than elimination from plasma. Concentrations of dazomet in portal plasma were at or near the limit of detection (0.5 µg/mL) at most of the time points examined, indicating that very little of the absorbed radioactivity was associated with parent compound which suggests that dazomet is decomposed during its passage across the gastrointestinal tract. Most of the radioactivity in portal vein plasma was detected as ^{14}C-labelled volatile compounds, which were considered likely to be MITC by the authors of the report. In methanol extracts of liver samples, dazomet could not be detected by radiochromatogram scanning of thin-layer chromatograms; most of the radioactivity in liver extracts was associated with polar material close to the origin.

2.1.2 Oral study

(Hawkins et al (1987) Huntingdon Research Centre. HRC/BSF 456/87983. Reg. Doc. No. BASF 87/0469. September, 1987).

Results from this study were presented in summary form. The rats, the dosing route and vehicle and the radiolabelled dazomet were as described for the above study.

Excretion of radiolabel: % of administered radioactivity

	10 mg/kg[a]	100 mg/kg
Urine (0-168 h)	60.1	64.5
Faeces (0-168 h)	3.2	2.3
Expired air (0-72 h)	22.4	30.4
Recovery[c]	96.7	99.5
C_{max} (µgeq./mL)	1.84	10.3-16.7
T_{max} (h)	1.0	generally 0.25-1.0

a: mean of M and F (n/sex not stated)
c: 168 h; includes residues in tissues

Composition of radioactivity in expired air:

	Percentage of radioactivity in expired air (0-72 h)	
	10 mg/kg	100 mg/kg
MITC	5.8	5.5
CO_2	75.6	37.9
CS_2, COS	18.6	56.6

The following results were obtained in bile duct-cannulated rats (n=2/dose group):

	Percentage of administered radioactivity	
	10 mg/kg	100 mg/kg
Bile (0-48 h)	7.4	6.7
Urine (0-48 h)	52.7	44.3
Faeces (0-48 h)	3.1	1.7
Residues in tissues (48 h)	5.1	6.4
Recovery (including cage wash)	68.5	60.6

Biliary excretion accounted for approximately 7% of radiolabel, while faecal excretion accounted for 2-3%, suggesting that enterohepatic recycling may occur.
The above excretion data indicate that the pharmacokinetics of dazomet are not affected by a 10-fold increase in dose from 10 mg/kg to 100 mg/kg.

The half-life of plasma radioactivity was estimated to be 61 h in males and 69-71 h in females.

Residues in tissues (at 168 h after a single oral dose) represented 2.2- 3% of total administered radioactivity. Highest concentrations of radioactivity were found in the lungs, kidneys and liver, but significant concentrations were also found in the ovaries, adrenals, thyroid and blood (presumably at 168 h). The distribution of radioactivity was also examined after a 7-day oral administration of 10 mg/kg/d. At 240 h after the last dose, ^{14}C residues in the tissues examined were higher than in plasma. It was also reported that there were no differences in excretion pattern and tissue distribution following a single dose of [^{14}C]dazomet and following a 14-d administration of unlabelled dazomet and a subsequent single dose of ^{14}C-labelled compound.

Radioactivity was analysed by TLC in urine, bile, liver and kidneys. Five polar components were detected in urine, with only two (M4 and M5) exceeding 10% of the dose administered. A proposed metabolic pathway is shown in Figure 1.

Figure 1. Proposed metabolic pathway for dazomet.

The relative proportion of the 5 compounds with respect to total urinary radioactivity was not altered following incubation with -glucuronidase/sulfatase (refs. 10 and 11). M5 was deduced to be an adduct of MITC and glutathione, subsequently acetylated - N-acetyl-S-(methyl-thiocarbomyl)-L-cysteine. The metabolites M2 and M4 were regarded as conjugates of MITC with cysteine. M5 was not detected in bile, but was found in liver, although M9 was the major metabolite in liver. M1 and M2 were also detected in liver. In kidneys, the main metabolite was M2, but M1, M5 and M9 were also found.

2.2 Soil

***(Hawkins et al (1986) Huntingdon Research Centre, UK. Project no. HRC/BSF 434/86968. BASF Reg. Doc #87/033818. September, 1986*)**

In soil samples (of sandy texture) kept under aerobic conditions (in the dark at 25°C), ^{14}C-dazomet (applied a rate of 650 µg/cm^2, which is equivalent to a horticultural application of 5 mg/cm^2 incorporated in the top 10 cm of soil) degraded with an apparent half-life of 13.64 h. The major degradation product was MITC (found in the soil and in the ethyl acetate solvent traps and identified by gas chromatography-mass spectrometry), while only minor proportions were degraded to CO_2, COS and CS_2 (sodium hydroxide and Vile's reagent traps). The soil samples were contained in dishes kept in sealed glass containers. A stream of air saturated with water was passed through the containers and the effluent air was passed through a series of traps.

3. ACUTE TOXICITY

3.1 Lethal Dose Studies

The acute lethal dose toxicity (LD_{50}) of dazomet in several species is shown in Table 1.

Web users: the following tables may not printsatisfactorily. See http://www.affa.gov.au/nra/chemrev.html and select the attachment to Metham Sodium vol. 3

Table 1. Acute lethal dose toxicity of dazomet.

Species	Strain Reference	Sex (n/group)	Route	Vehicle	LD_{50}(
Rat	Sprague- & Gelbke (1980)	M	Oral	Carmellose	596
	Dawley	(10)		(0.5%)	(551 -
Rat	Sprague- & Gelbke (1980)	F	Oral	Carmellose	415
	Dawley	(10)		(0.5%)	(335 -
Rat	Sprague- Hoffman (1975c) Dawley	M & F	Oral	Tragacanth (4%)	about (
Mouse	ICR (1980a)	M	SC	Hexaethyl	248
		(10)		cellulose (0.1%)	(212 -
Mouse	ICR (1980a)	F	SC	Hexaethyl	248
		(10)		cellulose (0.1%)	(212 -
Rat	Wistar (1980b)	M	SC	Hexaethyl	470
		(10)		cellulose (0.1%)	(392 -

Rat	Wistar (1980b)	F	SC	Hexaethyl	550
		(10)		cellulose (0.1%)	(440 -
Rat	Sprague Hildebrand & Jackh (1981)	M&F	Dermal	Water (25% or 50%	>2000
	Dawley	(?)		aqueous preparation)	
Mouse	NMRI Hoffman (1975a)	M&F	IP	Tragacanth (4%)	about 2
		(5/sex/group)			
Rat	Wistar (1986)	M	Inhal.	Air	>8400
		(10)	(4 h exposure)		
Rat	Wistar (1986)	F	Inhal.	Air	7290 n
		(10)	(4 h exposure)		(6060

*the result is the mean of males and females, but the LD_{50} was lower in females (in the region of 550 r
900 mg/kg).

After oral administration to the rat, the main clinical signs were dyspnoea, apathy, staggering, trembling, piloerection, erythema, shaking and poor general state. In dead animals, congestive hyperaemia and dilatation of the heart were observed and in some sacrificed animals, the tip of the forestomach was fused with the liver and peritoneum.

After sc administration to rats and mice (Japanese studies), rapid breathing and reduction in physical activity were observed immediately. Lacrimation, salivation, hunchback posture and tonic and clonic convulsions were observed later. Lung congestion was observed in dead animals.

In the dermal study in rats, the preparation (as a 25% or 50% aqueous preparation at doses of 200, 400, 1000 and 2000 mg/kg) was applied to a 50 cm^2 area of clipped skin of the back and flank, presumably for 24 h. After 24 h, slight oedema was observed at 200 mg/kg, slight erythema and oedema were observed at 400 and 1000 mg/kg, and very slight, partly spotted-necrotic erythema and slight to severe oedema were observed at 2000 mg/kg. No changes were observed after 13 d.

Symptoms observed in the ip mouse study were dyspnoea, apathy, prostration, lacrimation, salivation, spasms and convulsions.

Symptoms observed in the rat inhalation study were trembling gait, reddish nasal discharge, abdominal fur with yellow smear, reddish encrustation of noses (blood test positive), piloerection, squatting posture, reddish urine (blood test positive), anaemia, dragging hindlimbs, with paresis at the high dose (8400 mg/m^3). Diminished pain reflex was also observed at the high dose (8400 mg/m^3). At the high dose, 1/10 males died and 7/10 females died, giving a total of 8/20 deaths and an approximate LD_{50} of 8400 mg/m^3.

In addition to the 'traditional' lethality studies, an acute inhalational study was conducted in rats (Hoffman (1975b) in which a stream of air was passed through the product (Basamid Granular) at a rate of 200 L/h. It was stated that the air was saturated with volatile components of the substance (kept at 20^oC). The treated air stream was then directed into chambers, each housing one Sprague-Dawley rat, with the exposure period lasting for up to 8 h (maximum). There were no measurements performed to determine the concentration of dazomet. It was stated that there were no deaths in the 12 treated rats, nor were there any symptoms of toxicity.

3.2 Eye Irritation Studies

3.2.1 Rabbit

(Zeller (1975) BASF Aktiengesellschaft. 27 August, 1975.)

Dazomet (50 mg, as Basamid Granular) was introduced into the conjunctival sac (2 rabbits tested) and the same amount of talcum was introduced into the control eye, with inspections taking place at 1 and 24 h, and 8 days. It is unclear why talcum was chosen as the control substance.

The initial introduction of dazomet into the eye caused slight irritation, consisting of moderate erythema of the conjunctival sac, with slight oedema at 1 h and slight erythema at 24 h which was not present on day 8. Similar findings followed the application of talc.

3.2.2 Rabbit

(Kirsch & Kieczka (1985a) BASF Aktiengesellschaft. Report no. 85/389. 27 November, 1985)

A single application (0.1 mL; 39 mg) of technical dazomet was made to the right eye of each of six (2M/4F) white Vienna rabbits. The treated eye was not washed and the left eye (untreated) acted as the control in each animal. Examination of the animals took place at 1, 24, 48 and 72 h after application of the test substance.

Well-defined redness and slight swelling of the conjunctiva were evident at 1 h in the treated eye of all animals and redness was still present at 24 h in all animals, and in one animal at 48 h, but swelling was not evident at 24 h. Corneal and iris changes were not observed. The test substance was considered a slight irritant.

3.3 Dermal Irritation Studies

3.3.1 Rabbit

(Zeller (1975) BASF, Aktiengesellschaft. 27 August, 1975)

Basamid Granular was tested for its potential irritancy at two dermal sites on the rabbit (2 animals tested), namely: Skin - shaved dorsal:A test patch (area not stated) saturated with a 50% aqueous preparation of Basamid Granular (volume not stated) was applied to the dorsal skin of the rabbit for periods of 1, 5, 15 min and 20 h. After the 1 - 15 min applications, treated skin was washed, but not after the 20 h application. Skin - pinna: A cottonwool carrier coated with 2 g Basamid Granular was bandaged to the pinna of the rabbits ear for 20 h. Findings were recorded after removal of the dressings and at 24 h and 8 days.

At 24 h after short term exposure (1 to 15 min) to the dorsal back skin, barely perceptible erythema was seen, while a longer exposure period (20 h) resulted in moderate erythema and oedema and sanguineous infiltration. There were no findings at 8 days following the 1 - 15 min exposure. At 8 days after the 20 h exposure, it would appear that there was slight inflammation. The pinna showed slight erythema at 24 h but at 8 days, this was barely perceptible, although slight scar tissue was observed. It was stated that at 8 days, "parts of the inflamed areas showed disturbance of the tissue function". Dazomet was a slight skin irritant under these experimental conditions.

3.3.2 Rabbit

(Kirsch & Kieczka (1985b) BASF Aktiengesellschaft. **Report no. 85/388.** ***27 Nov 1985)***

A dose of 0.5 g of dazomet in a 50% aqueous suspension was applied to an area of 2.5 cm x 2.5 cm on the intact dorsal skin of 6 (2M/4F) white Vienna rabbits for an exposure period of 4 hours. Each of the test sites was covered with a semi-occlusive dressing, and after the exposure period, the dressings were removed and the treated areas washed. The test sites were examined at 4, 24, 48 and 72 hours post-application. No symptoms were observed at any time during the study. Dazomet was not a skin irritant under these experimental conditions.

3.4 Dermal Sensitisation

3.4.1 Guinea pig

(Zeller (undated) BASF Aktiengesellschaft. Code no. XXI/131)

Female white guinea pigs were used in two studies. No justification of the doses used was given for either of the studies. Details of the studies are presented below.

Study 1: Test animals (n = 10) were conditioned by topical treatment on a shaved area of the right flank (25 cm^2) with a 20% solution of Basamid Granular in acetone. The treatment was repeated 10 times (5 times weekly). There were no signs of irritation during this conditioning period.

After an interval of 12 d, a 20% solution of the product was applied topically to the shaved left flank and the skin reaction was recorded at 12 h. There were no positive reactions in either the pretreated animals or the controls which had received no pretreatment (n = 3).

After a further interval of 20 d, the same animals (9 remaining; one died intercurrently, presumably not treatment-related, although this was not stated) were given an intracutaneous injection in the neck with 0.05 mL of a 0.1% solution of Basamid Granular in physiological saline. Skin reactions recorded at 12 h were negative in both the pretreated animals and in a group of controls (n = 6) that were not pretreated.

Study 2: Ten test animals received 9 conditioning intracutaneous injections of 0.05 mL of 0.1% Basamid Granular in saline on the back on both sides of the spinal column. There was no irritation following the first 7 injections. From the eighth injection onwards, there was slight reddening around the area of the injection.

After an 18 d interval, animals received an intracutaneous injection of 0.05 mL of 0.1% Basamid Granular in saline in the neck. There were no positive skin reactions in either the test group or in a control group of 3 animals which had not received the pretreatment.
Thus, the challenge dose of EUP, regardless of the route used for conditioning, did not induce any sign of skin sensitisation. Positive control compounds were not tested in either of these studies.

3.4.2 Guinea pig

***(Kieczka & Jirsch (186) BASF Aktiengesellschaft. Report no. 85/399 (project no. 30H318/85). 20 December, 1985 (plus Amendment to the Report; July, 1986*)**

Dazomet was tested for skin sensitising potential in female Dunkin Hartley guinea pigs.

In a pretest for dose selection, concentrations of up to 60% in olive oil were tested topically in 4 animals/group. The test substance was applied to the flank (over 2 x 2 cm under an occlusive dressing) twice for 24 h within a period of 96 h. Observation at 24 and 48 h after the beginning of the application did not reveal any irritation at any of the concentrations tested.

The main study:
intradermal induction: test animals (duplicate groups of 10) were given 6 intradermal injections into the shoulder (2 injections of Freund's adjuvant:water (0.1 mL:0.1 mL), 2 injections of 0.1 mL of 5% test substance in olive oil and 2 injections of Freund's adjuvant:5% test substance in olive oil (0.1 mL:0.1 mL). Controls (two groups of 10) were given the same injections without the test substance. Skin areas at the injection sites (also the area of percutaneous induction) was treated with 10% formulation of sodium dodecyl sulfate in white vaseline 24 h before percutaneous induction which was conducted one week after intradermal induction.

Percutaneous induction: (0.3 g of the test substance formulation (60% in olive oil) over 2 x 4 cm) was for a duration of 48 h under an occlusive dressing. Controls received analogous treatment without the test substance.

Challenge: animals were challenged by percutaneous application to one flank (right flank - challenge 1; left flank - challenge 2) of test substance (60% in olive oil; about 0.15 g of the test substance formulation over 2 x 2 cm under an occlusive dressing for 24 h), with the other flank receiving a concurrent application of olive oil alone. The first challenge was 19 days after the intradermal induction and the second challenge was a week later. For the first challenge, control group 1 received the test substance, but not control group 2. For the second challenge, both control groups received the test substance. Skin reactions were examined at 24, 48 and 72 h after the beginning of the application.

There were a number of mortalities in this study. Three animals had died by the time of the first challenge and 6 had died before examination following the second challenge. Cause of death was considered to be pneumonia in 4 cases, pulmonary emphysema in one and peritonitis in another. Although all mortalities were test animals, the company considered that mortality was not induced by the test substance.

Erythema was observed in both test and control animals following intradermal injection with olive oil/test substance or olive oil, and erythema and oedema were observed in both test and control animals following intradermal injection with Freund's adjuvant/test substance or Freund's adjuvant/water. Percutaneous induction resulted in incrustation, erythema and oedema in both test and control animals.

At 24 h after the first challenge dose, erythema was observed in 2/10 controls challenged with test substance and 11/17 test animals (right flank). Oedema was also observed in 4/17 test animals (right flank) at 24 h. However, erythema was also observed on the left flank in 4/17 test animals and oedema was observed on the left flank in 1/17 test animals. By 48 h (the standard time for evaluation of reactions) after the first challenge dose, no skin reactions were observed. The second challenge dose did not cause any skin changes in the treated animals.

No concurrent positive control compound was tested in this study, but it was stated that the strain of guinea pigs used in this study was tested with DNCB as a positive control in September 1985, directly before the sensitising test with dazomet, and that the animals were sensitive.

The test substance did not have skin sensitising activity under these experimental conditions.

4. SHORT-TERM REPEAT DOSE TOXICITY

4.1 Rat

4.1.1 3-Week Preliminary Dietary Study

(BASF Department of Toxicology, West Germany. Project no. 20C0318/8527. Date?)

A full report of this study was not provided but a summary of results was given in the report of the chronic rat study ***(Kuhborth et al 1989a).***

Rats (strain, sex and number per group not stated) were given dazomet in the diet at 0, 200, 800 and 3200 ppm for 3 weeks. Pronounced signs of toxicity (deteriorated general state, piloerection, squatting posture, paralysis of forelegs, pareses of hindlegs, strutting gait etc.) were reported at the two highest doses while no signs were reported at 200 ppm. At 800 and 3200 ppm food consumption was dramatically reduced, with reduced bodyweight gain or loss of bodyweight. No NOEL was set due to the summary nature of the report.

4.1.2 4-Week Preliminary Dietary Study

(BASF Department of Toxicology, West Germany. Project no. 15C0318/8543. Date ?) GLP: yes

A full report of this study was not provided but a summary of results was given in the report of the chronic rat study ***(Kuhborth et al 1989a).***

Rats (strain, sex and number per group not stated) were given dazomet in the diet at 0, 20, 60, 180 and 540 ppm for 4 weeks. Clinical signs of toxicity (motor disturbances including pareses of hindlegs, strutting gait, abnormal foreleg position etc.) were reported in high-dose females (and one 180 ppm female). There was reduced food consumption and bodyweight gain in high-dose females (also a trend in males) and also in 180 ppm females over the first 2 weeks. Decreased creatinine was reported in high-dose animals and decreased aspartate aminotransferase (AST) and plasma cholinesterase in high-dose females. At 60 ppm and above, decreased alanine aminotransferase (ALT) values were noted in females. Liver weights were increased, accompanied by intermediary- and centro-acinary fatty degeneration in the form of large fat droplets (180 and 540 ppm males, 540 ppm females). There were apparently no compound-related effects at 20 ppm.

4.1.3 3-Week Inhalation Study

(Morris & Clark (1976) Huntingdon Research Centre, UK. 25 June, 1976)

Wistar rats (10/sex/group) were exposed (whole body) to Basamid Granular dust for 6 h/day for 5 days/week over a 3 week period. The dust was generated by mechanical agitation of the test material, with a resultant concentration of 33 g dazomet/m^3 of air. A control group received filtered air only. Analysis of the size of airborne particles indicated that all were of respirable size (less than 5 m).

There were no observable signs of toxicity, nor were there any treatment-related effects on haematological parameters, urinalysis, clinical chemistry, organ weights or microscopic and macroscopic tissue analysis.

4.2 Rabbit

4.2.1 3-Week Dermal Study

(Kynoch et al (1976) Huntingdon Research Centre, UK. 25 June, 1976)

This study investigated the local and systemic toxicity of Basamid granular, topically applied to shaven abraded skin of New Zealand White rabbits at dose levels of 0, 0.5% and 5.0% (2 mL/kg/d to an area of 10 cm^2) in aqueous sodium carmellose. The study involved 7 d/week application of the test substance for a 3-week period. An occlusive dressing was applied and the exposure period was 6 h, after which the skin was washed. Parameters examined were clinical symptoms, bodyweight, haematology, blood chemistry, ophthalmoscopy, organ weights, macroscopic and microscopic pathology.

Slight to well-defined erythema and oedema were observed at the treated sites of all rabbits in groups receiving 0.5% and 5.0% dazomet. Cutaneous hardening and discolouration resembling chemical burns were seen in 8/10 animals receiving 0.5% and 10/10 animals receiving 5.0% dazomet.

There were no signs of systemic toxicity as a result of the dermal application of dazomet. Potassium levels were slightly elevated, but this change was within biological limits.

4.2.2 3-Week Dermal Study

(Mackensie (1987) Hazleton Laboratories America, Inc. 17 June, 1987) GLP/QA: yes

Dazomet, at doses of 0 (vehicle, 0.4% aqueous carmellose), 10, 100 and 1000 mg/kg/d, was administered dermally to Hra:(New Zealand White) SPF rabbits (5/sex/group) for 5 days/week for at least 21 days. Drug was applied to unabraded skin of the clipped dorsal trunk over an area of approximately 10% of the total body surface. The period of exposure was 6 h and the application area was occluded. The area was then washed.

At the end of the treatment period, satellite groups (5/sex), at the 0 and 10 mg/kg dose levels, were observed for reversibility, persistence or delayed occurrence of toxic effects over a 24 d treatment-free period.

Dose levels were based on a dose-range finding study. Conditions in this study were the same as in the main study except that the duration of the study was 5 days and doses of 0, 0.6, 1.2, 2.5, 5.0 and 10.0 mg/kg/d were used initially, with one animal/sex/group. As no effects were seen with these doses, the dose levels were increased to 0, 12, 24, 50, 100 and 200 mg/kg/d. After 2 days of dosing at the increased level, an additional group of 2 males receiving 1000 mg/kg/d and dosed for 5 days, was added. There were no abnormal observations (dermal irritation; no necropsy) in any of the treated animals.

There were no treatment-related clinical signs. One high-dose male was sacrificed in a moribund condition on day 6. It was stated that the health problems of this animal "did not appear to be test material related". There was no dermal irritation seen in any of the rabbits during the study.

Clinical chemistry examination at the 3-week sacrifice revealed the following significant changes in males: a decrease in AST at 1000 mg/kg and in creatinine at 10 and 1000 mg/kg. There were no haematological changes that were considered to be treatment-related. Small changes that were not considered biologically significant, were observed in the treated satellite groups. For both the main study groups and the satellite groups, there were no significant differences between control and treated groups with respect to body weights and organ weights (brain, kidneys, liver and testes (with epididymides)), nor with respect to macroscopic and microscopic findings (kidneys, liver and skin examined microscopically; microscopic examination not conducted on satellite groups). Food consumption was not affected by treatment.

It was concluded that the NOEL in this study was 1000 mg/kg.

5. SUBCHRONIC TOXICITY

5.1 Mouse

5.1.1 10-Week Dietary Range-Finding Study

(BASF Aktiengesellschaft. Project no. 15C0318/8543. Date ?)

A full report of this study was not provided but a summary of results was given in the report of the chronic mouse study **(Kuhborth et al (1989a).**

Mice (strain, sex and number per group not stated) were given 800 and 1200 ppm dazomet in the diet for 71 days. At the high dose it was reported that there was a slight reduction in bodyweight in males. At both doses, there was a reduction in Hb, RBC counts and Hct, increases in MCH and MCV, and (in females) reduced MCHC. No NOEL was established in this study.

5.1.2 3-Month Dietary Study

(BASF Aktiengesellschaft. Project no. 25C0318/8530. Date?)

A full report of this study was not provided but a summary of results was given in the report of the chronic mouse study **(Kuhborth et al (1989a).**

Mice (strain, sex and number per group not stated) were given 20, 60, 180, 360 and 540 ppm dazomet in the diet for 91 days. It was reported that there were no clinical signs which could be attributed to the test substance. At the two highest doses there was a temporary reduction of food consumption which did not affect body weight gain. Haematological analysis of 360 and 540 ppm groups revealed:

- reduced Hb and RBCs in both sexes and reduced Hct in males;
- reduced MCHC (both sexes at the high dose, females only at 360 ppm);
- increase in MCV, reticulocytes, and polychromasia;
- increased anisocytosis (both sexes at the high dose and 360 ppm females).
- increased macrocytes (540 ppm males only)

Also, at 360 ppm and above, a slight increase in splenic haemosiderin deposition (in females only at 360 ppm) was reported. An increase in liver weight (in most instances, both absolute and relative) was noted in males at 180 ppm and above and in high-dose females. The NOEL was 60 ppm (estimated compound intake of 9 mg/kg/d) on the basis of increased liver weight at the next highest dose.

5.2 Rat

5.2.1 3-Month Dietary Study

(Hellwig et al (1987a) BASF Aktiengesellschaft. Project no. 30C0318/8544, Doc. BASF:87/0448. 11 December, 1987). GLP/QA: yes.

Wistar rats (10/sex/group) were given dazomet in the diet at 0, 20, 60, 180 and 360 ppm for 3 months.

At the high dose there was a temporary slight reduction in food consumption in females, with reduced bodyweight gain from week 2 (females; 10-13%) and week 3 (males; about 7%). Total protein was decreased (180 ppm males and high-dose animals of both sexes). At the high dose there was a decrease in Hb (both sexes), triglyceride levels (males) and in creatinine, potassium and albumin (females). At necropsy increased liver weights, with fatty degeneration of liver cells, were seen at 60 ppm and above in males and at 180 ppm and above in females. On the basis of these liver findings, the NOEL was 60 ppm (about 4.6 mg/kg/d) in females, 20 ppm (about 1.8 mg/kg/d) in males.

5.3 Dog

5.3.1 3-Month Dietary Study

(Hellwig et al (1987b) BASF Aktiengesellschaft. Project no. 31D0318/8533, Doc. BASF:87/0456. 9 September, 1987). GLP/QA: yes.

Beagle dogs (4/sex/group) were given dazomet in the diet at 0, 25, 100 and 400/200 ppm for 3 months. Compound intakes were calculated as follows:- males; 0, 0.8-1.0, 3.3-4.0, and 6.8-7.8 mg/kg/d: females; 0, 0.9-1.0, 3.1-4.0 and 7.2-8.9 mg/kg/d.

At 400 ppm there were very large losses in bodyweight, with sporadic vomiting and marked lack of appetite; because of this, the high dose was reduced from 400 to 200 ppm on day 23 of treatment. At 200 ppm there was still inappetence in individual females, with reduced bodyweight gain. At 100 ppm there was only reduced food consumption in one female. Haematological effects (reduced Hb, RBC counts and Hct values) were seen at the high dose, as was a reduction in ALT (both sexes) and total protein, albumin and cholesterol (females). Platelets were increased in females. Relative liver weights were increased in the 200 ppm animals and the 100 ppm males, without any histopathological correlates. There was a slight increase in splenic haemosiderin deposition at the high dose. The NOEL was 25 ppm (0.8-1.0 mg/kg/d) for males and 100 ppm (3.1-4.0 mg/kg/d) for females, on the basis of increases in relative liver weights in males at the next highest dose.

6. CHRONIC TOXICITY

6.1 Mouse

6.1.1 78-Week Dietary Study

(Kuhborth et al (1989a) BASF Aktiengesellschaft. Project no. 65C0318/8585, Doc. BASF:89/0341. 22 September, 1989). GLP/QA: yes.

B6 C3 F1 mice (60/sex/group) were given dazomet (98.2%) in the diet at 0, 20, 80 and 320 ppm for 78 weeks. Compound intakes were estimated as follows:- males; 0, 4, 16 and 68 mg/kg/d: females; 0, 6, 22 and 93 mg/kg/d. 10/Sex/group were killed at 52 weeks for histopathology.

Survival was not affected by treatment and there were no noteworthy clinical signs, bodyweight or food consumption changes associated with dazomet intake. A number of changes occurred in the 1.5-year kills, some (but not all) of which were also observed in the 1-year kills. There was a significant elevation of liver weight (absolute and relative) at the high dose. At gross necropsy there was an increased number of mid dose and high dose animals with liver discolouration and liver masses. In these two groups there was an increased incidence of centrilobular lipid deposition. At the high dose, females showed a slightly increased incidence of hepatocellular adenomas (3, 0, 1 and 7 females, out of 50 in the control - high dose groups, respectively; not significant using Fisher's exact test, but positive trend using Cochran Armitage test) and a significantly increased incidence of basophilic foci in the liver. Increased splenic haemosiderin deposition and extramedullary haematopoiesis were noted at the mid dose (males only) and high dose (both sexes). Increased lipofuscin deposition in the transitional epithelium of the urinary bladder was noted in mid- and high-dose females. There was no increase in hepatocellular carcinomas. Three females from each dose group (0 from controls) had malignant lymphoma at one or more sites but because of the low incidence, lack of a dose-response, and lack of any effect in males, This is considered not to be directly compound-related. From these data, dazomet lacked carcinogenic potential in mice at the doses tested. NOEL, based on liver effects, was 20 ppm (about 4 mg/kg/d in males, 6 mg/kg/d in females).

6.2 Rat

6.2.1 2-Year Dietary Study

(Kuhborth et al (1989b) BASF Aktiengesellschaft; Pathology; Research & Consulting Co., Switzerland. Project no. 70C0318/8583, Doc. BASF:89/0276. 31 July, 1989) GLP/QA: yes.

Wistar rats (20/sex/group) were given dazomet (98.2%) in the diet at 0, 5, 20, 80 and 320 ppm for 2 years. Mean compound intakes were estimated as follows:- males; 0, 0.3, 1, 4 and 18 mg/kg/d: females; 0, 0.3, 1, 6 and 23 mg/kg/d.

Survival was not affected by compound administration. At the high dose there was a reduction in bodyweight gain. Ophthalmoscopy was negative. Clinical chemistry and haematological examinations revealed reduced total protein, albumin, globulins, triglycerides and serum cholinesterase (80 and 320 ppm females), decreased RBCs, Hb and Hct (80 and 320 ppm females) and increased platelets (320 ppm males, 80 and 320 ppm females). At the high dose, relative liver weights were increased in males while in females there was a slightly increased incidence and severity of hepatocellular fat deposition, which was associated with vacuolation in some animals. There were no substance-related effects at 5 or 20 ppm. There was no evidence of any oncogenic effect of dazomet at any of the doses tested. The NOEL was 20 ppm (about 1 mg/kg/d) for females and 80 ppm (about 4 mg/kg/d) for males.

6.2.2 Rat 2-Year Dietary Study

(Kuhborth et al (1989c) BASF Aktiengesellschaft; Pathology: Research & Consulting Co., Switzerland. Project no. 70C0318/8584. Doc. BASF:89/0277. 31 July, 1989) GLP/QA: yes.

Wistar rats (50/sex/group) were given dazomet (98.2%) in the diet at 0, 5, 20 and 80 ppm for 2 years. Mean compound intakes were estimated as follows:- males; 0, 0.3, 1 and 4 mg/kg/d: females; 0, 0.3, 1 and 6 mg/kg/d.

Survival was not affected by compound administration and there were no reported clinical signs. Bodyweight gain and food intake were also not affected. Haematological examinations revealed a possible increase in atypical lymphocytes in 80 ppm males but since this effect was slight and was not seen in the parallel toxicity study (ref. 17, see Section 6.2.1), it was considered incidental. No organ weight changes or gross lesions considered to be treatment-related were observed. In high-dose males, there was a slightly increased incidence of diffuse hepatocellular fat deposition and vacuolation. Incidences were as follows (n=50):

Dose (ppm)	0	5	20	80
Hepatocellular fat deposition, diffuse	3	2	1	8
Vacuolation	7	12	11	18

In high-dose females, there was a slightly increased incidence of mixed cell and basophilic cell foci. Incidences were as follows (n=50):

Dose (ppm)	0	5	20	80
Mixed cell foci	4	4	1	8
Basophilic cell foci	3	2	2	9

There were no substance-related effects at 5 or 20 ppm. There was no evidence of any oncogenic effect of dazomet. The NOEL was 20 ppm (about 1 mg/kg/d).

6.2.3 2-Year Dietary Study

(Weil & Palm (1960) Mellon Institute of Industrial Research, University of Pittsburgh, USA. 1 June 1960) GLP/QA: no

Dazomet (lot number BA-29; 90% purity) was administered to Carworth-Wistar rats (20/sex/group) at levels of 0, 10, 40, 160 and 640 ppm in the diet for 2 years. The rats were purchased from Carworth Farms and were 60 days of age when dosing was started. Satellite groups (5/sex) of the 0, 160 and 640 ppm dose levels were established at the beginning of the study to enable interim sacrifices after 6 and 12 months of treatment (kidney and liver weights were measured and histological examination conducted at these times). In addition, 4 males from the 40 ppm and control groups (from the original 20 males/group) were sacrificed after 6 months dosing and 4 males and 4 females also from the original groups were sacrificed after one year of dosing.

No data were presented on analysis of the diet for the test compound. Approximate dosages were as follows: males, 0, 0.4, 1.7, 6.4 and 28 mg/kg/d and females, 0, 0.5, 2.0, 7.4 and 31.8 mg/kg/d (ie. the average dose in females was about 13% higher than that in males). As is typical of these studies, the doses received were about 20% higher in the first 6 months of the study than in the remaining 18 months.

Mortality rates were not affected by treatment, although the mean days of age at death was reduced compared with controls in males given 40 ppm dazomet. Food consumption was measured over 28-day periods. Food consumption was reduced at doses of 160 and 640 ppm in both sexes, while food consumption at the 40 ppm dose was either higher than or comparable to the control level. Thus, mean food consumption over the period of the study in males and females at the 640 ppm dose was 97.0% and 90.6% of the control level. Corresponding figures for the 160 ppm dose were 95.0% and 96.2%. Food consumption at 10 ppm was comparable to the control level.

Rats were weighed once every other week for the first year and then at monthly intervals. For the first year of the study, group mean body weights were expressed as a percentage of the initial weights and compared to the corresponding control weights using the t-test. In both males and females of the 640 ppm group, 26 of the biweekly means were significantly below control values. The mean weight of the 640 ppm males at the end of the first year of the study was 89.8% of the control weight and the corresponding figure for females was 87.0%. The only other group that varied significantly from the control was the 160 ppm females, with 5 of the 26 values being significantly below control values. The mean weight of the 160 ppm females at the end of the first year of the study was 896.5% of the control weight. At the end of the study, body weights in this group did not show a statistically significant difference from controls, whereas body weights of both males and females of the 640 ppm group remained significantly below those of controls. The mean weights of both males and females at the 40 and 10 ppm dose levels were generally higher than their controls.

Liver and kidney weights were measured at sacrifice at 6 months, 1 year and 2 years. At 640 ppm, mean liver weights were significantly increased above controls at the 6 month (males only), and the one and two year sacrifices. At the end of the study, liver weight

(as a % of body weight) was increased by 32% in males and 23% in females at the 640 ppm dose. Mean kidney weights were significantly increased in the 640 ppm females at the one and two year sacrifices. At the end of the study, kidney weight (as a % of body weight) was increased by 22% in females at the 640 ppm dose.

Haematocrits were determined on 6 occasions during the 2-year study (days 31, 122, 210, 299, 480 and 672 of dosing) in ten rats from the control, 160 and 640 ppm dose groups. There was no clear effect of treatment on haematocrit.

The total incidence of neoplasms did not differ between the groups, nor did the incidence of any specific tumour type.

At the sacrifice after 6 months dosing, lung, kidney, liver, heart and adrenal were examined microscopically (4 - 9 animals per dose). There was some evidence of an effect of the chemical on the incidence of "cloudy swelling" of the kidney tubules. After 1 year of dosing, histopathological examination of lung, kidney, liver, stomach and urinary bladder was conducted on 3 - 7 animals per group. Again, there was evidence of an effect on the kidney, and also on the liver, as evidenced by an increased incidence of "hepatic focal fatty metamorphosis". At the end of the 2-year study, the following organs were examined histologically: lung, kidney, liver, heart, spleen, pancreas, stomach, duodenum, descending colon, testis or ovary, fallopian tube, urinary bladder and adrenal. At this final sacrifice, there was also an increased incidence of pathological lesions in the kidney and liver. Interpretation of the data was difficult because the results for the control females were not provided. The main lesion in the kidney resembled glomerular nephritis. Generally, it was characterised by some subcapsular "crescent formation", diffuse capsular adhesions and thickening of the glomerular basement membrane. Often the glomeruli showed a focal fibrotic or avascular appearance. Some glomerular cellulitis was present. Tubular changes ranged from frank necrosis of the proximal convoluted tubules to diffuse tubular cloudy swelling with focal fatty degeneration. The main lesions observed in the liver were focal necrosis and diffuse cloudy swelling of the hepatic cords. Central fatty metamorphosis was observed at the high dose. These results (final sacrifice) are summarised in the following table (the control data are for males only; numbers of animals/sex/group examined at sacrifice ranged from 5 to 15).

Percentage of animals (at final sacrifice) affected (in parenthesese, number of animals affected/number of animals examined)

Dose (ppm)	0	10	40	160	640
Liver					
animals examined	8	21	13	19	27
Focal necrosis	0 (0/8)	14 (3/21)	69 (9/13)	84 (16/19)	56 (15/27)
Cloudy swelling*	25 (2/8)	90 (19/21)	100 (13/13)	100 (19/19)	100 (27/27)
Kidney					
animals examined	8	21	13	19	27
Glomerular nephritis**	0 (0/8)	43 (9/21)	92 (12/13)	100 (19/19)	100 (27/27)
Crescent formation	25 (2/8)	38 (8/21)	31 (4/13)	63 (12/19)	93 (25/27)
Capsule adhesions*	13 (1/8)	71 (15/21)	77 (10/13)	100 (19/19)	85 (27/27)
Thick Basement membrane	88 (7/8)	100 (21/21)	100 (19/19)	100 (27/27)	100 (13/13)
Tubular necrosis (focal)	0 (0/8)	57 (12/21)	92 (12/13)	95 (18/19)	100 (27/27)
Tubular cloudy swelling*	50 (4/8)	100 (21/21)	100 (13/13)	100 (19/19)	100 (27/27)

* diffuse**;** ** subacute

The evaluator considers that there was no clear NOEL in this study (the authors concluded that 10 ppm dazomet in the diet for a period of two years was without evidence of permanent serious effect).

6.3 Dog

6.3.1 1-Year Dietary Study

(Hellwig et al (1989a) BASF Aktiengesellschaft. Project no. 33D0318/85118, Doc. BASF:89/0050. 24 February, 1989). GLP/QA: yes.

Beagle dogs (6/sex/group) were given dazomet (98.2%) in the diet at 0, 15, 50 and 150 ppm for one year. Mean compound intakes were estimated as 0, 0.5, 1.6 and 4.8 mg/kg/d.

Doses were chosen on the basis of several studies: two test feedings (dietary administration), a 3-month dietary study (see Section 5.4 above) and an old (1956/57) one-year study (capsule 5 d/week; dose level - 8 mg/kg) and a pilot study with administration by capsule. The first test feeding employed doses of 0, 750, 3000 and 12000 ppm. Vomiting, reduced feed consumption and loss of body weight were observed and the study had to be terminated after 5 days. The second test feeding employed doses of 0, 200, 400 and 600 ppm for 14 days. At the two higher doses, vomiting was observed during the first two days of test feeding and there was a reduction in food intake and body weight gain and in red blood cell parameters. In the one year capsule study, 'mild transitory kidney changes without serious permanent effect' were noted at histopathological examination. In the pilot study using dosing by capsule, dogs vomited very violently after doses of 80 and 320 mg/kg and showed unusual high-stepping gait and significant bodyweight loss. Hindlimb weakness was also observed at the high dose. Animals in these groups died, or were sacrificed after 5 doses. At 20 mg/kg, the incidence of vomiting was only slightly increased and there were no findings at necropsy (10 days).

In the main study, survival was not affected by compound administration. At the high dose there was a slight reduction in bodyweight gain. Although reduced bodyweight gain was very apparent in females over the first 3 weeks, these findings were largely attributable to obvious effects in one animal**.** Ophthalmoscopy showed only incidental findings. Clinical chemistry examinations revealed reduced albumin (high-dose females), AP, ALT and AST (some high-dose females). The one high-dose male which showed significant bodyweight effects had distinct changes in several clinicochemical and haematological parameters. Relative liver weights were increased in high dose males. Gross pathological findings included focal or diffuse discolouration of the liver in 2/6 high-dose females and granular surface of the liver in 1/6 high dose males (with slight jaundice). At histopathology, chronic liver lesions were apparent in 3 high-dose dogs; 2 females had moderate to severe chronic hepatitis and one male had severe cirrhosis. In addition, there was a minimal to moderate hepatocellular fatty change and marked hepatocellular hypertrophy in 2 of these animals. There was an increased deposition of iron-positive pigment in the liver at the mid dose (females only) and the high dose. Three high-dose males had minimal gastric erosions. There were no effects of the compound at the low dose. The NOEL, based on liver toxicity, was 50 ppm (about 1.6 mg/kg/d) for males and 15 ppm (about 0.5 mg/kg/d) for females.

7. REPRODUCTIVE TOXICITY

7.1 Rat

7.1.1 2-Generation Dietary Study.

(Hellwig et al (1989b) BASF Aktiengesellschaft. Project no. 71R0318/8597, Doc. BASF:89/0051. 22 February, 1989). GLP/QA: yes.

Dazomet (98.2%) was administered to immature Wistar rats (24/sex/group) in the feed at levels of 0, 5, 30 and 180 ppm for at least 70 d prior to mating to produce F_1a then F_1b litters; dosing was continuous throughout mating and lactation. Animals (24/sex/group) were selected from F_1a pups for mating and were maintained on compound-containing diets post-weaning; F_1b pups were retained only until weaning. During pre-mating (F_0 and F_1 animals) the intake of dazomet was about 0.49, 2.95 and 18 mg/kg/d in the respective groups; for females, these intakes increased significantly during lactation.

At the high dose (180 ppm), there was depression of body weights and/or bodyweight gains for F_1 males and F_0 and F_1 females (including gestation and lactation periods). At the mid dose (30 ppm), there were slightly reduced bodyweights/bodyweight gains in F_1 males. At the high dose, clinical chemistry revealed reduced ALT in F_0 (both sexes) and F_1 males, reduced serum globulin (F_0 and F_1 parental males), reduced albumin (F_0 and F_1 parental females) and reduced total protein (F_1 females). Relative liver weights were increased in F_0 males and F_1 animals of both sexes. At the mid dose and high dose, there was an increased incidence and severity of liver fatty change (increased intracellular neutral lipids), predominantly in F_0 and F_1 males, less so in females. There was no impairment of mating or reproductive performance and no adverse effect on reproductive organs. Various developmental and behavioural tests on pups were negative for compound-induced effects. Therefore the NOEL with respect to reproductive function in rats was equal to or greater than 180 ppm, while that for systemic toxicity was 5 ppm (about 0.49 mg/kg/d).

8. DEVELOPMENTAL TOXICITY

8.1 Rat

8.1.1 Oral Teratology Study

(Hellwig & Hidebrand (1987) BASF Aktiengesellschaft. Project no. 34R0318/8564, Doc. BASF:87/0457. 29 December, 1987). GLP/QA: yes.

Mated Wistar rats (25/group) were given dazomet (in olive oil) by gavage at doses of 0, 3, 10 and 30 mg/kg/d on day 6 to 15 of gestation. Dams were killed on gestation day 20.

There was reduced feed consumption during and after treatment at the high dose, with reduction in bodyweight for the first two days of dosing; thereafter animals gained weight but did not recover to control weights by day 20. Slightly reduced food intake was noted at the mid dose, only at the beginning of treatment, and was accompanied by a slight transient reduction in weight gain. Uterine weights were slightly reduced at the mid dose and high dose. There was also a higher incidence of runts at these doses, however, without a clear dose-response relationship. There was no evidence of teratogenic effects doses up to and including 30 mg/kg/d and there were no maternal or foetal effects at the low dose. The NOEL for maternal and foetal effects was 3 mg/kg/d. Under the conditions of this study, dazomet lacks teratogenic potential in the rat.

8.2 Rabbit

8.2.1 Oral Teratology Study

(Zeller & Merkle (1988a) BASF Aktiengesellschaft. Study no. jm-ru/db. 15 July, 1980)

Dazomet was administered by gavage to inseminated Himalayan rabbits (11 to 14 animals/group) at dose levels of 0 (two control groups, untreated and vehicle (carmellose) controls), 25, 50 or 75 mg/kg/d from day 6 to 18 of gestation (post-insemination). On day 30 of gestation the animals were sacrificed and their uterine contents examined.

Clinical signs of toxicity associated with dazomet treatment (severe diarrhoea, apathy and unsteady gait) were seen at 50 and 75 mg/kg/d and 2 dams died at 75 mg/kg/d. Food consumption was depressed at 75 mg/kg/d (38 to 45%) and to lesser extent at 50 mg/kg/d (3 to 26%) from day 6 through to 19. Food consumption was slightly but non-significantly depressed at 25 mg/kg/d during the treatment period. The body weights of dams treated with 75 mg/kg/d were significantly depressed (up to 10%) from day 7 to the end of the study, while dams treated with 50 mg/kg/d had significantly reduced body weights from day 26 to 30 of the study.

Both 50 and 75 mg/kg/d of the test substance had a profoundly detrimental effect on the number of live foetuses (about 80% reduction compared to vehicle controls). This embryolethal effect was associated with a correspondingly high number of dead implantations at 50 and 75 mg/kg/d.

There was no evidence of any treatment-related foetal abnormalities at any dose, but it is difficult to assess the results because of the low numbers of foetuses in the 50 and 75 mg/kg/d groups. The NOEL was 25 mg/kg/d for both maternal and embryo toxicity.

8.2.2 Oral Teratology Study

(Zeller & Merkle (1980b) BASF, Aktiengesellschaft. Study no. jm-ru/kb. 3 March, 1980)

Dazomet was administered by gavage to inseminated Himalayan rabbits (15/group) at dose levels of 0 (vehicle - carmellose), 6.25, 12.5 or 25 mg/kg/d from day 6 to 18 of gestation. On day 29 of gestation the animals were killed and their uterine contents examined.

There was no evidence of maternal toxicity at any dose level used in this study. Embryotoxicity was observed at 25 mg/kg/d, with significantly reduced numbers of live foetuses (15% reduction) associated with increased dead implantations (200% increase), and in particular, increased early resorptions. There were no treatment-related increases in developmental abnormalities at the doses employed in this study.

The NOEL for embryotoxicity was 12.5 mg/kg/d and 25.0 mg/kg/d for maternal toxicity.

9. GENOTOXICITY

9.1 Gene Mutation Assays

9.1.1 Bacterial Mutation (Ames) Test and Reverse Mutation Assay in *Saccharomyces cerevisiae*

(Shirasu et al (1977) Institute of Environmental Toxicology, Japan. 27 December, 1977)

The mutagenic activity of dazomet was studied in *E. coli* WP2 *hcr* and *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 (1 to 200 g/plate). The study was conducted in the presence and absence of metabolic activation (rat liver S9 mix). Positive controls, AF-2, -propriolactone, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene, gave the expected responses. The vehicle control was DMSO. Dazomet did not produce an increase in mutation frequency under the conditions of the study.

(Brusick & Weir (1976) Litton Bionetics, Inc., USA. 26 October 1976)

The mutagenic activity of "sample #100" (the sample was presumably dazomet, but this was not made clear), at concentrations of 0 (vehicle - DMSO), 0.1, 1.0, 10, 100 and 500 g/plate was studied in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and in *Saccharomyces cerevisiae* strain D4. Cytotoxicity was stated to be evident at the high concentration, and for some strains, at the highest two concentrations. The study was conducted in the presence and absence of metabolic activation (rat liver S9 mix). Positive controls, 2-nitrofluorene, quinacrine mustard and methylnitrosoguanidine (in the absence of metabolic activation) and 2-acetylaminofluorine, 8-aminoquinoline,and 2-anthramine (in the presence of activation), all used at 100 g/plate, except MNNG, at 10 g/plate, gave the expected responses. The test substance did not produce an increase in mutation frequency under the conditions of the study.

(Majeska et al (1980a) The In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Company, USA. 9 June 1980) QA: yes

Dazomet was tested for mutagenicity in the Ames test using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538. The studies were conducted in the presence and absence of metabolic activation. Three plates were used at each test concentration. The vehicle control was DMSO. The studies involving metabolic activation also included a medium control. Studies done in the presence of metabolic activation involved four systems: aroclor-induced rat liver S9 mix, phenobarbitone-induced rat liver S9 mix, aroclor-induced mouse liver S9 mix and phenobarbitone-induced mouse liver S9 mix. Concentrations tested were 3.7, 11.1, 33.3, 100 and 300 g/plate in the absence of metabolic activation and 12.3, 37.0, 111.1, 333.3 and 1000 g/plate, in the presence of metabolic activation, with the exception of the study using aroclor-induced rat liver S9 mix which used the 3.7 - 300 g/plate range but also included a second examination of strain TA100 at concentrations over the 12.3 - 1000 g/plate range. Cytotoxicity was observed at 300 g/plate and for some strains, also at 100 g/plate. Positive controls (2-anthramine at 5 g/plate, in the presence of activation, and sodium azide and 2-nitrofluorine at 10 g/plate and 9-aminoacridine at 5 g/plate, in the absence of activation) gave the expected responses. The test substance did not induce an increase in mutation frequency under any of the conditions studied.

(Andersen et. al. (1972) J. Agr. Food Chem. 20, 649 - 656)

This paper describes studies in which 110 chemicals were evaluated for mutagenicity in a number of tests, including the Ames test. The standard plate test was used, but the chemicals were not added to the top agar mixture, but rather "small crystals of solid test chemicals were applied directly to the surface of each plate after the top layer of agar, containing the mutant bacteria, had solidified" (the actual concentrations/amounts of chemical tested were not stated). The *S. typhimurium* strains used were not stated, but 8 different strains were used. No metabolic activation was used. Detailed results were not shown, but dazomet was reported as being negative in the Ames test. Positive controls were used and gave positive (no further details given) results.

(Moriya et. al. (1983) Mutation Research 116, 185 - 216)

Dazomet was one of 228 pesticides examined for mutagenic activity in the Ames test. *E. coli* WP2 *hcr* and *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were used. The study was conducted in the presence and absence of metabolic activation (S9 mix). Chemicals were tested at concentrations of up to 5000 g/plate, unless they showed cytotoxicity at lower concentrations. Detailed results were not presented, but dazomet was reported to be negative in this study.

9.1.2 Host-mediated Assay

(Shirasu et al (1977) Institute of Environmental Toxicology, Japan. 27 December, 1977)

The mutagenic activity of dazomet was studied in *S. typhimurium* G46 (50 and 100 mg/kg in mice; 6/group) in a host mediated assay. Dimethylnitrosamine was used as the positive control and showed the expected response. Dazomet did not produce an increase in mutation frequency under the conditions of the study.

9.1.3 Chinese hamster ovary cells - HGPRT locus

(Gelbke & Jackh (1986) BASF Aktiengesellschaft, Department of Toxicology, Germany. 18 August 1986)

The mutagenic effect of dazomet at the HGPRT locus of CHO cells was investigated. Preliminary range-finding cytotoxicity tests were conducted (as measured by cloning efficiencies in the attachment assay 24 h after exposure). Toxicity was achieved at >0.464<1.0 µg/mL. The standard test was conducted in which cells were pretreated in "HAT medium", then exposed to test substance for 4 h, with a proportion taken for cytogenicity determination. After a 9-day expression period (with subculturing), the cells were transferred into the selection medium containing 10 µM 6-thioguanine (5 flasks per concentration), with 7 days to colony formation. The study was conducted in the presence and absence of rat liver S9 mix and 3-methylcholanthrene, 0.01 mg/mL, as positive control in the presence of S9 mix and ethylmethanesulfonate, 0.3 mg/mL, in the absence. Positive control compounds produced elevated mutation rates.

Dazomet was tested in two experiments, each one being conducted both in the presence and absence of metabolic activation. In experiment 1, concentrations tested were 0 (culture medium), 0.00464, 0.01, 0.0215, 0.0464 and 0.1 µg/mL, and in experiment 2, concentrations tested were 0 (culture medium), 0.01, 0.0215, 0.0464, 0.1, 0.215 and 0.464 µg/mL.

The first experiment did not fully meet the criteria for acceptance that the authors had laid out (cloning efficiency in the negative controls was poor, both in the presence and absence of metabolic activation, and clear cytotoxicity was not observed in the presence of metabolic activation). Mutation rate was zero in the negative controls (both in the presence and absence of metabolic activation) and small positive mutation rates were observed at the 3 lowest doses in the presence of metabolic activation (not concentration related) and at the two highest doses in the absence of metabolic activation (also not concentration related).

In the second experiment, cloning efficiencies of the negative control, and cytotoxicity results were acceptable. Mutation rate was again zero in the solvent controls (absence and presence of metabolic activation). Positive mutation rates were observed at concentrations of 0.01, 0.0215 and 0.464 µg/mL in the presence of metabolic activation (ie. not clearly concentration related, but the authors considered it to be a triphasic response) and at 0.215 and 0.464 µg/mL in the absence of metabolic activation (ie. concentration related). The results of experiment 2 are summarised in the table below:

Metabolic activation present Metabolic activation absent

Concentration (ug/mL)	Mutation rate*	Cytotoxicity (%)	Mutation rate*	Cytotoxicity (%)
0	0	51.0	0	75.0
0.01	13.3	47.5	0	45.5
0.0215	25.3	44.0	0	42.0
0.0464	0	29.75	0	53.5
0.1	0	20	0	43.5
0.215	0	28.25	8.0	52.5
0.464	36.0	28.25	20.0	27.5

* number of mutants per 10^6 cells (not corrected for cytotoxicity)

Dazomet was considered to be mutagenic under the test conditions employed.

9.2 Chromosomal Effects Assays

9.2.1 Human - *in vitro* lymphocytes

(Gelbke (1989) BASF Aktiengesellschaft, Department of Toxicology, Federal Republic of Germany. 4 April 1989) QA: yes

A pretest was conducted to determine cytotoxicity. The highest concentration tested in the main assay was based on the quality of the metaphases and not on the mitotic index because disruption of chromosomes such that evaluation was no longer possible occurred at lower concentrations than the required reduction in mitotic index (about 50%). Concentrations tested were 0, 0.002, 0.01 and 0.05 µg/mL in the absence of activation and 0, 2.5, 12 and 25 µg/mL in the presence of metabolic activation.

Duplicate cultures were used for all experimental groups. After PHA stimulation and incubation at 37^0C for 48 h, cultures were treated with dazomet. In the absence of metabolic activation, cells were incubated with dazomet for 24 h and in presence of metabolic activation (rat liver S-9 mix), for 2 h, followed by 22 h in fresh medium. Cells were arrested in metaphase by the addition of colchemid 2-3 h prior to harvest. After staining, 100 cells from each culture (50 for the positive control) were examined for chromosomal aberrations.

Negative and solvent (DMSO) controls were included in the study. Positive controls (0.1 µg/mL mitomycin in the absence of metabolic activation and 6 µg/mL cyclophosphamide in the presence of metabolic activation (rat liver S-9 mix)) induced significant increases in the number of aberrant metaphases.

In the absence of metabolic activation, dazomet induced a significant increase in the number of metaphases with aberrations at the high concentration, and in the presence of metabolic activation, dazomet induced a significant increase in the number of metaphases with aberrations at the low and high concentrations tested, but only when gaps were included in the analysis. When gaps were excluded from the analysis, there were no differences the numbers of chromosome aberrations between groups. When compared to the control groups, there were no differences in incidences of aneuploides and polyploidies in the test groups. It was concluded that under the conditions chosen, dazomet was not clastogenic.

9.2.2 Mammalian cytogenetics (non human) - *in vivo* bone marrow

(Stetka & Brusick (1979) Litton Bionetics, Inc., USA. 22 August 1979)

Dazomet was administered by gavage to male Sprague-Dawley rats at doses of 0 (distilled water control), 1, 6, 20 and 60 mg/kg (either a single dose or 5 consecutive doses). Dose levels were based on the LD_{50} values, with the high dose being half the LD_{50}. The rats (8 per dose/time point group) were killed at 6, 24 or 48 h after the dosing (single dose study) and 6 h after the last dose (sub-chronic dosing study) and colchicine was injected IP 3 h prior to sacrifice. Tibial bone marrow was collected and about 50 bone marrow spreads were read for each dosed animal.

The positive control (TEM, 1 mg/kg IP; 2 animals killed at 24 h after the last dose) induced a significant increase in the number of cells with chromosome aberrations and in the frequency of structural aberrations. Dazomet at the lowest dose (single dose study) and the 24 h sacrifice induced a small but significant increase in the number of cells with aberrations (this was due to the presence of hyperdiploid cells and to the fact that there were no aberrations at all in the concurrent controls). At the mid dose (single dose study), 48 h sacrifice, there was a significant increase in the structural aberration frequency (number of structural aberrations per cell). The aberrations were all fragments or acentric fragments. There was no indication of a positive dose response for either percent of cells with aberrations or frequency of structural aberrations in either the single dose or the sub-chronic study, and therefore it is reasonable to conclude that under the conditions of the study, dazomet was not clastogenic.

9.2.3 Micronucleus test - *in vivo* mouse study

(Gelbke & Engelhardt (1985) BASF Aktiengesellschaft, Department of Toxicology, Federal Republic of Germany. 24 May 1985) **GLP/QA: yes**

Basamid granular was given as a single oral administration to NMRI mice (5/sex/group, except the high-dose group which consisted of 15 animals/sex, ie.5/sex/time point) at doses of 0 (vehicle - DMSO), 45, 90 and 180 mg/kg. Doses were based on findings in acute toxicity studies in which deaths were observed down to an oral dose of 215 mg/kg, but all animals survived a dose of 178 mg/kg (although it led to irregular respiration and excitation about 15 min after dosing). In the current study, clinical signs were observed at the mid (piloerection) and high (irregular respiration, excitation, squatting posture, piloerection and apathy) doses. Gross pathological examination of animals at the end of the study did not reveal any changes.

Bone marrow smears from the two femora from each animal were prepared after sacrifice at 24 h after dosing. For the high dose, groups of animals were also sacrificed at 16 and 48 h after dosing. From each animal 1000 polychromatic erythrocytes were evaluated for micronuclei.

The positive control, cyclophosphamide (40 mg/kg PO), resulted in a significant increase in the number of cells with micronuclei/1000 polychromatic erythrocytes whereas dazomet treatment did not affect the number of cells with micronuclei/1000 polychromatic erythrocytes. The ratio of polychromatic to normochromatic nuclei was also not affected by dazomet treatment.

Under the conditions of this study, dazomet did not reveal any clastogenic potential.

9.2.3 Mammalian cytogenetics (non human) - male germ cell studies

(Miltenburger et al (1985) Laboratory for Mutagenicity Testing of the Technical University, Darmstadt, Federal Republic of Germany. 14 November 1985) GLP/QA: yes

Dazomet was tested for its potential to induce chromosome aberrations in spermatogonia of Chinese hamsters. Male animals (10/group) were dosed by gavage with 0, (vehicle), 10, 33 and 100 mg/kg dazomet. Doses were determined in a dose-range finding study. Animals were sacrificed at 18 h after dosing. At the high dose, there were two additional sacrifice times, 42 and 66 h. The high dose induced clinical signs but was not lethal. Cells were arrested in metaphase by IP administration of colchicine prior to sacrifice. Both testes were taken for the preparation of spermatogonia and for each animal 50 metaphases were scored for structural chromosomal aberrations. The initial positive control used (cyclophosphamide, 100 mg/kg, presumably PO) did not elicit an increase in chromosome aberrations. A second experiment was therefore conducted in which doxorubicin HCl (30 mg/kg presumably PO) was used as the positive control compound, and it caused a large increase in the number of cells with chromosome aberrations. This second study also included a test on dazomet at the high dose with an 18 h sacrifice time. The result of this test was comparable with the result obtained in the first study for the same conditions.

Treatment with dazomet resulted in a small increase in the number of cells with chromosomal aberrations above control levels, but this increase was not considered to be toxicologically significant (statistical analysis was not performed). The results can be summarised as follows:

			% aberrant cells	
Test	Dose	h	incl. gaps	excl. gaps
Negative control	0	18	2.2	1.0
Cyclophosphamide	100	18	2.4	1.2
Dazomet	10	18	2.6	1.0
Dazomet	33	18	3.6	1.4
Dazomet	100	18	4.6	1.8
Dazomet	100	42	2.8	1.6
Dazomet	100	66	4.2	1.0
Doxorubicin	30	18	27.0	20.8
Dazomet	100	18	3.0	1.8

9.2.4 Multiple endpoint studies

Results from a mouse lymphoma multiple endpoint study (see Section 9.3.3) and an *in vitro* cytogenetic assay measuring sister chromatid exchange and chromosome aberrations (see Section 9.3.4) are presented below.

9.3 DNA Damage and Other Genotoxic Effects Assays

9.3.1 Bacillus subtilis rec assay

(Shirasu et al (1977) Institute of Environmental Toxicology, Japan. 27 December, 1977)

The mutagenic activity of dazomet was studied in *B. subtilis* M45 and H17. Basamid was dissolved in DMSO at concentrations of 10 to 200 µg/disk and a filter paper disk soaked in the compound solution was placed so as to cover the starting parts of the bacterial streaks. DMSO was used as the vehicle control. Kanamycin and mitomycin were used as positive controls, and gave the expected responses. The study was conducted both in the presence and absence of metabolic activation. Dazomet did not show any evidence of a genotoxic effect under the conditions of the study.

9.3.2 *Bacillus subtilis* rec assay

(Hazelton (1987) Hazleton Biotechnologies Veenendaal Laboratory, the Netherlands. Study no. E-9583. January, 1987)

This study assessed the potential of dazomet (0.1 to 10000 g/plate) to cause primary DNA damage in *Bacillus subtilis* strains H17 (*rec*+) and M45 (*rec*-). The study was carried out in the presence and absence of metabolic activation and included positive controls (methylmethane-sulfonate (5 µL/plate) in the absence of activation and sterigmatocystin (100 µg/plate) in the presence of activation) and negative (DMSO) controls. Three plates per dose level were used. The positive controls produced differences in inhibition zones of greater than 4 mm, although only marginally in the case of sterigmatocystin. Dazomet did not, at any of the concentrations tested, produce differences in inhibition zones of greater than 4 mm.

Dazomet did not exhibit genotoxic activity in this assay.

9.3.3 Mouse lymphoma multiple endpoint test

***(Tmajeska et al (1980b) he In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Company, USA. 20 November 1980*) QA: yes**

Dazomet was tested for genetic activity in the L5178Y mouse lymphoma multiple endpoint test which covers mutation frequency at the thymidine kinase locus and the induction of chromosome aberrations and sister chromatid exchanges.

Forward mutation assay at the TK locus

In the forward mutation assay, the cell line (TK^{+}/-) is treated with test compound, after which the suspended cells are incubated for 48 h to allow expression of the induced TK-/$^{-}$ phenotype, then cloned in soft agar, either in selective medium for TFT resistant cells or in non-selective medium for viable counts. Dazomet was tested in this assay in three different experiments, all involving the absence of metabolic activation and two involving the presence of metabolic activation. The following concentrations were used in the absence of metabolic activation - experiment 1: 0 (solvent control, DMSO), 0.15, 0.46, 1.37, 4.12, 12.35 µg/mL; and experiments 2 and 3: 0 (medium and solvent controls), 0.6, 0.8, 1.0, 2.0, 3.0 (experiment 3 only) and 4.0 µg/mL. The following concentrations were tested in the presence of metabolic activation (rat liver S9 mix) - experiment 1: 0 (solvent control), 1.37, 4.12, 12.35, 37.04 and 111.11 µg/mL and experiment 2: 0 (medium and solvent controls), 4, 6, 8, 10 and 20 µg/mL. Higher concentrations were tested in the presence of activation than in its absence because of the lower toxicity of dazomet in the presence of activation.

Dazomet did not increase the mutant frequency when tested in the presence of activation. The results obtained in the absence of activation are shown in the table below. No increase in mutant frequency was observed in experiment 1. In experiments 2 and 3, increases in mutant frequency were observed but were not concentration dependent. In experiment 2, an increase above the solvent control level, of 2.5-fold was observed at 1 µg/mL, of 1.5-fold was observed at 2 µg/mL and of 3.4-fold was observed at 4 µg/mL. In experiment 3, increases in mutation frequencies of about 3-fold were observed at concentrations of 0.6, 0.8, 1 and 4 µg/mL and of about 2-fold were observed at concentrations of 2 and 3 µg/mL.

Experiment 1

	Mutation frequency $x10^{-6}$ *	Measure of cytotoxicity**
Solvent control	25	100
Positive control	321	79
Dazomet (µg/mL)		
0.15	18	93
0.46	20	72
1.37	10	126
4.12	19	46
12.35	27	25

Experiment 2

	Mutation frequency $x10^{-6}$ *	Measure of cytotoxicity**
Medium control	30	116
Solvent control	46	100
Positive control	634	50
Dazomet (µg/mL)		
0.6	32	98
0.8	58	71
1.0	114	61
2.0	70	43
4.0	158	12

Experiment 3

	Mutation frequency $x10^{-6}$ *	Measure of cytotoxicity**
Medium control	46	89
	35	104
Solvent control	38	100
	47	100
Positive control	309	63

Dazomet (µg/mL)		
0.6	131	40
0.8	145	26
1.0	131	20
2.0	87	6
3.0	86	7
4.0	109	2

* mutant clones/viable clones x (2×10^{-4})
** relative suspension growth (% of control) x relative cloning efficiency (% of control)/100

Positive controls (ethylmethanesulfonate, 0.5 µL/mL in the absence of metabolic activation and BP, 6 µg/mL or DMH, 0.3 µg/mL, in the presence of metabolic activation) gave the expected responses.

It can be concluded that dazomet showed evidence of being weakly mutagenic under the conditions of this assay.

Chromosome aberration assay

For this assay, the cells were treated and then incubated for 24 h in the presence of 10^{-4} mM BrDU. Colchicine was added 3 h prior to harvest and after preparation and staining, 50 metaphases/test concentration were scored. Two experiments were conducted, both in the presence and absence of metabolic activation (rat liver S9 mix). Concentrations tested in the absence of metabolic activation were - in experiment 1: 0, 0.8, 1, 2, 4 and 5 µg/mL and in experiment 2: 0, 0.6, 0.8, 1, 2 and 4 µg/mL. Concentrations tested in the presence of metabolic activation were - in experiment 1: 0, 6, 8, 10, 20 and 30 µg/mL and in experiment 2: 0, 4, 6, 8, 10 and 20 µg/mL. Each experiment included both medium and solvent (DMSO) controls and positive controls which gave the expected results.

Dazomet did not increase the number of cells with chromosome aberrations when tested in the presence of activation. In the absence of activation, significant increases were observed in the number of cells with aberrations. The analysis did not include gaps.

Results are summarised in the following table:

Experiment 1:	% of cells with aberrations	
	Structural aberrations	Numerical aberrations
Medium control	0	0
Solvent control	0	0
EMS (0.5 µL/mL)	12	0
Dazomet (µg/mL)		
0.8	2	0
1.0	0	0
2.0	0	4
4.0	6	6
5.0	8	26

Experiment 2:

Medium control	0	2
Medium Control	0	0
Solvent control	0	2
Solvent Control	0	6
EMS (0.5 µL/mL)	10	4
Dazomet (µg/mL)		
0.6	2	4
0.8	0	6
1.0	0	2
2.0	2	6
4.0	8	10

These results were reported to be statistically significant (at 4 and 5 µg/mL in experiment 1 and at 2 µg/mL in experiment 2 (it is unclear to the evaluator why the results at 4 µg/mL in experiment 2 were not significant and whether the analysis referred to the structural or the numerical aberrations)). Results for positive controls were reported as significant in both experiments. Endoreduplication, a rare numerical aberration, was observed at most concentrations of dazomet. Translocations, triradials and quadriradial, which are rare structural aberrations, were observed at some concentrations.

It was concluded that dazomet can induce chromosome aberrations when assayed in the absence of metabolic activation. The data suggest that the metabolites, unlike the parent compound, are not clastogenic.

Sister chromatid exchange (SCE)

The study of the ability of dazomet to induce sister chromatid exchange was largely done under the same experimental conditions as the experiments described above for the study of chromosome aberrations except that cells taken for scoring sister chromatid exchanges were stained with Hoechst stain rather than Giesma and 15 to 20 cells per concentration were scored if possible (there were slight variations in the numbers scored at the different concentrations). The number of sister chromatid exchanges per cell or per chromosome was not significantly affected by treatment of the cells with dazomet. At the higher concentrations of dazomet in experiment 1 in the absence of activation and at the highest concentration in experiment 2 in the presence of activation, the number of cells which could be counted was less than 15 - 20.

9.3.4 *In vitro* cytogenetic assay measuring sister chromatid exchange and chromosome aberrations

(Lbrusick & Stetka (1979) itton Bionetics, Inc., Maryland, USA. March 1979)

Dazomet was evaluated for its ability to induce chromosome aberrations in L5178Y mouse lymphoma cells. Medium was used as the negative control, DMSO as the solvent control. Cytotoxicity (measured as loss in growth potential of cells given a 4-h exposure to the chemical, followed by a 24-h expression period) was assessed in preliminary studies to determine concentrations to be tested. Concentrations of dazomet tested, both in the presence or absence of metabolic activation (rat liver S-9 mix), were 0 (negative and vehicle controls), 1.56, 3.13, 6.25, 12.50 and 25.00 ng/mL. Cells were exposed to chemical for 4 h at 37^0C, washed, and then incubated for 20 h, with colchicine added 3 h before harvest. After staining, 50 cells per test point were examined for chromosome aberrations and 10 cells (about 370-380 chromosomes) per test point were examined for SCEs.

Both in the presence and absence of metabolic activation, increases in the number of cells with aberrations were observed, although in neither case was the increase concentration dependent. The results are shown in the following table.

	Percentage of cells with aberrations	
	Absence of S-9	Presence of S-9
Negative control	0	4
Solvent control	2	0
Positive control	30**	36**
Dazomet concn (ng/mL)		
1.56	14*	8*
3.13	2	4
6.25	12	10*
12.50	0	4
25.00	4	10*

* significant increase at the 5% level (** and at the 1% level) in the number of cells with aberrations.

Positive control compounds, ethylmethanesulfonate (0.5 µL/mL) in the absence of metabolic activation, and dimethylnitrosamine (0.3 µL/mL) in the presence of activation, induced significant increases in the number of cells with aberrations and in numbers of SCEs.

The lack of a dose response relationship for the dazomet results on chromosome aberrations makes the results equivocal, but the sizeable increase in number of cells with aberrations at 1.56 and 6.25 ng/mL in the absence of metabolic activation, the consistent increase in the number of cells with aberrations at all concentrations tested in the presence of metabolic activation, and the fact that several breakage-reunion type aberrations which are rare in controls (translocation, triradials and complex rearrangement) were observed in the presence of dazomet (mainly in the absence of metabolic activation), suggest that dazomet is weakly clastogenic.

In the absence of metabolic activation, numbers of SCEs/chromosome were comparable to control levels for all concentrations of dazomet tested, except the highest concentration at which there was a significant increase (5% level) from 0.244 (negative control) and 0.257 (solvent control) to 0.352 (25 ng dazomet/mL). In the presence of metabolic activation, SCE numbers were comparable for all groups. Thus, the results in the absence of metabolic activation suggest that dazomet may induce SCEs, but there is no clear evidence for any SCE-inducing potential.

9.3.5 Unscheduled DNA synthesis - rat primary hepatocytes

(Cifone & Myhr (1985) Litton Bionetics, Inc, Maryland, USA. June 1985) GLP/QA: yes

Three experiments were conducted, but experiment 2 was terminated because of insufficient toxicity. In all the experiments, 5 cultures were subjected to each treatment, two of which were used for cytotoxicity measurements (viable cell counts by trypan blue exclusion determined at 23.5 h after the addition of test compound). Treatment lasted for 18-19 h in the presence of test compound and ^{3}H-thymidine. Fifty cells were counted from each culture, giving a total of 150 cells examined per test concentration. In both experiments presented, the positive control (2-acetyl aminofluorene, 0.05 µg/mL) was not highly toxic (81.7 - 102.8% survival compared to solvent controls), but induced significant and acceptable increases in unscheduled DNA synthesis as measured by the average net nuclear grain counts and average % nuclei with >6 and >20 grains.

Experimental conditions were satisfactory in both experiments as indicated by the following (experiment 1/experiment 2): viability of hepatocytes collected by perfusion with collagenase was 84.9%/84.1% (>50% is acceptable) and viability of the monolayer cell cultures was 77.2%/89.9% (>70% is acceptable). Viability of cells in the solvent control at the end of the study was 84.3%/86.9% (>50% is acceptable). Labelling of the nuclei in the solvent control was also within acceptable limits for both experiments.

Dazomet was tested in this experiment at concentrations of 0 (vehicle - acetone), 0.125, 0.25, 0.5, 1.25, 2.5, 5.0, 12.5, 25.0, 50.0 and 500 µg/mL. Concentrations of 50 and 500 µg/mL were completely lethal and 25 µg/mL was highly toxic (10.7% cell survival compared to the solvent control, resulting in insufficient cells to evaluate). At 12.5 µg/mL, cell survival was 25% compared to the solvent control and the cells were rounded indicating cellular toxicity. At 5 µg/mL, survival was 76% and the cells appeared normal.

The results of experiment 1 are shown in the following table because they show increases in unscheduled DNA synthesis.

	Concn. (µg/mL)	Av. net nuclear grain counts	Av. % nuclei with >6 grains	Av. % nuclei with >20 grains
Solvent control	0.05% acetone	1.12	2.0	0.0
Positive control	0.05 2-AAF	19.42	94.7	45.3

Dazomet	25.0	excessive toxicity; insufficient cells to evaluate		
	12.5	1.71	4.7	0.0
	5.0	2.71	16.0	0.0
	2.5	2.32	11.3	0.0
	1.25	2.08	8.7	0.0
	0.5	1.73	2.7	0.0
	0.25	1.04	1.3	0.0
	0.125	1.84	6.0	0.0

If the test compound satisfied any one of the following criteria it was considered positive in the assay:

(i) mean nuclear grain count to exceed 6 grains/nucleus after subtraction of the solvent control - ie. mean net nuclear count of 7.12 for exp. 1;
(ii) the percent of nuclei having 6 or more net grains to exceed 10% after subtraction of the solvent control - ie. 12% for exp. 1; and
(iii) the percent of nuclei having 20 or more net grains to exceed 2%.
A dose-related increase in unscheduled DNA synthesis for 2 consecutive concentrations is also desirable.

For criterion 2, the response at 5 µg/mL exceeded criterion (ii) and the response at 2.5 µg/mL was elevated, but did not exceed any of the criteria. There was also a dose response for criteria (i) and (ii) over the concentration range 1.25 to 5 µg/mL.

Another experiment was performed because of the positive results found in this experiment. Concentrations tested ranged from 1.5 to 20 µg/mL and the results are presented in the following table.

	Concn. (µg/mL)	Av. net nuclear grain counts	Av. % nuclei with >6 grains	Av. % nuclei >20 grains
Solvent control	0.05% acteone	0.54	0.7	0.0
Negative control	0.58	0.7	0.0	
Positive control	0.05 2-AAF	8.15	63.3	4.7
Dazomet	15 & 20	excessive toxicity; insufficient cells to evaluate		
	10.0	1.36	3.0	0.0
	7.5	1.84	10.0	0.0
	5.0	2.03	11.3	0.0
	3.0	1.48	5.3	0.0
	1.5	1.29	4.7	0.0

Survival ranged from 49.9% to 97.4% over the concentration range 1.5 to 10 µg/mL. As occurred in experiment 1, one concentration (5.0 µg/mL) induced nuclei with 6 or more grains that exceeded the criterion for a positive response. The response at 7.5 µg/mL was also elevated, although the minimum criterion was not met. There was also a dose response over the range 1.5 to 5 µg/mL. The results of this experiment therefore confirm the activity observed in the first experiment.

It can be concluded that dazomet showed weak activity in this assay.

9.3.6 Unscheduled DNA synthesis - rat primary hepatocytes

(Cifone (1986) Hazleton Biotechnologies Company, Maryland, USA. September 1986) GLP/QA: yes

Rats (3/dose; strain and sex not specified) were administered dazomet by gavage at doses of 0 (vehicle - DMSO), 37.5, 75, 150 and 300 mg/kg and the animals were sacrificed at 4 h after dosing. This choice of dose and sacrifice time was based on a knowledge of the LD_{50} and a preliminary experiment in which one animal was administered a dose of 350 mg/kg, and another animal, a dose of 250 mg/kg. The animal that received 350 mg/kg showed a very toxic reaction and was sacrificed at 2 h after dosing, while the animal receiving 250 mg/kg showed a toxic reaction but appeared normal at sacrifice (15 h). Slides "prepared for unscheduled DNA synthesis" (presumably liver cells were prepared and assayed for UDS) from these animals were said to be comparable. Dimethylnitrosamine (10 mg/kg IP) was used as the positive control (3 rats treated).

The assay was conducted with an 18-19 h labelling period and the criteria for designating a compound as positive in the assay were as described in the above section (Section 9.3.5). Eight cultures were prepared from each animal. Net nuclear grain count was determined for 50 cells from three cultures per animal (ie. 50 x 3 = 150 cells). Experimental conditions were satisfactory as indicated by the following: viability of hepatocytes collected by collagenase perfusion was 72.4 - 90.3%, viability of the monolayer cell cultures was 90.5 - 96.7% and viability of cells in the solvent control at the end of the study was 66.4 - >100%.

While the positive control was clearly positive for all the criteria for a positive compound, none of the dazomet treatments were positive for any of the criteria (they did not result in nuclear labelling significantly different from the vehicle control). It was concluded that dazomet was inactive in the *in vivo* rat hepatocyte unscheduled DNA synthesis assay.

9.4 Cell transformation

9.4.1 Cell transformation studies: BALB/c-3T3 cells

(Matheson & Brusick (1978) Litton Bionetics, Inc., Maryland, USA. 2 June 1978)

Dazomet was tested for its ability to induce morphological transformation in BALB/c-3T3 cells *in vitro*. Dazomet was incubated with the test cells for 72 h at 37^0C, following which the cells were for 3-4 weeks in fresh medium. Generally, ten replicate plates per test concentration were scored. Concentrations tested were 0 (solvent control - DMSO), 0.078, 0.156, 0.312, 0.625 and 1.25 µg/mL. Test concentrations were chosen on the basis of a preliminary test which showed significant cytotoxicity at 2.5 and 5 µg/mL, hence the highest concentration chosen was 1.25 µg/mL. Dazomet did not induce an increase in the number of foci (transformed clones) above solvent control levels. In contrast, the positive control, 3-methylcholanthrene (5 µg/mL), caused a significant increase in number of foci per plate. It was concluded that dazomet did not induce morphological transformation under the conditions of this assay.

9.4.2 Cell transformation studies: BALB/c-3T3 cells

(Matheson & Majeska (1980) Stauffer Chemical Company, USA. 5 December 1980)

Dazomet was tested for its ability to induce morphological transformation in BALB/c-3T3 cells *in vitro*. Dazomet was incubated with the test cells for 72 h, following which the cells were incubated for 4-6 weeks in fresh medium. Fifteen replicate flasks per test concentration were scored. Concentrations tested were 0 (solvent control - DMSO), 0.025, 0.050, 0.10, 0.20 and 0.40 µg/mL. Test concentrations were chosen on the basis of a range-finding study in which cytotoxicity (reduction in clonal survival) was observed at all the concentrations tested (0.15 µg/mL - 3.0 mg/mL), with zero cell survival at 4.12 µg/mL and above. The positive control, 3-methylcholanthrene (5 µg/mL), caused an increase in number of foci per flask, but no colonies were observed in any flasks in which the cells had been treated with dazomet. It was concluded that dazomet did not induce morphological transformation under the conditions of this assay.

10. HUMAN STUDIES

10.1 Dermal Sensitisation Study

Jung *et al.* (1987) utilised case reports from the international literature to form an opinion on acceptable patch test concentrations for human experimentation. A particular case report mentioned contact dermatitis resulting from exposure to dazomet, but no further details were supplied.

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List of Abbreviations/Acronyms

mg	Milligram
kg	Kilogram
mL	Millilitre
L	Litre
ng	Nanogram
µg	Microgram
m	Metre
d	Day
h	Hour
s	Second
iv	Intravenous
po	Oral
sc	Subcutaneous
id	Intradermal
ip	Intraperitoneal
im	Intramuscular
NOEL	No Observed Effect Level
ADI	Acceptable Daily Intake
MRL	Maximum Residue Limit
TGAC	Technical Grade Active Constituent
EUP	End Use Product
FASD	First Aid and Safety Directions
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
GLP	Good Laboratory Practice
QA	Quality Assurance
PACC	The former
PACSC	The former Pesticides and Agricultural Chemicals Standing Committee of the NHMRC

APPENDIX I

Structures of dazomet and impurities

Dazomet:

($C_5H_{10}N_2S_2$; RMM = 162.3)
Tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-thione
CAS-Reg.-No.: 533-74-4
Internal code LAB-No.: 9328 (equivalent to 14 531)

"[3+5]-dehydro-dimer of Dazomet":

($C_{10}H_{18}N_4S_4$; RMM = 322.5)
3,3'-Ethylenedi[tetrahydro-5-methyl-1,3,5-thiadiazine]-2,6'-dithione
Internal code LAB-No.: 277 940

"[3+3]-dehydro-dimer of Dazomet":

($C_{10}H_{18}N_4S_4$; RMM = 322.5)
3,3'-Ethylenedi[tetrahydro-5-methyl-1,3,5-thiadiazine-2-thione]
Internal code LAB-No.: 277 455

Structures of dazomet and impurities (cont.)

"[5+5]-dehydro-dimer of Dazomet":

($C_{10}H_{18}N_4S_4$; RMM = 322.5)
5,5'-Ethylenedi[tetrahydro-3-methyl-1,3,5-thiadiazine-2-thione]
Internal code LAB-No.: 277 939

NB: 3,3'-ethylenedi[tetrahydro-5-methyl-1,3,5-thiadiazine]- 2,6'-dithione is the same compound as 3',5-dimethyl-3,5'-ethylenedi[tetrahydro-1,3,5-thiadiazine-2-thione]

METHYLISOTHIOCYANATE (MITC)

SUMMARY

Introduction

Methylisothiocyanate (MITC) is a pre-plant soil fumigant for control of nematodes, soil fungi and insects. It is a relatively volatile compound, which therefore evaporates from the soil into the air. It is currently in schedule 6 of the SUSDP. No ADI has been set. It is not currently registered in Australia because the application was withdrawn by the company and has never been considered by the PACC or PACSC.

Metabolism and Toxicokinetics

Evidence was presented that orally administered MITC is excreted in rats as a mercapturic acid conjugate. This is likely to proceed via glutathione, cysteinylglycine and cysteine conjugates. Excretion in the rat was largely urinary and no unchanged MITC was present in 24 h urine. A tissue distribution study revealed highest concentrations of radioactivity in the thyroid and pituitary at 7 d postdose. The available data in rats suggest that MITC is likely to be completely metabolised to single carbon compounds which are incorporated into the general metabolic pool.

Urine was also the major route of excretion in the dog, but a large proportion of administered radioactivity (about 20%) remained in the tissues at 7 d postdose, with liver and thyroid having the highest concentrations. Urinary metabolic profile differed considerably between rats and dogs.

Acute Toxicity

MITC is a moderately toxic compound. The oral LD_{50} is 72 mg/kg in rats and 90 mg/kg in mice. The acute oral NOEL in dogs is approximately 0.1 mg/kg based on necropsy findings at 0.5 mg/kg. In monkeys, the acute oral NOEL was < 10 mg/kg. In rabbits, the acute oral NOEL was > 10 mg/kg. Haemorrhagic lesions were observed in the stomach in monkeys and dogs. The dermal LD_{50} was 1870 mg/kg in mice, approximately 1000 mg/kg in rats and 33 mg/kg in rabbits. Inhalation LC_{50} was 540 mg/m^3 in rats (4 h exposure).

MITC was shown to be a severe eye and skin irritant in rabbits. In maximization tests in guinea pigs, MITC showed weak skin sensitising potential.

Short Term Repeat Dose Studies

No NOEL was demonstrated for skin effects after repeated daily application of MITC to rats for 1 month. The lowest dose tested was 1 mg/kg/d. Erosion/necrosis of the skin was observed at doses of 100 mg/kg and above.

Subchronic Toxicity

Several subchronic (3 month) gavage studies have been conducted at doses up to 20 mg/kg/d in mice, 40 mg/kg/d in rats and 2 mg/kg/day in dogs. In rats and mice, corrosive effects on the stomach were observed. In all species tested, changes were observed in the liver, in particular fatty changes. "Spermatogenic disorder" was observed in rats and mice and a decrease in testes weight in dogs. In some studies a NOEL could not be demonstrated. The NOEL in the mice (gavage) study was 0.7 mg/kg based on increased liver weight at 1 mg/kg/d. The NOEL in the dog (gavage) study was 0.04 mg/kg/d based on decreased testes weight and increased liver effects at 0.4 mg/kg/d.

In a 3 month inhalation study (nose only 4 h/d) the NOEL was 10 ppm (30.7 mg/m^3) based on clinical signs (apathy, salivation and nasal discharge) observed at 45 ppm.

Chronic Toxicity

Chronic (2 year) studies with MITC administered in drinking water have been conducted in mice and rats. There was no carcinogenic effect observed in either species. The major toxicological findings were decreased body weight gain and decreased food or water intake. The NOEL for the mice study was 20 ppm (3.48 mg/kg/d) in drinking water (the next highest dose being 80 ppm) and the NOEL for the rat study was 10 ppm (0.5 mg/kg/d) in drinking water (the next highest dose being 50 ppm).

Reproductive Toxicity

In a three-generation oral reproduction study, rats were dosed at 0, 1, 3 and 10 mg/kg (5 d/week) beginning at 28 d of age in all generations. Histopathological examination revealed increases in the incidences and severity of mucosal acanthosis and hyperkeratosis in the forestomach (non-glandular portion of the stomach) of treated animals (F_0, F_1 and F_2 generations). Parameters of reproductive performance and the incidences of gross fetal abnormalities were not altered by treatment for any generation. No NOEL was observed in this study because the forestomach lesions occurred at all dose levels (ie. NOEL < 1 mg/kg/d).

Developmental Toxicity

Two gavage teratology studies were conducted in rats. Doses of MITC that did not cause maternal toxicity were not associated with fetal toxicity, nor teratogenicity. In the first study, at the highest dose tested (25 mg/kg/d) there was fetal growth retardation, presumably secondary to decreased maternal weight gain and decreased maternal food intake. The NOEL was 5 mg/kg, based on decreased fetal size at 25 mg/kg/d. In the second study, there was an increase in the number of runts at the high dose (30 mg/kg/d), presumably secondary to decreased maternal weight gain. NOEL for embryo/foetotoxicity in this study was 10 mg/kg/d. There was no clear NOEL for maternotoxicity because of a reduction in "corrected" body weight at all doses tested.

Three oral teratology studies were conducted in rabbits. In the first study (0, 1, 3 and 10 mg/kg/d), there was high mortality of does at the high dose and body weight was deleteriously affected at the mid and high doses. At the high dose, there was an increase in the number of resorptions and a decrease in the number of live pups at birth, in pup birth weight and in 24-h pup viability. There was a dose-related increase in the percent of fetuses with incompletely ossified sternum sections. NOEL for maternal toxicity was considered to be 1 mg/kg/d, while that for fetal toxicity was 3 mg/kg/d (if the increase in the percent of fetuses with incompletely ossified sternum sections is not considered, otherwise no NOEL was determined for fetal toxicity).

In the second study (doses of 0, 1, 3 and 5 mg/kg/d), numbers of fetuses were higher in the test groups than in the control group and there was a significant reduction in weight and length of high-dose fetuses . There was a increased incidence of minor anomalies of the heart or major blood vessels in high-dose fetuses , probably due to fetal growth retardation, and although the total incidence of fetuses with skeletal variants was comparable for all groups, there was an increase in the incidence of fetuses with an extra pair of ribs in the high-dose fetuses . The NOEL for both maternal and fetal toxicity was considered to be 5 mg/kg/d.

In the third study (doses of 0, 1, 3 and 10 mg/kg/d), there were no effects on the fetuses . Thus the NOEL for embryo/fetotoxicity was 10 mg/kg/d. The NOEL for maternotoxicity was 3 mg/kg/d because of a reduced body weight gain over the treatment period in the high-dose does.

Genotoxicity

MITC was tested in the Ames test in a number of laboratories: Inveresk Research Laboratories, Schering laboratories, BASF Laboratories, Germany and the Institute of Environmental Toxicology, Tokyo. All studies were conducted both in the presence and absence of metabolic activation and used 4 or 5 strains of *S. typhimurium*, and the latter additionally used an *E. coli* strain. MITC was tested at up to toxic concentrations in all studies. There was no evidence of a mutagenic potential of MITC in any of these studies or in a test for gene mutation at the HGPRT locus in Chinese hamster V79 cells.

MITC was negative in two rec assays using *B. subtilis*, and it did not induce unscheduled DNA synthesis in an assay in primary rat hepatocytes.

Whilst MITC did not show evidence of a chromosome damaging effect in an *in vivo* mouse micronucleus test employing an oral dose at the LD_{10}, or in an *in vitro* chromosome aberration test in human lymphocytes, it was positive in an *in vitro* chromosome aberration test in Chinese hamster V79 cells. In the *in vitro* test, MITC induced an increase in the percentage of aberrant cells (both including and excluding gaps), and caused nuclear disintegration, both in the presence and absence of metabolic activation at a harvest conducted at 28 h after the start of treatment. A large proportion of the aberrations induced were chromosome breaks and chromosome exchanges. Minimal or no increases were seen at the other harvest times (6 h and 12 h).

DISCUSSION

MITC (technical) is severely corrosive to tissues and is thus a severe eye and skin irritant. Corrosive effects on the stomach were observed in rats, mice, dogs and monkeys following oral dosing. MITC was shown to have skin sensitisation potential. MITC is of moderate acute toxicity via the oral, dermal and inhalational routes. High doses of MITC appeared to cause some nervous system effects such as convulsions, piloerection and changes in posture.

The liver is a target organ and to a lesser extent, the testes. Thus, in 3-month oral mouse studies, increased liver weights were observed at doses of 0.7 mg/kg and above and fatty degeneration of the liver was observed at 20 mg/kg. In a 3-month oral dog study, there was an increase in the incidence and severity of periportal hepatocyte vacuolation and lipid deposition at 0.4 and 2 mg/kg. "Spermatogenic disorder" was observed in a 3-month oral study in mice at a dose of 20 mg/kg, but not at 5 mg/kg, and in a 3-month oral rat study at 40 mg/kg (10/10 animals affected) and at 10 mg/kg (1/10). There was no evidence of an effect on reproductive performance in rats, but the reproductive study only employed doses (oral) of up to 10 mg/kg (stomach, but not liver or testicular lesions were observed in this study).

MITC was not carcinogenic in mice at doses of up to about 27 mg/kg/d in the drinking water for 2 years, nor in rats at doses of up to 1.6 (females) or 2.7 mg/kg/d (males). The major toxicological findings in the chronic studies were decreased body weight and decreased food or water consumption. Hepatotoxicity and testicular toxicity were not observed in the 2-year mouse study, possibly due to a different strain being used compared to that in the 3-month study. No histopathological effects were observed in the 2-year rat study, probably because of the relatively low doses used. MITC was not teratogenic at oral doses of up to 25 mg/kg in the rat and at oral doses of up to 10 mg/kg in the rabbit.

There were adequate data to establish an NOEL. The dog appears to be the most sensitive species. The lowest NOEL of about 0.04 mg/kg/d was established in a 3-month dog study (gavage). This was based on decreased testes weight and increased liver effects at 0.4 mg/kg/d. In the 2-year rat study (compound administered via drinking water), an NOEL of about 0.5 mg/kg/d was established based on decreased body weight gain at the next highest dose.

There was no evidence of a mutagenic effect of MITC in a number of tests covering the major end-points. These testes included Ames tests (*S. typhimurium* and *E. coli*), a test for gene mutation at the HGPRT locus, a test for chromosome aberrrations in human lymphocytes *in vitro* and tests for DNA damage (rec assays in *B. subtilis* and unscheduled DNA synthesis assay in primary rat hepatocytes). MITC was negative in an *in vivo* clastogenicity test (mouse micronucleus test), but was clearly positive in an *in vitro* clastogenicity test (chromosome aberration test in Chinese hamster V79 cells) at one time point, but not at other time points. MITC could be considered to have equivocal clastogenic potential.

DRAFT RECOMMENDATIONS

1. Whilst a considerable number of additional studies have been reviewed in this evaluation, no change in the previously established NOEL is required. The lowest NOEL is 0.04 mg/kg/d based on decreased testes weights and liver effects seen in the 3 month gavage study in dogs.

2. The scheduling of MITC in schedule 6 is appropriate on toxicological grounds.

3. The existing SDs for MITC are:

Methyl isothiocyanate	All strengths	130 131 132 133 206 162 161 163 164 210 211 220 222 230 279 280 283 290 292 294 298 301 330 331 332 342 340 341 342 343 340 341 343 370

Information on formulations and use pattern/application methods for each product are usually used in developing SDs. However, given that MITC is not marketed in Australia, such information is not availableand so SDs, if prescribed, would have to be based on the 96% TGAC. Given the toxicological profile of technical MITC, the following SDs, based on hazard, may be appropriate (Worksafe would prescribe SDs related to PPE):

Amendments

Methy isothiocyanate - amend entry to read:

Methyl isothiocyanate	All strengths	100 130 131 132 133 205 206 162 220 222 223 230 207 164 340 343 330 332 340 342 **...** **...** PPE from Worksafe) **...** **...** **...** **...** **...** **...** **...** **...** **...** **...**

No change to the existing First Aid Instructions for MITC is recommended.

4. WSA should consider by-stander exposure to gaseous MITC.

SUMMARY OF TOXICOLOGICAL HAZARD (TGAC)

Date of preparation:	July 1996
Chemical name:	Methylisothiocyanate
Worst oral LD_{50} in rats:	72 mg/kg
Worst oral LD_{50} in other species:	90 mg/kg in mice
Worst dermal LD_{50}:	33 mg/kg in rabbits
Worst inhalation LC_{50}:	540 mg/m^3 in rats
Skin irritation:	severe in rabbits
Eye irritation:	corrosive in rabbits
Skin sensitisation:	weak skin sensitisation in guinea pigs
Remarks:	
T-value:	7
NOEL:	The lowest NOEL was 0.04 mg/kg/d, based on decreased testes weights and liver effects seen in a 3-month gavage study in dogs.

METHYLISOTHIOCYANATE

1. INTRODUCTION

1.1 Historical Background

Methylisothiocyanate (MITC) is manufactured by Schering Pty Ltd for use as a pre-plant soil fumigant for control of nematodes, soil fungi and insects. It is currently in schedule 6. No ADI has been established due to its lack for potential residues in food. The company withdrew the application for TGAC clearance of MITC because of problems with the other active constituent (1,3-dichloropropene) in their product (Di-Trapex) containing MITC. It should be noted that former PACSC had examined the data submitted and had recommended to the Public Health Committee of the National Health & Medical Research Council that the TGAC for MITC be cleared.

The current Ad Hoc Review consists of a consolidation of previous evaluations, together with evaluations of a number of studies not previously considered, namely metabolism studies (rats, dogs and soil), acute toxicity study in rabbits, 3-generation reproductive study in rats, two developmental toxicity studies in rabbits, genotoxicity studies and a study of allergic contact dermatitis in humans.

1.2 Chemical Identity

IUPAC name: methylisothiocyanate

Chemical name: isothiocyanatomethane

CAS No: 556-61-6

Synonyms: isothiocyanic acid methyl ester, methyl mustard oil, SN 32866,

Empirical formula: CH_3NCS

Structural formula: $\mathbf{CH_3\text{-}N{=}C{=}S}$

Molecular weight: 73.12

1.3 Chemical and Physical Properties

Colour: Colourless when pure; brownish/yellow as TGAC

Odour: pungent horseradish-like; smell threshold @ 23^oC = 16 mg/L

Physical State: brown oily or clear yellow liquid (TGAC)

Vapour Pressure: 20.7 mm Hg @ 20^oC

Melting Point:	35^{o}C
Boiling Point:	119^{o}C
Flash Point:	30-40^{o}C
Density:	1,069 mg/ml @ 37.2^{o}C
Solubility:	8.2 g/L for water; readily soluble in most organic solvents
Octanol/Water Partition Coefficient:	10.5 @ 25^{o}C
Hydrolysis:	Hydrolysed under acid and basic conditions
Stability:	pH 5 $t_{1/2}$: 85 h pH 7 $t_{1/2}$ 490 h pH 9 $t_{1/2}$ 110 h
Reactivity:	reacts readily with inorganic acids, bases, metal salts, halogens; natural and synthetic rubber and PVC; and is corrosive to metals (Zn, Fe, Al, Mg) especially in presence of water; severely affected by MITC

(Major degradation products are methylamine and N,N-dimethylthiourea).

2. METABOLISM AND TOXICOKINETICS

2.1 Rat

2.1.1 Oral metabolism study

(Mennicke, et al., 1983)

Male Wistar rats were given a single oral dose of 10 mg MITC suspended in olive oil. Urine was collected and metabolites were identified by TLC. MITC was reported to be excreted as a mercapturic acid, with the structure of a dithiocarbamidic ester, formed by the addition of the isothiocyanate group to the SH group of the cysteine component. Evidence for this was obtained following the synthesis the N-acetylcysteine conjugate (dithiocarbamidic ester) and the achievement of identical TLC results to those for urine samples. The metabolite was also isolated from urine and its i.r. and ^{1}H-n.m.r. spectra were identical with those of the synthetic substance.

2.1.2 Oral metabolism study

(Needham et al (1986) FBC Limited Chesterford Park Research Station, England. Report no METAB/86/12. 6 May 1986).

MITC, ^{14}C-labelled in the methyl group (20 µCi/mg), was administered as a single oral (gavage) dose of 10 mg/kg (vehicle - corn oil) to Sprague-Dawley rats (5/sex). Mass-balance data were similar for males and females. Excretion data are summarised below:

Route	**Time**	**% dose excreted**
Urine	24 h	77.0
Urine	7 d	81.0
Faeces	7 d	1.9
Volatiles	7 d	5.5
Tissue residues	7 d	4.1
Total recovery	7 d	92.5

Thus, the predominant route of excretion was urine, with a large proportion of the radioactive dose being excreted in 24 h.

Tissue concentrations of radioactivity at 7 d (expressed as mg MITC equiv./kg tissue) were highest in the thyroid (about 1.0) and pituitary (about 0.8). Levels in liver, kidney, heart, lungs, spleen, adrenals, muscle and ovaries were generally in the range 0.3 - 0.5, whilst those in testes, fat, eyes, brain, bone, gastrointestinal tract, blood, plasma and residual carcase were generally in the range 0.1 - 0.3 mg MITC equiv./kg tissue.

HPLC analysis of 24 h urine indicated that unchanged MITC was not present and that a major metabolite accounted for 65 - 86% of the total urinary radioactivity. The remaining radioactivity consisted of 3 more polar materials in approximately equal proportions. Co-chromatography of the major metabolite with the synthetic standard , N-acetyl-S-(N-methylthiocarbamoyl)-L-cysteine revealed that they were the same compounds, thus confirming that the major route of metabolism involves conjugation of MITC with mercapturic acid as shown below.

Such formation of mercapturic acid conjugates is considered to proceed via glutathione, cysteinylglycine and cysteine conjugates and it is likely that these intermediates may account for one or more of the 3 minor metabolites observed.

When liver taken from rats at 12 h postdose was heated with dilute sodium hydroxide, approximately 70% of radioactivity was converted to volatile material, almost certainly methylamine. At this stage, radioactivity is present either as MITC and/or the mercapturic acid, both of which decompose to yield methylamine under these conditions. However, at 7 d postdose, the same process yielded no methylamine, indicating the absence of MITC or closely related conjugates at this time. Extraction studies with a variety of agents suggested that, at this time, low levels of radioactivity were associated with the free amino acid pool and that a large proportion of radiolabel may be associated with soluble and insoluble proteins. Taken together, the data suggest that MITC is likely to be completely metabolised to single carbon compounds which are incorporated into the general metabolic pool.

2.2 Dog

2.2.1 Oral metabolism study

(Campbell & Needham (1986) FBC Limited Chesterford Park Research Station, England. Report no METAB/86/10. 6 May 1986).

MITC, ^{14}C-labelled in the methyl group (40 µCi/mg), was administered as a single oral (gavage) dose of 0.5 mg/kg (vehicle - corn oil) to 6 overnight-fasted dogs (beagles/mongrels not stated; 2M, 4F). Radioactivity in blood and plasma was measured in 5 animals and was similar in males and females, peaking at between 3 and 6 h. The half-life of plasma radioactivity was long, being 178 ± 45 h, from 72 h post dose onwards. Tissue levels of radioactivity were measured in 5 animals at 7 d postdose. Levels were highest in liver (about 0.5 mg MITC equiv./kg tissue), followed by thyroid, and were lowest in CSF (about 0.01 mg MITC equiv/L) and then bone. Levels in most other tissues were in the range 0.1 - 0.2 mg MITC equiv/kg. The radioactivity remaining in the tissues at 7 d was estimated to represent a substantial portion of the dose (16 - 25%). Over 7 d, 57 - 70% of the dose was recovered in the excreta, most being in the urine (50 - 56 % of the dose) and little (3 - 8% of the dose) in the faeces. Most of the urinary excretion (43 - 48% of the dose) occurred in the first 48 h. The release of radioactivity into volatile radioactivity was determined approximately in one dog. It was estimated that about 7.1% of the dose is excreted in this form. Assuming this figure for all dogs, total recovery of radioactivity was 88 - 96%. An investigation of the contents of the ethanolamine traps indicated that only a small proportion of the volatile radioactivity was $^{14}CO_2$. The nature and source of the volatile radioactivity is unknown. The volatile radioactivity may be excreted via the lungs or may arise from the excreta in the cage.

Dog urine was analysed by TLC. Comparable profiles were seen for males and females. Profiles for dogs were quite different from profiles obtained for rats in another study (dose 5 mg/kg). The two major metabolites in dog urine were not separated for identification. Further work was conducted in an effort to determine whether MITC is converted to formic acid (which would result in the ^{14}C methyl group being incorporated into the metabolic pool). Attempts to detect ^{14}C-formic acid in liver and urine samples and ^{14}C-amino acids in plasma from the dogs on study were unsuccessful, although this does not constitute proof of their absence.

2.3 Soil

(Willenbrink et al., (1966) and Krotter (1966))

In a study (ref. 36) conducted in desiccators filled with compost soil (water content 20%, pH 6.8) into which ^{35}S-MITC (as a 20% xylene solution) had been injected to a depth of 5 cm, 37% of the applied MITC was evaporated into the air in 22 d. The daily amount evaporated into the air depended largely on the velocity of the air exchange. A second study (ref. 37), also conducted in desiccators, similarly revealed that with high air exchange rates, more MITC (from both formulations tested, TRAPEX and DI-TRAPEX, the latter being 20% MITC + 80% dichloropropene-dichloropropane) is given off from the soil to the air than when the circulation is low. MITC escaped more quickly from a soil with little moisture (10%) than from a soil with a higher water content (18%), about 61% escaping compared with 40% in 21 d. The two formulations were comparable in the evaporation of MITC into the air. The volatile product analysed almost entirely as MITC (97 - 99%).

3. ACUTE TOXICITY

3.1 Lethal Dose Studies

The LD_{50} of MITC (tgac and EUP) in several species is shown in Table 1. Clinical signs of poisoning included an initial decrease in movement, lethargy followed by vigorous movement and tonic convulsions; accompanying these were nasal discharge, lacrimation, salivation, piloerection, changes in posture and vocalisation. Except for convulsions which only appeared following oral, ip or inhalational exposure these symptoms were present via all dose routes.

Table 1: Acute lethality of MITC[a]

Species	Strain	Sex	No of gps/ anim/sex/gp	Route	LD_{50} (mg/kg)[b]	Ref
Rat	Sprague	M		PO	220 (110 - 440)	Vernot et al (1977)
Rat	Donryu	M	5/10	PO	175 (147 - 205)	Tokyo (1970b)
Rat	Donryu	F	5/10	PO	72 (63 - 83)	Kondo (1974a)
Rat	Wistar	M/F	6/5	PO	95 (86 - 104)	Rosskamp et al (1979)
Rat	Wistar	M	4/5	PO	approx. 163	Kirsch & Kieczka (1986)
Rat	Wistar	F	4/5	PO	approx. 147	Kirsch & Kieczka (1986)
Mouse	dd	M	5/10	PO	90 (70 - 123)	Tokyo (1970b)

Mouse	CF-1	M	?/?	PO	110 (40 - 300)	Vernot et al (1977)
Mouse	dd	F	6/10	PO)	104 (88 - 123)	Kondo (1974b)
Mouse	NMRI	M	3/5	PO	approx. 120	Hildebrande & Kirsch (1987a)
Mouse	NMRI	F	3/5	PO	approx. 100	Hildebrande & Kirsch (1987a)
Rat	Donryu	M	6/10	Dermal	2780 (2260 - 3419)	
Rat	Wistar	M	4/5	Dermal	approx. 1000[d]	Hidebrande & Kirsch (1987b)
Rat	Wistar	F	4/5	Dermal	1930	Hidebrande & Kirsch (1987b)
Mouse	dd	M	5/10	Dermal	1870 (1290 - 2712)	

Rabbit	NZ White	F	?/3	Dermal	33[e] (18 - 64)	Vernot et al (1977)
Rabbit	NZ White	M/F	3/5	Dermal	263[e]	Kynoch & Parcell (1986)
Rat	Donryu	M	5/10	SC	60 (54 - 67)	Kondo (1974a)
Rat	Donryu	F	5/10	SC	59 (53 - 66)	Kondo (1974a)
Mouse	dd	M	5/10	SC	75 (63 - 89)	Kondao (1974b)
Mouse	dd	F	5/10	SC	89 (78 - 101)	Kondo (1974b)
Rat	Donryu	M	5/10	IP	54 (49 - 60)	Kondo (1974a)
Rat	Donryu	F	5/10	IP	56 (49 - 64)	Kondo (1974a)

Mouse	dd	M	5/10	IP	82 (73 - 93)	Kondo (1974b)
Mouse	dd	F	5/10	IP	89 (78 - 101)	Kondo (1974b)
Rat	CFY	M/F	5)/5	Inhalation	1900[c]	Clark & Jackson (1977a)
Rat	Sprague	M	6/5	Inhalation	540[c]	Jackson et al (1981)
Rat	Sprague	F	6/5	Inhalation	540[c]	Jackson et a (1981)
VORLEX 77						
Rat	CFY	M/ F	3 (+ ctl)/2	PO	0.18 (0.16 - 0.21)*	Kynoch & Lloyd (1977a)
Rabbit	NZ white	M/ F	3 (+ ctl)/1	Dermal	0.09 (0.07 - 0.12)*[f]	Kynoch & Lloyd (1977b)
Rat	CFY	M/ F	3 (+ ctl)/2	IP	0.15 (0.12 - 0.17)*	Kynoch & Lloyd (1977c)
Rat	Sprague	M/ F	4 (+ ctl)/5	Inhalation	5600[g]	Clark & Jackson (1977b)

* mL/kg

a. Clinical symptoms include lethargy, nasal discharge, lacrimation, salivation, piloerection, changes in posture, vocalisation, vigorous movement and convulsions.

b. 95% confidence interval in parentheses.

c. The result from Clark & Jackson (1977a)referes to a 1 h $_{50}$ (mg/m^3). Hyperactivity was observed in all five test groups (concentration of MITC from 0.6 - 3.1 g/m^3) during the first 5 min of exposure. Eye irritation, dyspnoea and hypoactivity were observed during the remainder of the exposure period. Convulsions, followed by death, were observed in most rats at the 3.1 g/m^3 level. Necropsy of animals that died revealed congestion of the lungs, often associated with small areas of haemorrhage. Necropsy observations of survivors were normal.

The result from Jackson et al (1981) refers to a 4 h LC_{50} (mg/m^3). Clinical signs noted during exposure were consistent with marked irritation and included closure of the eyes, lacrimation and peripheral vasodilation in all esposed rats, and a hunched posture in the majority of exposed rats. Peripheral vasodilation persisted some hours following exposure. Rales was heard in exposed rats for several days following exposure. Opacity of the eyes was observed in rats given doses of 0.5 g/m^3 and above. Macroscopic and histopathological examination of animals that died revealed congestion, oedema, bronchiolitis, interstitial pneumonitis and intra-alveolar haemorrhage in the lungs, stomach and intestines distended with gas (attributed to the gasping noted in these animals prior to death), and focal necrosis in the liver. In all rats that died, there was a marked increase in the lung to bodyweight ratio.

d. In the study reported in Hildebrand & Kirsch (1987b), the application site was covered with a semi-occulsive dressing for 24 h and site was rinsed after the dressing was removed. Local findings included erythema, oedema and scaling. Necropsy findings in animals that died were general congestion and bloody ulcerations in the glandular stomach.

e. The reason for the difference in these two studies is not clear especially since the study reported in Vernot et al (1977) involved a 4 h application and the study reported in Kynoch & Parcell (1986) involved a 24 h application.Vernot et al (1977) provided no information other than the LD_{50}. In Kynoch & Parcell (1986), compound was applied to the clipped dorsal surface (10% of body surface) under an occlusive dressing and was washed off after 24 h. LD_{50} (M + F) was estimated as 263 (207 - 322) mg/kg. This estimate was for both sexes combined, but females were slightly more sensitive than males (LD_{50}s being in the region of 200 and 320 mg/kg, respectively). Clinical signs at all dose levels included gasping, piloerection, salivation, lethargy, unsteady gait, "collapsed state", pallor of extremities and iridial inflammation. For most animals, recovery was complete between d 8 and 11.

Severe dermal reactions, with necrosis and well-defined to moderate oedema, were seen at the site of application in all animals. These persisted throughout the observation period. Terminal autopsy revealed bruising and oedema of the subcutaneous tissue at the treatment site in surviving animals. Other necropsy findings were normal.

f. Exposure was for 24 h and was occluded.

g. Groups of Sprague-Dawley rats (5/sex) were exposed (whole body exposure; inhalational route of administration) continuously for 1 h to the vapour produced from liquid MITC (VORLEX 77). Air samples were taken from the chamber during exposure to analyse the atmospheric concentration of VORLEX 77. Periods of hyperactivity were observed in the four test groups during the first 20-30 min of exposure. Eye irritation, vasodilation, laboured breathing and hypoactivity were observed subsequently during the exposure. Abnormalities seen in animals that died were moderate to severe lung congestion, areas of lung haemorrhage and distended gas-filled stomachs and small intestines.

In addition to the 'traditional' lethality studies, there have been three dose ranging studies to determine no effect levels for adverse effects following acute oral exposure. These are detailed below:

(a) Dose Ranging Study in Dogs

(Reno (1976a) Hazleton America. 28 April, 1976)

Beagle dogs (n = 8) were orally dosed with technical MITC dissolved in corn oil and given in gelatine capsules at 0, 0.1, 0.5, 1.0, 4.64, 10.0, 21.5, 46.4, 100 mg/kg (n = 1 per dose, except for 10.0 and 21.5 where 2 and 3 dogs, respectively received these doses; thus, four dogs received two different doses (interval between doses not stated)). No signs of toxicity were observed at doses of up to 0.5 mg/kg. Clinical signs observed at doses of 1.0 - 46.5 mg/kg included emesis, sanguineous emesis, retching, salivation, soft or dark coloured stools, decreased activity and generalised weakness. In surviving animals signs of poisoning abated two days after dosing and surviving dogs showed slight to moderate weight gain. An animal given 46.4 mg/kg survived, but one of two dogs given 21.5 mg/kg and one dog given 100 mg/kg, died. In the two dogs which died, additional clinical signs included ataxia, hyperpnoea, miosis and phonation. Necropsy findings that were common to the two dogs that died included congested liver and kidneys, and haemorrhagic lesions in many organs, particularly, the stomach and intestines. Foci of reddening were observed in some organs in surviving dogs, including the dog given 0.5 mg/kg (necropsy at 14 d). The NOEL was 0.1 mg/kg based on necropsy findings.

(b) Dose Ranging Study in Monkeys

(Reno (1976b) Hazleton America. 28 April, 1976)

Juvenile male rhesus monkeys (*Macaca mulatta*) were dosed with technical MITC dissolved in corn oil via gavage at 10, 21.5, 46.4 and 100 mg/kg (n = 1 per dose). Clinical signs were as follows: at 10 mg/kg - diarrhoea; at 21.5 mg/kg - none; at 46.4 mg/kg, emesis, mydriasis and diarrhoea followed by very firm stools; and at 100 mg/kg, salivation, sanguineous emesis, sanguineous diarrhoea, epistaxis, dyspnoea, inappetence, depression, difficult movement followed by inability to move due to weakness, with death occurring at d 2. Necropsy findings (d 7) were unremarkable in survivors. In the animal that died, necropsy revealed mottled liver, necrotic and haemorrhagic areas on the stomach mucosa and blood in the stomach. No NOEL was demonstrated in this study.

(c) Dose Ranging Study in Rabbits

(Reno (1976c) Hazleton America. April, 1976, 1976)

One New Zealand White rabbit received a single oral (gavage) dose of 10.0 mg/kg (vehicle - corn oil) and a second rabbit received a dose of 21.5 mg/kg. No signs of toxicity were observed over the 14 d observation period. It was concluded that the NOEL of MITC in rabbits was greater than 21.5 mg/kg.

In Conclusion, MITC is a moderately toxic compound following oral administration. Gross pathology of the stomach and intestines at autopsy is typical of a severe irritant.

3.2 Eye Irritation Studies

3.2.1 Rabbit

(Baker (1974) Industrial Biotest Laboratories Inc.. 18 February, 1974)

Instillation of 50 mg MITC undiluted into the eye (unwashed) of the albino rabbit (strain unstated; n = 6) caused immediate vocalisation lasting for 1 minute. In all 6 animals, miosis was observed at 1 min and 1 h. No reaction to light was observed in all 6 animals from 1 min until 7 or 14 d. All animals had a maximum score of 10 for iris irritation, at least in the first day following dosing, and in most animals, throughout the 14 d observation period. Chemical burn was observed in all 6 animals, with conjunctival scores for redness, swelling and discharge of 16 or 18 out of a possible maximum of 20. In two animals, corneal ulceration and vascularisation were observed on observation d 7 and 14, and in another two, vascularisation. These 4 animals had corneal irritation scores of 60 or 80 out of a possible maximum of 80. MITC was corrosive and a severe eye irritant under the conditions of this assay.

3.2.2 Rabbit

(Kynoch & Liggett (1976) Huntingdon Research Centre, UK. 6913/134D/76. 23 December, 1976)

Instillation of 0.1 mL MITC into the rabbit (albino, strain unspecified) eye (unwashed; n = 2) caused severe iritis, corneal opacities involving the whole corneal surface and considerable conjunctival swelling in both animals. Diffuse crimson reddening of the conjunctivae, considerable conjunctival swelling, and in one animal, complete closure of the eye, were observed. Due to the severity of the eye effects both rabbits were killed on day 2.

MITC was corrosive to the eye and a severe eye irritant under the conditions of this assay.

3.3 Dermal Irritation Studies

3.3.1 Rabbit

(Liggett & Parcell (1986) Huntingdon Research Centre, UK. Tox/85/203-17. 20 January, 1986)

A single semi-occlusive application of 0.5 mL technical MITC applied under a 2.5 cm^2 gauze pad to New Zealand White rabbit skin (intact, clipped) for 4 h (followed by washing) caused moderate to severe erythema and moderate oedema which persisted throughout the 14 d observation period. Some discolouration of the skin and blanching was seen. There was desquamation of the stratum corneum from d 10. Due to the severity of response, only one animal (female) was used. Thus, MITC is a severe skin irritant.

3.4 Dermal Sensitisation

3.4.1 Guinea pig

(Kieczka & Kirsch (1986) Department of Toxicology, BASF Aktiengesellschaft, Federal Republic of Germany. Project No. 30H231/85. December 1986). GLP:no. QA: yes.

In a guinea pig maximisation test based on the OECD Guidelines, MITC was tested in female Pirbright White and Dunkin Hartley guinea pigs (10 per each of two control groups and 20 per test group). Intradermal induction (6 injections; 2 of 0.1 mL of Freund's adjuvant emulsified in water (1:1), 2 of 0.1 mL of substance formulation (or vehicle for controls) and 2 of 0.1 mL of Freund's adjuvant emulsified in substance formulation (or vehicle for controls (1:1))) was conducted in the shoulder region one week prior to percutaneous induction. Vehicle was olive oil DAB 8. Percutaneous induction involved a 48 h application of filter paper strips (soaked in 2% substance formulation (or vehicle for controls) to the shoulder under an occlusive dressing.

Each group was challenged with a non-irritant concentration (0.5%) of MITC (right flank), as determined by a pretest, and with vehicle (left flank), except control group 2 which was not challenged with test substance at the first challenge. The first challenge was about 14 days after percutaneous induction and the second challenge was one week later. Filter paper strips soaked in test formulation or vehicle for controls were applied to the clipped flank under an occulsive dressing for 24 h.

The number of animals with skin findings after challenge (at 24 h after removal of the dressing) is shown in the following table:

	1st challenge		2nd challenge	
	0.5% test substance	vehicle	0.5% test substance	vehicle
Control group 1	0/10	0/10	0/10	0/10
Control group 2	N/A	0/10	0/10	0/10
Test group	12/20	0/20	13/20	1/20

It was concluded that MITC had a weak sensitising effect on the skin of the guinea pig under the conditions of this study.

3.4.2 Guinea pig (MITC formulated as TRAPEX 40)

(Ullman & Sachse (1986) Research & Consulting Company, Switzerland. PF-85.848. 7 March 1986). GLP/QA: yes.

In the maximization guinea pig hypersensitivity test (protocol according to US EPA guidelines), MITC (formulated as TRAPEX 40; no further details given of this formulation) exhibited weak skin sensitising potential and was a moderate skin irritant. Dunkin-Hartley guinea pigs (5/sex in the control group and 10/sex in the test group) were used. A preliminary study was conducted to determine an irritant test compound concentration for the induction phase of the main study and a non-irritant concentration for the topical challenge. Skin reactions were assessed after intradermal injections and topical application of the test compound at concentrations of 0.1, 0.5 and 1%.

In the main study, induction was as follows: three pairs of intradermal injections (0.1 mL/site) were made in the dorsal skin of the scapular region. Injections were of Freund's complete adjuvant 50:50 with corn oil, the test compound diluted to a final concentration of 1% emulsified in a 1:1 mixture of Freund's adjuvant and corn oil. One week later, a filter paper patch, saturated with 1% test compound, was placed over the injection sites and secured in place for 48 h. The guinea pigs of the control group received identical testing, but without the test compound. Challenge, at 2 weeks after topical induction, was as follows: a filter paper patch saturated with 1% test compound was applied to the flank for 24 h. Sites were assessed for erythema and oedema immediately upon, and 24 and 48 h after, removal of the patch. The 1% concentration was said to be non-irritating (results of the preliminary study were not presented). Control animals were treated with vehicle alone. A second challenge, on the opposite flank to the first challenge, was performed two weeks after the first challenge. The control animals were challenged with 1% test compound. No positive control compound was tested.

It is not clear if the concentration of the test compound (1%) used for epidermal challenge was the maximum subirritant concentration, as it was the highest concentration tested in the preliminary study. It would appear that an irritant induction concentration of test compound was not used, as it was reported that, following induction, animals of the test group showed the same dermal reactions as those of the control group. It is unclear why such low concentrations of test compound were tested in the preliminary (and main) study, as in the maximization test, liquids should be used tested up to 100% concentration for epidermal induction and up to 5% for intradermal injection.

The incidences of positive reactions (slight erythema) after challenge were as follows:

	First challenge						Second challenge					
	After 0 h		After 24 h		After 48 h		After 0 h		After 24 h		After 48 h	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
TRAPEX 40	11	9	4	16	3	17	2	18	1	19	5	15
Vehicle	0	10	0	10	0	10	0	10	0	10	0	10

In conclusion, under the conditions of this study, which did not appear to use maximum concentration of test compound at induction, and may also not have used maximum subirritant concentrations at challenge, MITC, formulated as TRAPEX 40, had weak skin sensitising potential.

4. SHORT TERM REPEAT DOSE TOXICITY

4.1 Rat

4.1.1 1-Month Dermal Studies

(a) Tsubura et al (1975) Nara Medical College.

Crystalline MITC (purity not stated) was dissolved in benzol at 1.5, 3 and 6 g/15 mL and 0.3 mL applied daily to the back (clipped) of Sprague-Dawley rats (individually housed) for one month. Calculated applied dose was approximately 120, 240 and 480 mg/kg. All doses caused flare and erosion of the skin. During week 2 there was change from erosion to ulceration, with crust formation occurring during week 3. Histology showed infiltration of inflammatory cells in the area of application. This was localised and did not involve spleen or lymph nodes. There were no systemic changes. Although many animals showed enlargement of peribronchial lymph nodes this is likely to be the result of respiratory infection since one control and one mid dose animal died of pneumonia. No NOEL was demonstrated.

(b) Schobel et al (1975) Schering Report 4411. 1975.

MITC at doses of 1, 10 and 100 mg MITC/kg (purity not stated) dissolved in sesame oil was applied 7 d/week for 31 d to shaved skin of Wistar rats (10/sex/group). There was irritation (desquamation and erythema) at 1 and 10 mg/kg and severe necrosis at 100 mg/kg. There was a decrease in food consumption and body weight gain in the high-dose group. All animals at 100 mg/kg also had depressed plasma cholinesterase. There was a dose dependent decrease in serum albumin after 1 mg/kg and a slight increase in serum LDH in some animals at 10 and 100 mg/kg. These changes are consistent with the slight hepatic lesions sporadically observed in treated animals. No NOEL was demonstrated.

In conclusion, no NOEL for skin effects after repeated daily application of MITC for 1 month was demonstrated (lowest dose 1 mg/kg).

5. SUBCHRONIC TOXICITY

5.1 3-Month Oral Studies

A series of studies has been carried out using mice, rats and dogs. The details of these studies in terms of dosing regimens and animal details are summarised in Table 2 below. The findings for each of the studies are also detailed.

Table 2: Summary of dosing schedules for three month oral toxicity studies with MITC.

Reference	Species	Strain	Dose (mg/kg/d)*	n/sex/group
Kajimoto (1973)	Mice	dd	0, 2.5, 5, 10	10
	Rats	Wistar	0, 5, 10, 20	10
Kato et al (1973)	Mice	dd	0, 1, 5, 20	10
	Rats	Wistar	0, 2, 10, 40	10
Tsubura et al (1976)	Mice	ddY	0, 0.35, 0.5, 0.7, 1.0	12
Harling et al (1986)	Dog	Beagle	0, 0.04, 0.4, 2.0[a]	4

* By gavage; vehicle - sesame oil for rat and mouse studies and corn oil for the dog study

a. Total daily doses in dogs after administering b.i.d.

(a) Rats and Mice

(Kajimoto (1973) Department Pharmacology, Tokushuma University.1973). GLP/QA: no

There were no clinical signs or mortalities reported. Body weights did not appear to be affected by treatment. In rats and mice there was a dose-related increase in white blood cell counts in males only. There were no changes in clinical chemistry or clear changes in organ weights. Apparently, there were no changes except in the liver. In mice, it was reported that "2 to 4 cases in each of the 2.5 - 5.0 mg/kg groups of both sexes had a picture of recovery". In rats, "recovery" was observed in several animals at 5 mg/kg/d, and 20% of males at 20 mg/kg/d had fatty degeneration of the liver.
It was not clear from the methods section which organs were examined in this study (9 organs in mice and 16 in rats were weighed), but it would appear that the stomach was not evaluated in either species.

The incidences of pathology findings (macroscopic or histopathological) in the treatment groups were not presented. No individual animal data were presented. Due to the above inadequacies and poor reporting, the importance of the effects at low doses could not be evaluated, hence NOELs cannot be established.

(b) Rats and Mice

(Kato et al (1973) Toho University, Shionogi.. GLP/QA: no.)

In mice, there was no change in body weight gain or food consumption. In females, a slight increase in ALT at 5 and 20 mg/kg/d, and increased protein in urine at 20 mg/kg/d, were observed. There was no change in organ weights except for a decrease in ovary weight (absolute and relative) in all treatment groups. Histopathology revealed increased thickening of the gastric wall and hyperplasia of the forestomach in 6/10 males and 3/10 females at the high dose. There were also dose-related increases in central vein inflammatory response in the liver of high-dose males and females. "Spermatogenic disorder", accompanied by interstitial oedema, was identified in 6/10 males at 20 mg/kg/d. There were a few cases in every treatment group of hyperplasia of adipose tissue of the adrenal zona reticularis.

In rats, there were 8 (4M and 4F) deaths at the high dose probably due to perforative peritonitis. Clinical signs observed in the high-dose group included anorexia and inhibition of motility. Food consumption and body weight gain were decreased in the high-dose group and water intake increased in all treatment groups. There were changes in clinical chemistry (increased glucose, decreased BUN and protein) in mid- and high-dose groups. There were no changes in urinalysis.

The incidences of pathology findings (macroscopic or histopathological) in the treatment groups were not presented. No individual animal data were presented. In the high-dose group there was considerable thickening of the forestomach wall and perforative ulceration of the mucosal surface in all animals. In almost all animals severe adhesions occurred between the stomach and other organs. At 10 mg/kg/d, there was considerable thickening of the forestomach wall, and hyperplasia and cornification of the squamous epithelium. There were no histopathological changes at 2 mg/kg/d. An increase in ovary weight (absolute and relative) was observed in all dose groups (dose-related). A "spermatogenic disorder" was observed in all 40 mg/kg/d animals and in one 10 mg/kg/d animal.

In summary, the most notable changes following treatment occurred in the stomach. A NOEL could not be established because of decreased ovary weights in mice and increased ovary weights in rats at the low dose.

(c) Mice

(Tsubura et al 1976). GLP/QA: no.

This study was primarily conducted to determine the effects of MITC on ovary weight in mice. There were no changes in the standard parameters except for a slight (not significant) decrease in body weight gain and a dose-related increase in liver weight (absolute and relative), significant at 1 mg/kg/d (both sexes) and at 0.7 mg/kg/d in females. Histopathological examination revealed testicular atrophy in one high-dose male. NOEL was 0.7 mg/kg/d based on increased liver weight at 1 mg/kg/d.

(d) Dogs

(Harling et al (1986) Huntingdon Research Centre, UK. Tox/86/203-218. 25 March, 1986). GLP/QA: yes).

There were no mortalities. There was an increased frequency of vomiting in the dogs treated with 2 mg/kg/d. These animals also had decreased body weight gain (females) and decreased serum calcium and total protein and/or total globulin (males and females). Mean activated partial thromboplastin time was increased in the high-dose group. Results of ophthalmological examinations and urinalysis were unremarkable. Mean testes weight was slightly, but significantly, decreased at 0.4 and 2 mg/kg/d and mean pancreas weight of females was increased at these doses. The incidence and severity of periportal hepatocyte vacuolation and lipid deposition were increased at 0.4 and 2 mg/kg/d. A degree of thymic involution was observed in 2 mid-dose dogs and 3 high-dose dogs. No macroscopic or histopathological changes were observed in the stomach. NOEL was 0.04 mg/kg/d based on organ weight changes and liver effects observed at 0.4 mg/kg/d.

5.2 Rat: 8-Month Oral Study (with 6 Months Recovery)

(Nelson et al (1978) Industrial Bio-Test Laboratories Inc.USA. 622-07392. 27 July, 1978)

A letter from IBT prefixing the study indicated it had not been analysed by the usual Quality Assurance procedures. This, together with inconsistency in dosing and reporting, and changes in study protocol from original design, limit the value of the study. The original study was planned for 2 years but was altered because all dose groups had gastric irritation.

Albino rats (60/sex/group) were dosed with technical MITC via gavage (vehicle - corn oil) at 0, 3, 10, 30 mg/kg/d, 5 d/week for 8 months, followed by a 6 month recovery period. At 5 and 8 months of dosing, 10/sex/group were sacrificed and 5/sex/group were killed at monthly intervals during the recovery period, with the remainder killed at the end of the recovery period. The recovery assessment relates only to gastric lesions.

Animals dosed with 30 mg/kg/d had decreased body weight gain, excess salivation, rapid and erratic movement, increased ALT, AST and AP. Liver weights were decreased at 10 and 30 mg/kg/d.

Gross pathological lesions, consisting of thickening of the mucosa of the forestomach were noted in mid- and high-dose animals at the 5 and 8 month sacrifices. Histopathological examination at the 5 and 8 month sacrifices revealed acanthosis and hyperkeratosis of the forestomach in all treated groups. The incidence and severity of these lesions were dose-related. At the 8-month sacrifice, all animals in the 10 and 30 mg/kg groups were affected (both acanthosis and hyperkeratosis); severity of lesions was minimal in the 3 mg/kg group, mild to moderate in the 10 mg/kg group and moderate to marked, with focal mucosal erosion and ulceration involving 9/20 animals, in the 30 mg/kg group. Also, an increase in the incidence (10/20) of animals exhibiting submucosal cysts which contained keratinised debris was observed at the 8-month sacrifice. None of these changes was observed in control animals. During the recovery period, the incidence of the gross lesion, thickening of the mucosa of the forestomach, decreased with time, such that it was found only in one animal in the high-dose group (out of a total of 25 high-dose animals) at the 6-month sacrifice. At this time, there were no stomach lesions observed histopathologically in the 3 mg/kg group (only 1/sex examined). Acanthosis, hyperkeratosis and submucosal cyst were observed at incidences of 4/10, 3/10 and 0/10, respectively, at the 10 mg/kg dose and 7/10, 4/10 and 2/10, respectively, at the 30 mg/kg dose, and severity of the lesions was minimal to mild.

In conclusion, no NOEL was demonstrated. The study demonstrated that the stomach lesions induced by MITC are reversible. The quality assurance status of this IBT study remains to be validated.

5.3 Rat: 3-Month Inhalation Study

(Rosskamp et al (1978) Schering Report 374/77. 26 September, 1978). GLP/QA: no

Wistar rats (10/sex/group) were exposed nose only to MITC for 4 h/d for 12-13 weeks at MITC atmospheric concentrations of 0, 1, 10 and 45 ppm (0, 3.16, 30.67 and 137 mg/m^3). There were no compound-related effects on the eyes and only the high dose groups exhibited clinical symptoms (apathy, increased salivation and nasal discharge and vocalisation). This group also had decreased food consumption and body weight gain. There were no changes in haematology, urinalysis, blood chemistry, necropsy or histology (performed in the control and high dose groups only). While lungs, trachea and bronchi were examined, the nasal turbinates were not. NOEL was 30.67 mg/m^3 based on clinical signs and decreased body weights.

6. CHRONIC TOXICITY

6.1 Mouse

6.1.1 2-Year Drinking Water Study

(Kashima (1985) Haruma Laboratory of Nippon Experimental Medical Research Institute. April, 1985). GLP: no; QA: yes

ICI:JCR mice (70/sex/group) were given drinking water (changed daily) containing 0, 5, 20, 80 and 200 ppm MITC for 106 weeks. There were interim kills of 6/sex/group at 26 and 52 weeks. The average compound intake was 0, 0.87, 3.48, 12.43 and 27.37 mg/kg/d (no difference between males and females).

Mortality was comparable for all groups (35-56%). No specific toxic symptoms were observed, but raised hair and dull coat were observed at 80 and 200 ppm. There was decreased body weight gain in males and females at 200 ppm and in males at 80 ppm. There were no differences in food consumption. There was decreased water intake at 80 and 200 ppm. At 52 and 106 weeks there were some changes in the differential count at 80 and 200 ppm, although values were within the normal range. Although there were differences in biochemical findings at 26 and 106 weeks (not 52 weeks) for 80 and 200 ppm groups, these were not consistent with time, and there was no abnormal tissue histopathology. There were no differences in incidences (50-60% for all groups) or time to appearance for type of tumour between the treatment groups.

In conclusion, no carcinogenic effect of MITC when administered to mice for 2 years in drinking water was demonstrated. The NOEL was 20 ppm (3.48 mg/kg/d) based on body weight gain suppression and changes in differential blood counts at 80 ppm (12.43 mg/kg/d).

6.2 Rat

6.2.1 2-Year Drinking Water Study

(Brown (1984) Hazleton Europe. 2611-14/IR. February, 1984). GLP/QA: yes

CD rats (60/sex/group) were administered 0, 2, 10 and 50 ppm MITC in drinking water. There were an extra 10 animals for each sex for an interim kill at 52 weeks (n = 5) and for a recovery experiment at this time (n = 5). The purpose was to detect any lesions present at 52 weeks and to assess their reversibility; there were no lesions present at 52 weeks.

Because MITC has an appreciable vapour pressure, considerable rapid loss of MITC from the drinking water into the head space and from the sipper tube occurred. Hazleton Laboratories redesigned the drinking bottles which minimised this loss and yielded measured concentrations of MITC in the water which were acceptably close to the nominal values. The actual average dose of MITC over the two year study was for males 0, 0.08, 0.37 and 1.60 mg/kg/d and for females 0, 0.12, 0.56 and 2.65 mg/kg/d.

There were no differences between any of the groups for the standard range of parameters measured. The only observable effect of MITC was decreased body weight gain and decreased water intake for males at 50 ppm. Food intake was comparable to controls.

In conclusion, the NOEL was 10 ppm (equivalent to approximately 0.47 mg/kg/d) based on slight decrease in body weight gain in high-dose males. In this study MITC exhibited no chronic toxicity nor carcinogenicity in rats.

7. REPRODUCTIVE TOXICITY

7.1 Rat

7.1.1 3-Generation Oral Study

(Pflaum et al (1978) Industrial Bio-Test Laboratories Inc., USA. Report 623-07393. 27 July, 1978). GLP/QA: no

A three-generation reproductive study was conducted in Charles River CD strain albino rats, with oral (gavage; vehicle - corn oil) treatment beginning at 28 d of age in all generations. Initially, there were three test groups (3, 10 and 30 mg/kg) and a control group of F_0 parents, with 10 M and 20 F/group, but at 5 weeks, the 30 mg/kg group was terminated because of toxicity (no further details given) and a 1 mg/kg group was initiated (with treatment commencing at about 40 d of age). Dosing was on 5 d/week until sacrifice. Animals from each generation were allowed to reach maturity, mate and produce 2 litters.

Mating was initiated when parental animals were 100 d old. After weaning of the first litter, parental females were given a 10-d rest period and mated again. Progeny from the first litter were sacrificed at weaning. Ten M and 20 F weanlings (survival permitting) were selected from the progeny of the second litter of each group, as parental animals for the succeeding generation. After weaning of the second litter, the parental animals were sacrificed and subjected to gross pathological examination. Histopathological examination was performed on 5 parental animals/sex/group. The study terminated with the weaning of the F_3 progeny. F_2 progeny from the first litters were examined grossly, but F_1 and F_3 progeny from the first litters were not. Gross pathological examination was also conducted on F_1 and F_2 progeny from the second litter that were not selected as parents (in the latter group, at 90 days) and on all F_3 progeny from the second litter (10/sex/group of the latter were also examined histologically).

Body weights and weight gains were generally comparable for all groups at each generation. There were several mortalities in all generations, but none was attributable to treatment. There were no clinical signs. Parameters of reproductive performance were not altered by treatment for any generation. For each generation, there were no differences in the numbers of progeny delivered, in survival of progeny or in body weights of weanlings (d 21 postpartum) that could be attributed to treatment. The incidence of gross fetal abnormalities was comparable for all groups at each generation. Organ weights (liver, kidneys, spleen, heart, brain and gonads) and ratios measured for F_0, F_1 and F_2 parental animals were generally comparable for all groups.

There were no remarkable findings upon gross examination of parental animals or weanlings. Histopathological examination of F_0, F_1 and F_2 parental animals (5/sex/group) revealed lesions in the forestomach which were dose-related in incidence and severity. The lesions consisted of mucosal acanthosis and hyperkeratosis. No treatment-related histological changes were observed in the F_3 weanlings examined. No NOEL was observed in this study because the forestomach lesions were observed in all dose groups.

8. DEVELOPMENTAL TOXICITY

8.1 Rat

8.1.1 Oral Teratology Study

(Irvine (1983) Hazleton and Schering Report 3191-14/10. 1983). GLP: presumably, but not stated. QA: yes.

CD rats were given MITC by gavage in corn oil at 0, 1, 5 and 25 mg/kg/d on d 6-15 of gestation (n = 24-28/dose). Analysis of the dosing preparation revealed actual MITC concentrations to be 92-101% of nominal values. There was no difference in pregnancy incidence in the groups (82-89%) and there were no clinical changes except for staining of fur in high-dose group. There was a dose-related depression of food intake, but this was only significant at 25 mg/kg.

This group also had significantly decreased weight gain during pregnancy. There was a slight, but not significant, decrease in body weight gain at the mid dose. Dams in the high-dose (24 of 27 animals) had thickening of the stomach wall and some had adhesions of viscera to the stomach. At the mid dose, 1/28 dams had thickening of the stomach lining.

There was no difference between controls and any of the dose groups for the standard litter parameters, nor for the incidences of major external/ visceral or skeletal defects. There was a slight increase in the incidence of minor external/visceral defects at the high dose compared with the control, which was due to an increase in the incidence of bilateral ureter dilation (2/270 in controls and 11/337 at the high-dose). However, the incidence of bilateral ureter dilation at the high-dose was said to be within the historical control range, and the increase was not significant. At 25 mg/kg, fetuses were significantly smaller (fetal weight and crown-rump length) than control fetuses . These fetuses had an increased incidence of delayed ossification (especially of the skull) and consequently an increased incidence of minor skeletal defects.

In conclusion, doses of MITC that did not cause maternal toxicity were not associated with fetal toxicity, nor teratogenicity. At the highest dose level tested (25 mg/kg/d), there was fetal growth retardation, presumably secondary to decreased maternal food intake and weight gain. The NOEL was considered to be 5 mg/kg based on decreased fetal size at 25 mg/kg.

8.1.2 Oral Teratology Study

(Hellwig & Hildebrand (1987) BASF Aktiengesellschaft, Department of Toxicology, Federal Republic of Germany. Project no. 34R0231/8537. 2 Sept 1987). GLP/QA: yes

Mated Wistar rats (25/group) were given MITC by gavage in corn oil at 0 (vehicle), 3, 10 and 30 mg/kg/d on days 6-15 of gestation. Animals were sacrificed on day 20 of gestation.

Mean food consumption was considerably reduced in the high-dose dams during the treatment period and body weight and body weight gains were significantly reduced during the treatment and post-treatment periods. Significantly reduced body weight gains were observed on days 8 - 10 of gestation in the mid-dose group. "Corrected" body weight gain (body weight on day 20 minus body weight on day 0 minus uterus weight) was also significantly reduced in all the treated groups. The reduction in "corrected" body weight at the low-dose was considered incidental by the company because of one "outlier" reducing the value. Statistical analysis by the evaluator confirmed the lack of a significant difference between means for the low dose and control groups when the "outlier" value was omitted from the analysis.

There were no mortalities or major clinical signs. At the end of the treatment period some high-dose dams showed reddish snout. Water consumption, which was not measured, but roughly estimated during the study, seemed to be increased in individual dams at the mid and high doses. Uterus weights were comparable for all groups, but mean placental weights were significantly lower in the high-dose group. There were no significant differences between groups with respect to mean numbers of corpora lutea, total implantations, live fetuses , dead implantations, calculated values for pre- and post-implantation losses and fetal sex distribution.

Mean fetal weights were comparable for all groups but the number of runts (fetuses weighing 75% of the mean fetal weight per litter) was increased in the high-dose group. One foetus from each of the low- and high-dose groups exhibited pseudoankylosis. One foetus in the mid-dose group exhibited unilateral anophthalmia.

It was concluded that the NOEL was 10 mg/kg/d for the fetuses but was not clearly established for the dams. MITC was not teratogenic at the doses tested.

8.2 Rabbit

8.2.1 Oral Teratology Study

(Ladd & Smith (1976) Industrial Biotest Laboratories Inc, USA. Report no. 651-07457. 27 July, 1976). GLP/QA: no

Inseminated New Zealand White rabbits (17/group) were dosed orally with MITC at 0 (empty gelatine capsules), 1, 3 and 10 mg/kg/d from days 6 to 18 of gestation and were sacrificed on day 29. Numbers of pregnant animals were 10, 13, 15 and 17 in the control, low-dose, mid-dose and high-dose groups, respectively. Statistical analysis did not appear to have been conducted on the results of this study.

Deaths of does were as follows: 1, 1, 2 and 7 in the control, low-dose, mid-dose and high-dose groups, respectively. All high-dose does that died had enlarged gall bladders and multiple red foci on their liver surfaces and the deaths appeared to be treatment-related. One of the two mid-dose deaths appeared to be treatment-related, with the animal having multiple red foci on the surface of its liver. Multiple red foci were noted on the surface of the liver and/or kidneys in 4 high-dose animals at sacrifice. There were no clinical signs observed.

Mid-dose does had a slightly reduced body weight gain over the gestation period. High-dose does showed a small mean body weight loss over the gestation period. This loss occurred over the dosing period, with a gain, comparable to that in controls, being observed from day 18 to day 29 of gestation.

One mid-dose and one high-dose animal aborted. The historical control rate of abortion in New Zealand White rabbits from this laboratory was 2.4% (data from 47 groups). Mean numbers of implantation sites/doe were comparable for all groups. In the HD group, there was an increase in the number of resorptions (22 cf 9 in controls) and a decrease in the number of live young (40 cf 64 in controls) (the number of implantation sites was the same for these two groups). Two high-dose does accounted for a large proportion of the resorptions.

Mean body weights of fetuses at caesarean section were reduced at the high dose (33.1 g cf 40.6 g in controls). There were no treatment-related changes in the incidences of fetal external abnormalities. The 24-h viability index of fetuses was slightly reduced at the high dose (80% cf 93.8% in controls). Two of the 6 litters in this group accounted for this reduction. Dissection of the fetuses did not reveal any visceral abnormalities. Skeletal examination of fetuses did not reveal any treatment-related skeletal abnormalities, but there was a dose-related increase in the percent of fetuses with incompletely ossified sternum sections (the incidence at the low dose (45/74 or 60.8%) was slightly greater than that in the control group (35/64 or 54.7%) ; the incidence at the high dose (29/40 or 72.5%) was reported to be at the high end of the range normally observed for control New Zealand White rabbit fetuses in that laboratory). Based on the increase in the percent of fetuses with incompletely ossified sternum sections at the low dose, it would be considered that a NOEL was not determined in this study; if this effect is disregarded, the NOEL for fetal toxicity would be considered to be 3 mg/kg/d, based on the increase in resorptions and decrease in fetal body weights and fetal viability at the high dose.

8.2.2 Oral Teratology Study

(Irvine (1984) Hazleton Laboratories, Europe. Report no. 3687-14/30. June, 1984). GLP/QA: yes

Mated female New Zealand White rabbits (16/group) were dosed orally (gavage) with MITC at 0 (vehicle - corn oil), 1, 3 and 5 mg/kg/d from d 7 to d 19 of gestation and were sacrificed on d 29. Numbers of pregnant animals were 14, 15, 16 and 16 in the control, low-dose, mid-dose and high-dose groups, respectively.

There were no mortalities of does between d 7 and 29 of gestation. One mid-dose doe aborted (d 27) and one low-dose doe aborted one foetus (d 29), the others being alive at necropsy. Total litter loss was observed in one mid-dose (not considered to be associated with treatment) and 2 control does. Clinical signs were observed in 2, 2, 5 and 5 animals in the control, low-dose, mid-dose and high-dose groups, respectively. These were commonly nasal exudate or perianal staining. There were no significant differences between groups in mean body weight gain during gestation (adjusted for gravid uterus weight). However, high-dose does did show a mean body weight loss at the onset of dosing (day 7 - 10 of gestation). In this group, mean food consumption was lower (not significant) than that of controls during the treatment period and there was a compensatory increase in food consumption between the end of dosing and the end of gestation. Gross pathological examination of does at sacrifice did not reveal any differences between groups.

Mean numbers of implantations and numbers of fetuses /doe were higher in the test groups than in controls, while numbers of resorptions/doe were lower in the test groups than in controls. For these parameters, incidences in the test groups compared favourably with normal background incidences, whilst control incidences were unusually low/high. Sex ratios (all groups) were within the normal range. Thus, MITC did not appear to affect these parameters. Mean weights and mean crown-rump lengths of fetuses in the treated groups were lower than those of control fetuses , significantly at the HD. This possibly reflected in part, the higher number of fetuses /doe at the HD, but probably was also treatment-related, being associated with the effect on maternal growth during the treatment period. Fetal viability at 24 h was slightly, but not significantly, reduced in the treated groups compared with controls, possibility reflecting the smaller size of the fetuses in these groups.

There was no effect of treatment on the incidence of major defects (external/visceral and/or skeletal). The incidence of minor external/visceral defects was higher than normal in **all** groups, which was due to a large proportion of fetuses with lens opacities. The incidence of minor skeletal defects was also higher than normal in all groups, which was due to an unusually high proportion of fetuses with irregularities of the frontal bones in **all** groups. The reason for the increases in incidence of lens opacities and irregularities of the frontal bones in this study is not clear, but, as both also occurred in the control group and incidences were not dose-related, they would not appear to be due to treatment. Discounting the fetuses with lens opacities, the incidence of fetuses with minor external/visceral defects was slightly higher in the high-dose group than in the other groups. This was due to a higher incidence of minor anomalies of the heart or major blood vessels in the high-dose group (see table below) and this may be related to treatment. Discounting the fetuses with irregularities of the frontal bones, the nature and incidences of other minor skeletal defects was comparable for all groups. The total incidence of fetuses with skeletal variants was comparable amongst groups. However, the incidence of fetuses with an extra pair of ribs in the high-dose group was higher (51.5) than in the control group (34.2), and was higher than historical control incidences.

Percentage incidences in fetuses of minor anomalies of the heart or major blood vessels are summarised below:

Anomalies	Control	LD	MD	HD
Persisting ductus arteriosus	0	0	0	3.7
Abnormal common carotid	2.5	0.8	2.8	4.4

Persisting ductus arteriosus may be related to general fetal growth retardation, rather than be due to a teratogenic effect of the chemical, because it is a functional defect resulting from failure of the ductus arteriosus to close at the time of independent fetal respiration. The fetuses with this abnormality were generally those of lower fetal weight.

The NOEL for both maternal and fetal toxicity was considered to be 5 mg/kg/d. This was based on a decrease in food consumption during treatment and body weight loss at the onset of treatment in the high-dose does. In fetuses , it was based on a decrease in fetal weights and crown-rump length and an increase in the incidence of minor external/visceral defects at the high dose.

8.2.3 Oral Teratology Study

(Becker et al (1986) RCC Research & Consulting Company AG, CH 4452 Itingen/Switzerland. Project no. 056687. RZ-No. 86/395. 5 September 1986).GLP/QA:yes.

Mated Chinchilla rabbits (16/group) were given MITC by gavage in corn oil at 0 (vehicle), 1, 3 and 10 mg/kg/d on days 6-18 of gestation. Doses were based on the results of a preliminary study (results not presented). Animals were sacrificed on day 28 of gestation.

One high-dose doe died on day 10 of gestation, apparently from intubation injury. No clinical signs were observed. There were no treatment-related macroscopic findings upon necropsy of the does.

Mean body weight gain over the period from day 6 to 28 of gestation was comparable for all groups, but for the treatment period, was lower in the high-dose group than for the control group, and higher in the mid-, and particularly, the low-dose group than the control group. This was associated with a reduction (compared with control) in food consumption in the high-dose does, and an increase (compared with control) in food consumption in the low-dose does, over this period, but particularly over days 6 to 11. The high-dose group showed a compensatory increase in food consumption in the period 19-24 days, with food consumption being higher in this group than in the other groups over this period; body weight changes reflected these changes in food consumption. Over the experimental period, there were no significant differences between the treatment groups and the control group with respect to food consumption. "Corrected" body weight gains (body weight on day 28 minus body weight on day 6 minus uterus weight) were comparable for all groups.

There was no effect of treatment on the mean number of implantations, number of live and number of dead fetuses (there were no dead fetuses), embryonic/fetal death and calculated values for preimplantation and postimplantation losses. Fetal weights and sex distribution were comparable for all groups.

One low-dose fetus had an omphalocoele and unilateral microphthalmia. Two fetuses from one mid-dose doe had a range of abnormal external findings including hydrocephalus, coelioschisis and agenesia of the tail. One fetus each from the low- and mid-dose groups had hydrocephalus. There were no abnormal external findings and no abnormal findings upon cranial investigations in fetuses from the control or high-dose groups. Investigations of the body cavities of fetuses revealed hemidiaphragm in one low- and one high-dose fetus . All these findings were considered to be incidental. Skeletal investigations of fetus es revealed a number of fetus es in each group with minor abnormal findings, but the incidence of abnormal findings was comparable for all groups. Stage of development (degree of ossification) was also comparable for all groups.

It was concluded that the NOEL for maternotoxicity was 3 mg/kg/d and for embryo/ foetotoxicity was 10 mg/kg/d. There was no evidence of a teratogenic potential of MITC.

9. GENOTOXICITY

9.1 Gene Mutation Assays

9.1.1 Bacterial Mutation (Ames) Test

(Glass et al (1976) Inveresk Research International, Scotland. Report no. 615. October, 1976). GLP/QA: no

MITC was tested for mutagenicity at concentrations of 0 (solvent control - DMSO), 5, 25, 125 and 500 µg and 2.5 mg/plate in the Ames standard plate test using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98, both in the presence and absence of metabolic activation (mouse liver S9 mix). For all strains except TA1535, MITC was toxic at the highest concentration tested, both in the presence and absence of activation, as evidenced by a thin (or very thin) lawn or a reduced number of colonies. It would appear that one plate was used at each test concentration. There was no evidence of a mutagenic potential for MITC. In the presence of activation, the positive control compound, 2-aminoanthracene (0.5 µg/plate), elicited expected responses in strains TA 1535, TA100, TA1538 and TA98, but did not increase the frequency of mutation in strain TA 1537. No appropriate positive control was used in the absence of metabolic activation. The study was also repeated with a new strain of TA 100, again revealing no evidence of a mutagenic potential of MITC.

9.1.2 Bacterial Mutation (Ames) Test

(Lang & Redmann (1978) Schering, Agrochemical Division, Federal Republic of Germany. Report no. PF T17. 11 April, 1978). GLP/QA: no

MITC was tested for mutagenicity at concentrations of 0 (solvent control - acetone), 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1 and 5 mg/plate in the Ames standard plate test using *Salmonella typhimurium* strains TA 1535, TA100, TA1537, TA1538 and TA98, both in the presence and absence of metabolic activation (rat liver S9 mix). DI-TRAPEX and its other main component (1,3-dichloropropene-1,2-dichloropropane (DD)) were also tested at concentrations of 0.02 - 2 µL/plate and 0.01 - 20 µL/plate, respectively. Positive controls, 2-aminoanthracene (2 µg/plate or for strain TA1537, 10 µg/plate), in the presence of activation, and MNNG, in the absence of activation (tested in strain TA1535 only), showed the expected responses. Data were means of 3 plates. The study was repeated for MITC in strains TA1535, TA1537 and TA1538, using the same concentrations but only up to 0.5 mg/plate. MITC was toxic to the bacteria (both studies, all strains, and both in the presence and absence of metabolic activation) at concentrations of 0.2 mg/plate and above. In some strains, toxicity was also observed at 0.1 mg/plate. There was no evidence of a mutagenic potential of MITC or DI-TRAPEX. DI-TRAPEX was tested up to toxic concentrations in all strains, both in the presence and absence of metabolic activation. The concentration at which toxicity was observed varied between strains from 0.5 - 2 µL/plate.

DD had a concentration dependent mutagenic effect from 1 µL/plate on strains TA1535 and TA100, both in the presence and absence of activation, but not on the other strains. The high concentration of DD tested was toxic in all strains, both in the presence and absence of metabolic activation. It would seem reasonable to conclude that it was not possible to detect the mutagenic activity of DD within DI-TRAPEX because of the toxicity of DI-TRAPEX (largely attributed to MITC). Thus, DD only had a mutagenic effect on strains TA1535 and TA100 from 1 µL/plate, whist DI-TRAPEX showed a toxic effect on these strains at 1 - 2 µL/plate (0.8 - 1.6 µL DD/plate).

9.1.3 Bacterial Mutation (Ames) Test

(Shirasu et al (1978) Institute of Environmental Toxicology, Japan. 1978). GLP/QA: no

MITC was tested for mutagenicity at concentrations of 0 (solvent control - DMSO), 0.5, 1, 5, 10, 50, 100, 500 and 1000 µg/disk in the Ames plate test using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98, and *Escherichia coli* strain WP hcr, both in the presence and absence of metabolic activation (rat liver S9 mix). Top agar containing the bacteria and the S9 mix, when required, was poured onto a minimal agar plate, but the test compound was not added to the top agar. Instead, a paper disk was soaked with 0.1 mL of the solution of the compound and was placed on the inside of the lid of the plate. The plate was then incubated for 2 or 3 d. MITC did not induce an increase in the numbers of revertant colonies in any of the strains tested either in the presence or the absence of metabolic activation. The positive control (2-amino-anthracene, 10 µg/plate) gave the expected results in the presence of activation. In the absence of activation, positive controls (9-aminoacridine, 2-nitrofluorine, -

propiolactone and AF-2, depending on the strain) also gave the expected results. MITC showed evidence of toxicity to the tester strains at concentrations of about 50 µg/disk (in some instances at 10 µg/disk)

9.1.4 Bacterial Mutation (Ames) Test

(Moriya et al., 1983, and Shirasu et al., 1982). GLP/QA: no

A total of 228 pesticides, including MITC, was tested for mutagenicity in the Ames test using the same strains as in Lang & Redmann(1978). A negative result was obtained for MITC. It was said that compounds were tested at concentrations of up to 5000 µg/plate. It is probable that the results presented in this paper for MITC were those obtained in the above study Shirasu et al (1982), although the methods in the paper Moriya et al (1983) are not identical to those reported for the above study.

9.1.5 Bacterial Mutation (Ames) Test

(Gelbke & Engelhardt (1986) BASF Atkiengesellschaft, Research and Development, Federal Republic of Germany. 28 January 1986). GLP: no QA: yes

MITC was tested for mutagenicity in two experiments using the Ames plate test. In experiment 1, it was tested at concentrations of 0 (solvent control - DMSO), 20, 100, 500, 2500 and 5000 µg/plate. In experiment 2, it was tested at concentrations of 0, 30, 60, 125, 250 and 500 µg/plate. Both experiments used *Salmonella typhimurium* strains TA1535, TA100, TA1537 and TA98 and both were done in the presence and absence of metabolic activation (rat liver S9 mix). The positive controls (2-amino-anthracene, 10 µg/plate, in the presence of metabolic activation, and 9-aminoacridine, 100 µg/plate, 4-nitro-o-phenylenediamine 10 µg/plate and N-methyl-N'-nitro-N-nitroso-guanidine, 5 µg/plate, in the absence of metabolic activation) gave the expected results. MITC showed evidence of toxicity to the tester strains at concentrations of about 500 µg/plate. MITC did not induce an increase in the number of his^+ reveratants above control levels and it was concluded that MITC was not mutagenic in the Ames test under these experimental conditions.

9.1.6 Chinese hamster ovary cells - HGPRT locus

(Miltenburger (1985a) Laboratorium fur Mutagenitatsprufung, LMP Darmstadt. 22 March, 1985). GLP/QA: yes

MITC technical grade was tested for mutation potential at the HGPRT locus in the Chinese hamster cell line V79, both in the presence and absence of metabolic activation (rat liver S9 mix) in two independent experiments. Concentrations tested (based on preliminary toxicity tests) were 0 (solvent (DMSO) control), 0.1, 0.25, 0.5 and 1.0 µg/mL in the absence of activation and 0, 0.25, 0.5, 1.0 and 2.5 µg/mL, in the presence of S9 mix. There was also a control group of untreated cells. At the highest concentrations of MITC tested, survival ranged between 25% and 45% based on plating efficiencies at test concentrations compared with plating efficiencies in solvent controls. Treatment was for 4 h, after which cells were subcultured into selective medium. There was one flask per experimental point. The positive controls, ethylmethanesulfonate

(without metabolic activation) and 9,10-dimethyl-1,2-benzanthracene (DMBA) (in the presence of metabolic activation) produced the expected results. MITC did not induce an increase in the number of mutant colonies.

9.2 Chromosomal Effects Assays

9.2.1 *In vitro* chromosome aberration test in the Chinese hamster cell line V79

(Miltenburger (9185b) Laboratorium fur Mutagenitatsprufung, LMP Darmstadt. 22 March, 1985). GLP/QA: yes

MITC technical grade was tested for potential to induce chromosomal aberrations in Chinese hamster cells of the V79 cell line. Cells were exposed to the test compound for 4 h, at concentrations (chosen on the basis of preliminary toxicity tests) of 0 (DMSO; solvent control), 0.1, 0.5 and 1.0 µg/mL in the absence of metabolic activation, and of 0, 0.25, 0.75 and 2.5 µg/mL in the presence of activation (rat liver S9 mix). There was also a control group of untreated cells. In the preliminary toxicity test, survival of the cells at 1 µg/mL (absence of S9 mix) was 35% and at 1 and 5 µg/mL (presence of S9 mix) was 63% and 0%, respectively. After treatment, cells were cultured in Minimal Essential Medium plus 10% calf serum and were harvested at 12 h after the start of treatment (and also 6 and 28 h for controls and at the highest concentration), with colcemid being added 2 h before harvest. 400 Metaphases were scored per experimental group (100 metaphases for each of 4 flasks). The positive controls, ethylmethanesulfonate (without metabolic activation) and cyclophosphamide (in the presence of metabolic activation) produced the expected results.

At the 6 h and 12 h harvests, there were some small increases in incidences of aberrant cells in the test groups as follows, but the biological relevance of these increases is unclear:

		Percent aberrant cells	
		including gaps	excluding gaps
At the 6 h harvest (presence of S9 mix)	Solvent control	5.00	1.50
	Test compound (2.5 µg/mL)	4.50	3.00
At the 12 h harvest (absence of S9 mix)	Negative control	2.75	1.00
	Solvent control	1.75	0.25
	Test compound (0.1 µg/mL)	1.75	0.75
	Test compound (0.5 µg/mL)	2.50	0.50
	Test compound (1.00 µg/mL)	4.25	1.25
At the 12 h harvest (presence of S9 mix)	Negative control	3.25	0.75
	Solvent control	3.75	0.75
	Test compound (0.25 µg/mL)	3.75	1.50
	Test compound (0.75 µg/mL)	3.75	1.50
	Test compound (2.5 µg/mL)	5.00	2.00

However, at the 28 h harvest, there were clear increases in the incidences of aberrant cells, as follows:

		Percent aberrant cells	
		including gaps	excluding gaps
(absence of S9 mix)	Solvent control	1.0	0
	Test compound (1.0 µg/mL)	7.5	5.25
(presence of S9 mix)	Solvent control	2.75	1.5
	Test compound (2.5 µg/mL)	6.5	4.0

At the 28 h harvest, the types of aberrations observed are detailed below (out of a total of 400 metaphases scored):

Type of aberration	Test compound (1.0 µg/mL) (absence of S9 mix)	Test compound (2.5 µg/mL) (presence of S9 mix)
Gap	10	14
Iso-gap	0	0
Break	11	11
Iso-break	1	1
Fragment	1	0
Iso-fragment	0	0
Deletion	1	0
Multiple aberration	6	1
Exchange	8	9
Chromosomal disintegration	1	0

It can be seen from the above table that MITC induced quite high numbers of chromosome breaks and chromosome exchanges. The numbers of exchanges induced were comparable to the number induced by the positive control compounds (12 h harvest). Furthermore, in contrast to the solvent controls, mitotic index could not be determined in the test substance groups at the 28 h harvest because of nuclear disintegration induced by the test substance.

MITC was therefore classified as clastogenic under the test conditions employed in this study.

9.2.2 Mouse micronucleus test

(Allen at al (1985) Huntingdon Research Centre, UK. 3 December, 1985). GLP/QA: yes.

Technical MITC was administered orally (gavage) at doses of 0 (vehicle - corn oil) and 110 mg/kg (LD_{10}) to CD-1 outbred mice of Swiss origin (15/sex/group) and there were three sampling times (24, 48 and 72 h). Bone marrow smears were made from each femur and it would appear that 1000 polychromatic erythrocytes were score per experimental point. The positive control compound, mitomycin, was administered ip to 5 mice/sex (24 h sampling time). A preliminary toxicity test had been conducted to determine the LD_{10} (72 h). The incidence of micronucleated polychromatic erythrocytes (and the incidence of micronucleated monochromatic erythrocytes) in the treated mice was comparable to that in controls at all three sampling times, whereas mitomycin resulted in significant increases in the incidence of micronucleated polychromatic erythrocytes. It was concluded that there was no evidence of a mutagenic potential of MITC under these experimental conditions. The ratio of polychromatic to normochromatic erythrocytes (in at least 1000 erythrocytes) in treated animals was comparable to controls at 24 h, but was significantly reduced at 48 h, indicative of bone marrow cell toxicity. It was slightly reduced in MITC-treated animals at 72 h. It was significantly reduced in mitomycin-treated animals.

9.2.3 Mammalian cytogenetics (human) - *in vitro* lymphocyte

(Glebke & Engelhardt (1987) BASF Atkiengesellschaft, Department of Toxicology, Federal Republic of Germany. 26 May 1987). GLP: no QA: yes

MITC was tested for its ability to induce chromosomal aberrations in human lymphocytes. Based on a preliminary cytotoxicity test, concentrations of 0.1, 0.5 and 1.0 µg/mL (in the presence of S9 mix) and of 0.05, 0.1 and 0.5 µg/mL (in the absence of S9 mix) were tested. The concentrations tested were limited by the quality of the metaphases, with higher concentrations severely affecting the chromosomes, thus no longer allowing evaluation. The lymphocytes were stimulated to divide with PHA, incubated at 37^0 C for 48 h and then treated with MITC for 24 h (or for experiments in the presence of S9 mix, for 2 h, followed by 22 h in fresh medium). Cells were arrested in metaphase by treatment with colchicine at 2-3 h prior to harvest and, after preparation and staining, 100 metaphases (50 for positive controls) from each culture (there were duplicate cultures for each experimental point) were scored. MITC treatment did not significantly increase the number of aberrant metaphases (excluding gaps) above that observed in negative (untreated and solvent (DMSO)) controls, either in the presence or absence of metabolic activation. However, in the presence of metabolic activation, although not statistically significant and not dose related, there was an increase in the number of aberrant metaphases (excluding gaps) above control levels at all concentrations tested. Additionally, both in the presence and absence of metabolic activation, the number of aberrant metaphases (including gaps) was increased at all concentrations tested (not dose related; significant at the lowest concentration tested).

The positive control compounds (0.3 µg/mL mitomycin in the absence of S9 mix and 6 µg/mL cyclophosphamide in the presence of S9 mix) significantly increased the number of aberrant metaphases. Since there were no significant increases in the numbers of aberrant metaphases (excluding gaps) induced by MITC, it was concluded that MITC did not have clastogenic activity in this assay.

9.3 DNA Damage and Other Genotoxic Effects Assays

9.3.1 *Bacillus subtilis* rec assay

(Shirasu et al (1978) Institute of Environmental Toxicology, Japan. 1978.). GLP/QA: no

MITC at 0 (DMSO control), 20, 100, 200, 500, 1000 and 2000 µL/disk did not produce any inhibitory zone for either *B.subtilis* strain M45 or strain H17 after overnight incubation. The positive control, mitomycin (0.1 µg/disk), produced a 10 mm difference in the lengths of the inhibitory zone, whereas the negative control, kanamycin (10 µg/disk), produced similar lengths of inhibitory zones.

9.3.2 *Bacillus subtilis* rec assay

(Jagannath (1989) Hazleton Laboratories America, Inc. HLA Study no. 10538-0-404. BASF Project no. 70M0231/859178. 13 March 1989). GLP: no QA: yes

MITC was tested for its ability to cause DNA damage in the standard *B. subtilits* rec assay, both in the presence and absence of rat liver S9 mix. Concentrations of MITC tested were 1, 10, 100, 500, 1001, 2502, 5004 and 10008 µg/plate. MITC was toxic to both strains at concentrations of 5004 µg/plate and higher. Zones of inhibition were measured after 24 h incubation at 37^0C in the presence of test substance. Three plates per test concentration were used and the entire assay was performed twice. Positive control compounds (methylmethane, 10 µL/plate in the absence of metabolic activation and diethylnitrosamine, 100 µL/plate in the presence of metabolic activation) induced significant increases in the zones of inhibition of the M45 culture compared with the H17 culture, while the negative control (kanamycin, 250 µg/plate) induced significant increases in the zones of inhibition of both strains, with no differential between the two strains. MITC did not alter the ratio of the zones of inhibition of the two strains and it was concluded that under these test conditions, it did not have DNA damaging activity.

9.3.3 Unscheduled DNA synthesis in rat primary hepatocytes

(Cifone (1985) Litton Bionetics Inc., USA. Project no. 20991. March, 1985) GLP/QA: yes

Primary rat hepatocytes were exposed to MITC technical grade for 18 h at eight concentrations over the range 0.253 to 30.3 µg/mL. At 30.3 µg/mL, the test compound was lethal, at 15.2 µg/mL survival was 39.7% relative to the solvent control and at 10.1 µg/mL, survival was 66.7%. 2-Acetyl aminofluorene was the positive control and gave the expected response. The viability of the hepatocytes prepared by perfusion, and of the monolayer cultures, was satisfactory. The data were obtained by autoradiography and the grain counts were expressed as the average net nuclear grain counts (nuclear counts minus cytoplasmic counts for a nuclear-sized area) for 150 cells. In the solvent control (DMSO) there were 0.72 grains/nucleus (0.7% of nuclei having 6 grains and no nuclei having 20 grains). No effects which indicated unscheduled DNA synthesis were observed at the concentrations of MITC tested and no dose-related effects were seen. Under the conditions of this study, MITC did not induce unscheduled DNA synthesis in primary rat hepatocytes.

10. SPECIAL STUDIES

10.1 Effect on the Digestive Tract in Rats

(Kajimoto (1975) University Tokushima). GLP/QA: no

The acute and subacute effects of MITC on the digestive tract of Wistar rats was investigated at gavage doses (in sesame oil) of 0, 50, 100, 150 mg/kg and 0, 25, 50 and 100 mg/kg/d for 10 d, respectively (n = 10/sex/dose for both acute and subacute experiments). In the acute study, survivors were sacrificed 24 h and 7 d after dosing (results also refer to a 72-h sacrifice, not documented in the methods), and in the subacute study, at 24 h after the last dose.

Documentation of this study was poor, with discrepancies between the text and tables, as well as ambiguities in the tabular presentation of the results (eg no distinction between animals with respect to time of sacrifice). In the acute study mortality was stated to be 0, 5, 25 and 100%, respectively (males and females combined) in the text, but corresponding values in the table were 0, 15, 65 and 100%. All animals of the 150 mg/kg group died within 1 h and had pronounced hypereamia and haemorrhage of the stomach. At the 24 h sacrifice, pronounced hyperaemia was observed in all animals - in the apex of the "proventricular" portion of the stomach the 50/mg/kg group and throughout the proventricular portion in the 100 mg/kg group. In the latter group, hyperaemic spots were observed sporadically in the jejunum and duodenum. At the 72-h sacrifice, the hyperaemia was less severe. In the 100 mg/kg group, there were adhesions of the digestive tract to the spleen and areas of mucosal thickening and muscle thinning in the stomach. At the 7 day sacrifice, adhesions of the digestive tract to abdominal tissues was observed, areas of the stomach mucosa were thickened and ulcers were present at both the 50 and 100 mg/kg doses.

In the subacute study, dosing at 100 mg/kg/d was stopped after the second dose due to toxicity and an autopsy was performed 48 h later. The stomach of every dosed rat was swollen with thickened mucosa. There was a decrease in stomach elasticity, hyperaemia and haemorrhage of the stomach and hypertrophy of the duodenum. Stomach ulceration and adhesions of the gastrointestinal tract to abdominal organs occurred in all groups, increasing in incidence and severity with dose.
In conclusion, single doses of MITC of 50 mg/kg and above, and repeated doses of 25 mg/kg and above, are extremely irritating to the gastrointestinal tract.

11. HUMAN STUDIES

11.1 Allergic Contact Dermatitis in Humans (*Richter, 1980)*

From observations on nine patients with occupational dermatitis from MITC, Richter (1980) concluded that MITC causes primarily a toxic dermatitis, but induces sensitisation as well. Although all the patients had contact with the soil disinfectants (dazomet or metham-sodium) for a few days or less, 8 of the 9 patients showed positive patch test reactions to VAPAM (metham-sodium) at a non-irritant concentration of 0.05% in water, and the results could be elicited 1 year later.

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