

# Existing Chemical Hazard Assessment Report



Australian Government  
Department of Health and Ageing  
NICNAS

Methyldibromo glutaronitrile (MDBGN)

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# Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

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This assessment of the chemical methyl dibromo glutaronitrile (MDBGN) was initiated in 2007 following reports of contact allergy in consumers from products containing MDBGN as a preservative. In 2008, NICNAS made a submission to the National Drugs and Poisons Schedule Committee (NDPSC) recommending the scheduling of the chemical under the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

As a result, the NDPSC resolved to list MDBGN in the SUSDP in Schedule C for cosmetic use and products intended to be in contact with the skin, in Schedule 6 for other uses, and in Appendix F, Part 3 specifying warning statements and safety directions. The implementation date for these new scheduling requirements is 1 January 2010.

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More information on NICNAS can be found at the NICNAS web site:

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Other information on the management of workplace chemicals can be found at the web site of Safe Work Australia:

<http://www.safeworkaustralia.gov.au/swa/>

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# Acronyms and Abbreviations

2-PE	2-phenoxyethanol
AICS	Australian Inventory of Chemical Substances (NICNAS)
AO	Arachis oil
bw	bodyweight
CAS	Chemical Abstracts Service
CCET	cumulative contact enhancement test
CHO	Chinese hamster ovary
CO <sub>2</sub>	carbon dioxide
DMF	dimethyl formamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EC	European Community, or European Commission
EU	European Union
FCA	Freund's Complete Adjuvant
HRIPT	human repeat insult patch tests
IC(NA) Act	<i>Industrial Chemicals (Notification and Assessment) Act 1989</i> (Cwlth)
IVDK	Information Network of Departments of Dermatology
kg	kilogram
L	litre
LC <sub>50</sub>	median lethal concentration
LD <sub>50</sub>	median lethal dose
LLNA	Local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MDBGN	methyldibromo glutaronitrile
MEK	methylethylketone
mg	milligram

mg/kg bw/d	milligram per kilogram bodyweight per day
µg	microgram
ml	millilitre
NDPSC	National Drugs and Poisons Schedule Committee
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOHSC	National Occupational Health and Safety Commission
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OO	olive oil
PE	phenoxyethanol
ppm	parts per million
ppb	parts per billion
ROAT	repeated open application test
SI	Stimulation index
SLS	sodium lauryl sulfate
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TEWL	transepidermal water loss
UDS	unscheduled DNA synthesis
USEPA	United States Environmental Protection Agency
UV	Ultra-violet
UVCB	unknown or variable composition, complex reaction products, biological
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight

# Summary

Methyldibromo glutaronitrile (MDBGN) is used as a preservative and biocide in a wide range of aqueous-based products, including cosmetics. In Australia, it has been reported as a component of adhesives and coatings in addition to various personal care products (sunscreens, shampoos, shower gels and wet wipe hand towels).

In the mid 1980s, MDBGN began to be used as a preservative in cosmetics and the first case reports of contact sensitivity due to MDBGN-preserved cosmetics were reported in the late 1980s and early 1990s. Several research groups have demonstrated that the prevalence of contact sensitivity to MDBGN in various European countries has increased since the early 1990s. Animal studies have also demonstrated that MDBGN is a sensitising agent. As a result, use of this preservative is no longer permitted in cosmetics within the EU. However, in the USA, MDBGN can be formulated in cosmetics at up to 0.025% in leave-on products and 0.06% in rinse-off products.

Allergy clinics in Australia have reported cases of allergy (combined prevalence of 0.7%) associated with the use of MDBGN as a preservative, most commonly in hand cleaner. Due to use in consumer products and its oral toxicity, skin and eye irritation effects and skin sensitising potential, in 2008 NICNAS made a submission to the National Drugs and Poisons Schedule Committee (NDPSC) recommending the scheduling of the chemical under the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

The NDPSC considered the scheduling of MDBGN in its meeting of June 2008 and again in its October 2008 meeting. As a result, the NDPSC resolved to list MDBGN in the SUSDP in Schedule C for cosmetic use and products intended to be in contact with the skin, in Schedule 6 for other uses, and in Appendix F, Part 3 specifying warning statements and safety directions. The implementation date for these new scheduling requirements is 1 January 2010.

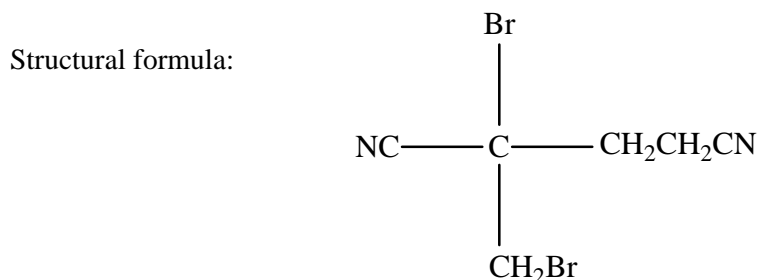
# 1. Introduction

This hazard assessment of MDBGN uses information from previously published reports of the Cosmetic Ingredient Review (CIR) Expert Panel (1996), the United States Environmental Protection Agency (USEPA) R.E.D Facts (1996) and the National Toxicology Program (NTP) Technical Report (2008). Three opinions, by the Scientific Committee on Cosmetic Products and Non-food Products Intended for Consumers (SCCNFP) (2002) and the Scientific Committee on Consumer Products (SCCP) (2005 and 2006), and literature searches conducted until March 2007, provided relevant supplementary studies.

In this report, references marked with an asterisk denote a secondary citation from a review article, while an unmarked reference denotes an original article examined for this assessment.

## 1.1 Chemical identity

Common name: Methyl dibromo glutaronitrile (MDBGN)



Molecular formula:  $\text{C}_6\text{H}_8\text{Br}_2\text{N}_2$

Molecular weight: 265.94

CAS registry number: 35691-65-7

IUPAC chemical name: 1,2-dibromo-2,4-dicyanobutane

AICS chemical name: Pentanedinitrile, 2-bromo-2-(bromomethyl)-

## 1.2 Regulatory information

### Australia

MDBGN is listed in:

- The Australian Inventory of Chemical Substances (AICS)
- The National Drugs and Poisons Schedule Committee has resolved to list the chemical in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) in Schedule C for cosmetic use and products intended to be in contact with the skin, in Schedule 6 for other uses; and in Appendix F, Part 3 (Warning statement 28 – “Repeated exposure may cause sensitisation” and Safety Directions 1, 4 and 7 – “Avoid contact with eyes”, “Avoid contact with skin” and “Wash hands thoroughly after use”). The implementation date for this decision is January 2010 (NDPSC, 2008a; NDPSC, 2008b).

MDBGN is not listed in the Hazardous Substances Information System (ASCC, 2008).

## Overseas

Until recently in the EU, MDBGN was permitted in rinse-off cosmetic products at a maximum concentration of 0.1% and was prohibited to be used in cosmetic sunscreen products at a concentration of > 0.025% (CIR 2006). However, based on a SCCP opinion in 2006 that MDBGN is a skin sensitiser and that no safe use levels in cosmetic leave-on and rinse-off products could be established, MDBGN was removed from Annex VI of the EU Cosmetics Directive (List of Preservatives which Cosmetic Products May Contain) and is no longer allowed to be present in any cosmetic products in the EU.

Regulations in the USA currently permit the use of MDBGN in cosmetics at up to 0.025% in leave-on products and 0.06% for rinse-off products (CIR 2006, NTP 2008).

### 1.3 Physical properties of MDBGN

MDBGN is off-white to tan crystalline powder, which melts at 50-53°C. It has a vapour pressure of  $3.3 \times 10^{-5}$  kPa at 25°C. At 20°C, its solubility in water is 0.212 g/100mL. It is also soluble in diethyl ether, ethanol and methanol. MDBGN is very soluble in acetone, benzene, chloroform, dimethylformamide, and ethyl acetate (Calgon Corporation 1994\*; O'Neil 2006).

### 1.4 Uses of MDBGN

MDBGN is reported to be used as a preservative and biocide in a wide range of products, including paints, emulsions, dispersed pigments, adhesives, joint cements, metalworking fluids, cosmetics, paper, inks, waxes and household detergents (Technical Resources International 1996; Scientific Committee on Consumer Products 2006).

In the USA, MDBGN is supplied to cosmetic manufacturers as a 10% solution in dipropylene glycol or as a 20% solution in 2-phenoxyethanol (2-PE). In 1994, industry reported to the Food and Drug Administration that MDBGN was found in cosmetic formulations at concentrations ranging from 0.0075 to 0.06% (as active ingredient). Subsequent literature reports noted the use of MDBGN increasing (Technical Resources International 1996). Cosmetic product containing MDBGN may be applied or come in contact with skin, eyes, hair, nails and mucous membranes. During 1992, seven of 20000 products (0.04%) on file with the Food and Drug Administration's Voluntary Cosmetic Registration Program contained MDBGN, and this proportion increased marginally by 1996 when 56 of 22287 registered products (0.25%) contained the chemical (De Groot et al. 1996a; 1996b).

In the Netherlands, 13% of cosmetic products on the market contained MDBGN in 1990, which increased to 25-35% by 1996.

In Australia, MDBGN is imported as mixtures and as a raw chemical for local formulation. Following a call for information from industry on the Australian use of MDBGN in 2007, MDBGN was reported in products that varied from adhesives and coatings to personal care products, including sunscreens, shampoos, shower gels and wet wipe hand towels. Limited information was provided on the final use concentration in certain products. MDBGN was reported to be present in shower gels and shampoos at between 0.003 and 0.004% and in sunscreens at 0.04%.

## 2. Toxicology

### 2.1 Toxicokinetics

#### Experimental animals

The CIR Expert Panel (1996) reviewed and summarised a range of studies on the toxicokinetics of MDBGN.

In an in vivo study, <sup>14</sup>C-MDBGN was administered to rats using one of the following protocols, single oral dose of 5mg/kg bw or 20mg/kg bw, 15 days of oral dosing at 5mg/kg bw/day or a single dose of 5mg/kg bw administered intravenously (Inveresk Research International 1990\*). In all dosing regimens, most of the radioactivity (>64.6%) was excreted in urine. At 7 days after exposure the blood had the highest levels of radioactivity in the body. In animals administered a single oral dose of 5 mg/kg, <sup>14</sup>C label in the blood peaked at 8 hours, and the tissues with the highest level of radioactivity were the gastrointestinal tract and the kidneys. In another in vivo study, 5mg/kg bw <sup>14</sup>C-MDBGN was applied to the skin of rats and 4 hours later it was estimated that 13% of the label had been absorbed and 4.1% was found in the excreta. At 96 hours the estimates were ~22% absorbed and 19.4% excreted (Inveresk Research International 1990\*). Male Sprague Dawley rats were percutaneously administered 0.1mL of <sup>14</sup>C-MDBGN at a concentration of 1 mg/mL. When the fate of the label was determined at 72 hours, ~30% of the radioactivity remained at the application site while ~40% was accounted for by excretion and distribution within the body (Cosmetic Toiletry and Fragrance Association 1982\*).

Other studies not reviewed by the CIR included a study where the fate of <sup>14</sup>C-MDBGN was investigated in male Fischer 344 rats administered by one of the following routes, intravenously (iv) at 8 mg/kg bw, 80 mg/kg bw orally or dermal doses of 25 mg/kg bw (Sauer et al. 1998). Animals were placed into metabolism cages and the fate of MDBGN monitored for 72 hours. Radioactive MDBGN was never recovered from the animals following iv administration. Instead, its de-brominated metabolite 2-methyleneglutaronitrile (2-MGN) peaked at a blood concentration of 7.3 µg/mL and quickly decreased to undetectable levels within an hour. At 72 hours, 63.6% of the label had been excreted in the urine, 4.1% was eliminated through faeces, 12.5% was circulating in the blood, 4.9% was distributed throughout the tissues and 6.6% had been exhaled as <sup>14</sup>CO<sub>2</sub>. Following oral dosing, 72.0% was recovered in urine, 9.7% in faeces, 2.6% in blood, 3.5% in tissues and 7.5% exhaled as CO<sub>2</sub> at 72 hours. Only a fraction of the dose was absorbed via the dermal route (<12%), however once inside the body the pattern of elimination for oral and dermal administration was virtually identical to that for the iv route. Regardless of the administration method, the urinary metabolites were similar with MDBGN ultimately converted to *N*-acetyl-*S*-(2,4-dicyanobutane)-*L*-cysteine and *N*-acetyl-*S*-(2,4-dicyanobutan-2-ol)-*L*-cysteine. Radiolabel was recovered from the body as 2-MGN and it was evenly distributed throughout the tissues. The above studies demonstrated that MDBGN was extremely labile in blood, plasma or glutathione-containing solutions, and in each case 2-MGN was produced. The formation of 2-MGN was inhibited by *N*-ethylmaleimide, a sulfhydryl-alkylating agent.

In a parallel study by the same authors, the biotransformation and binding of MDBGN to blood was investigated (Bao et al. 1998). At 48 hours after the injection of

radiolabelled MDBGN into rats, 12% of the label was covalently bound to blood, with 20% of this being bound to the plasma fraction and the remainder (80%) to erythrocytes. When added to whole blood at 37°C in vitro, MDBGN was converted to 2-MGN within 30 seconds. The study demonstrated that the conversion of MDBGN to 2-MGN is mediated by a free sulfhydryl-dependent biotransformation pathway and that 2-MGN is the reactive species responsible for binding to macromolecules following exposure to MDBGN.

## **Humans**

In an in vitro study reported by CIR Expert Panel (1996), <sup>14</sup>C-labelled MDBGN was applied (0.08 mg/cm<sup>2</sup>) to excised rat and human skin in either water or a sunscreen formulation, and passage of the <sup>14</sup>C label across the skin was measured for a 6 hour period (Bushy Run Research Center 1990\*). Female rat skin was more permeable than human skin to MDBGN in the sunscreen formulation; 1.5% of the radioactivity passed through the skin at 6 hours compared to ~0.9% for human. However, absorption was slightly greater in humans using the aqueous vehicle, with the amount of MDBGN absorbed after 6 hours contact being ~33% for human and 25% for female rat skin.

## **2.2 Acute toxicity**

The CIR Expert Panel (1996) and the USEPA (1996) reviewed and summarised a range of studies on the acute toxicity of MDBGN.

Administered orally the LD50 of MDBGN was 770 mg/kg for male Wistar Albino rats and 515 mg/kg for females (MB Research Laboratories 1991b\*, USEPA 1996). In New Zealand White rabbits, the LD50 dermal value was >5.0 g/kg (Biosearch 1982a\*, USEPA 1996). The toxicity of inhaled MDBGN was investigated in two studies using Sprague Dawley rats. In the first study, the LC50 was determined as >13.01 mg/L (MB Research Laboratories 1992\*, USEPA 1996). Some unexplained discrepancies were observed in this study. There was no mortality when rats were exposed to 13.01 mg/L MDBGN for 4 hours, but mortality was observed in 3 out of 5 animals that received a 4-hour exposure to 4.76 mg/L. The second study determined a LC50 of >5.09 mg/L (USEPA 1996).

## **2.3 Skin irritation**

There was no irritation observed in the 48 animals tested when MDBGN was applied to the skin of guinea pigs at concentrations of 0.5, 1, 2.5 and 5% in acetone, ( Hill Top Research 1981a\*). When the same concentrations were applied using 80% ethanol and 20% water as vehicles, irritation was observed in 24 animals (Hill Top Research 1981a\*).

In another study, there was no irritation observed in eight guinea pigs tested with 10 and 100% MDBGN in water. No further details were provided (IBR 1988b\*; IBR 1988a\*).

MDBGN (98%) was applied to the skin of 6 New Zealand albino rabbits via an occlusive patch for 4 hours (MB Research Laboratories 1991a\*). Skin reactions were scored at 0.5, 1, 24, 48 and 72 hours after removal of the wrapping. Erythema (very slight to moderate) and eschar formation, as well as oedema (present to well-defined) was noted at 30 and 60 minutes after removal of the patch in 5 animals. Erythema persisted through all the observation periods, while oedema began to subside by 48

hours and had disappeared in all but one animal by 72 hours. All skin reactions had dissipated by 7 days.

## **2.4 Eye irritation**

Instillation of MDBGN powder (98%) to the conjunctival sac of New Zealand White rabbits resulted in severe ocular irritation in all of the 6 animals tested (Biosearch 1982b\*). Severe irritation continued to be observed at 21 days post-instillation.

However, only slight irritation was observed in the eyes of 6 rabbits (strain not specified) when 2% MDBGN in aqueous solution was tested (MB Research Laboratories 1983\*). By day 3 post-instillation, irritation effect was further reduced. Conjunctival irritation was the primary response observed but no corneal opacity or iritis was noted.

## **2.5 Skin sensitisation**

### **2.5.1 Animal studies**

Euxyl K 400 is a commercially available mixture of 20% MDBGN and 80% phenoxyethanol (PE) marketed as a cosmetic and toiletry preservative. As the general population is commonly exposed to this mixture, Euxyl K 400 test conditions are often run in parallel with MDBGN in experimental studies and on occasion Euxyl K 400 acts as a surrogate for MDBGN (Jackson and Fowler 1998). In the following studies, the aforementioned ratio applies for Euxyl K 400 unless otherwise stated.

The studies described below are summarised in Table 1.

### **Non-adjuvant tests**

A sensitisation study was conducted on MDBGN using 19 guinea pigs induced with a closed patch containing 5% MDBGN in ethanol/water (80:20) for 6 hours, once a week for 3 weeks (with 5 to 9 days between exposures) (Hill Top Research, 1981d\*). The induction period was followed by a non-treatment period of 2 weeks. Non-exposed sites were challenged with 5% MDBGN w/v in acetone. Twelve days later, a re-challenge of 7 animals with 5, 2.5 and 1% MDBGN in acetone was conducted. One animal showed a grade 1 reaction during the initial challenge. No skin reactions were reported at rechallenge and in the control group.

Twenty Dunkin Hartley guinea pigs were induced with 5% MDBGN in ethanol/water (80:20) and challenged with 5% MDBGN w/v in acetone (Hill Top Research, 1982a\*). One animal had a response of grade 1 (scale 0-3). Rechallenge with 2% MDBGN in water returned a negative result for both dosed animals and control groups. Cross challenge with 2% MDBGN in water or 5% MDBGN in acetone from a different lot of test material also produced no skin reactions in dosed animals and control group.

Two groups of 20 Hartley guinea pigs were induced with closed patches containing 0.2% MDBGN in distilled water (Hill Top Research (1982b\*). Each patch was left in contact with the skin for 6 hours, once a week for 3 weeks (with 5 to 9 days between exposures). Induction was followed by a non-treatment period of 2 weeks. Non-exposed sites were challenged with either 0.2% MDBGN in water or 5% MDBGN in acetone. At the 0.2% challenge concentration, 3/20 had a score of 0.5 (scale 0-3) at 24 hours, which persisted in 2 animals for 48 hours. In the 5% challenge group, 6 animals had a score of 0.5 at 2 hours, which persisted in 4 animals for 48 hours. The overall

result was interpreted as negative because the scores were <1. After the primary challenge, 2 groups of 6 animals with scores of 0.5 (slightly patchy erythema) at 24- or 48-hour observation period were cross-challenged with a new lot of MDBGN. No irritation was observed at the 0.2% (mean score of 0.1 at 24 hours) and 5% (mean score of 0.2 at 48 hours) challenge groups.

In 20 Hartley guinea pigs induced and challenged with MDBGN (98%) in 0.2% aqueous solution, MDBGN did not show sensitisation reactions (Hill Top Research (1982c\*). In a follow-up study, the animals were induced with 5% MDBGN in ethanol/water and challenged with 5% MDBGN in acetone (Hill Top Research (1982c\*). Positive skin reactions were observed in both the test and control groups. However, no skin reactions were noted when the animals were re-challenged with 5% MDBGN in acetone.

In another study, 10 Dunkin Harley albino guinea pigs (5/sex) were induced and challenged with 5% MDBGN in aqueous solution (Biosearch Incorporated, 1982c). There were no sensitisation reactions observed. The positive control (1-chloro 2,4-dinitrobenzene) showed strong sensitisation reactions.

The components of Euxyl K 400 were examined individually for their skin sensitising potential using guinea pigs in 4 sensitisation test series (2 series with PE and 2 series with MDBGN) (Bruze et al. 1988). For each of the test series, 42 (12 control group; 24 test group and 6 positive control group) female Dunkin Hartley guinea pigs were induced intradermally and topically with a concentration of 0.5% w/v MDBGN in propylene glycol, and then challenged with a 0.1% w/v MDBGN. Positive reactions were similar for animals treated with MDBGN to that of control animals treated with vehicle (propylene glycol) alone. Ten percent (5/48) of the test animals showed sensitisation reactions.

In conclusion, available non-adjuvant tests in animals show no, or only minimal evidence of skin sensitising potential for MDBGN.

### **Adjuvant tests**

In a study using the Magnusson-Kligman maximisation method, 5% MDBGN in 80:20 ethanol/water was injected alone or in combination with Freund's Complete Adjuvant (FCA) to groups of 10 male Dunkin Hartley albino guinea pigs (Biosearch Incorporated, 1982d\*). At 24- or 48-hours post challenge, MDBGN or MDBGN with FCA did not induce sensitisation reactions. The positive control, 1-chloro-(2,4-dinitrobenzene) showed positive reactions in 9/10 animals at 24 hours- and 0/10 animals at 48 hours post challenge.

A sensitisation study was also conducted using 20% MDBGN in 2-PE on 20 Pirbright guinea pigs (IBR, 1988b\*). Initial induction consisted of injections of each of the following: 10% dilution in water, 10% dilution in FCA (1:1) and FCA in water (1:1). The effective concentration of MDBGN used for induction was 2%. A week later, an occlusive patch with 20% MDBGN was applied to the injection site for 24 hours. The challenge was conducted after three weeks using another occlusive dermal application of 20% MDBGN. No reactions were noted after patch removal and 24 hours later.

An additional 20 Pirbright guinea pigs (IBR, 1988a\*) were tested using 20% MDBGN that had been UV irradiated for 8 hours (details not provided). Induction of test animals consisted of intradermal injections of each of the following: 10% dilution in Arachis oil (AO), 10 % dilution in FCA, and undiluted FCA. After 7 days, occlusive patches with

undiluted MDBGN were applied on the same test sites for 48 hours. Control animals were induced using similar protocols but in the absence of MDBGN. After 3 weeks, the test and control animals were challenged with occlusive patches of undiluted MDBGN for 24 hours on one side of the spine and the other side with AO. No sensitisation reactions were noted at 24 and 48 hours after patch removal.

In another study, 10 female guinea pigs were injected with MDBGN dissolved in FCA: saline (50:50) or Euxyl K 400 (20 females) in FCA: saline (50:50) into the dermis on days 1, 5 and 9 (Hausen 1993). On day 20 the animals were challenged by topical application (non-occlusive) of 0.3% and 0.1% MDBGN or 3% and 1% Euxyl K 400. Skin reactions were evaluated 24, 48 and 72 hours later. At the end of the observation period (72 hours), 7/10 and 5/10 animals treated with 0.3% and 0.1% MDBGN showed positive results, respectively. For Euxyl K400, there were 7/10 animals treated with 3% and 4/10 animals treated with 1% that showed positive results. The allergic reactions observed were described as distinct but weak (slight erythema). However, this study lacked a suitable negative control required to determine background skin reactions.

A GPMT reportedly carried out according to OECD guidelines was used to examine the skin sensitisation potential of Euxyl K 400 and MDBGN (99.1% purity) alone (Wahlkvist et al. 1999). In this study, 15 female Dunkin Hartley guinea pigs were induced by the injection of MDBGN into the dermis on day 0 (0.1% in AO) and on day 7 by occlusive topical application of MDBGN (10% in AO) for 24 hours. The guinea pigs were then challenged on day 22 with occlusive patches containing 1%, 0.3% or 0.1% MDBGN (in olive oil, OO) or Euxyl K 400 diluted with PE (equivalent to 1% and 0.3% MDBGN in PE) for 24 hours. Test reactions were read at 48 and 72 hours after challenge. Negative control animals were induced and challenged in the same manner as the test animals but were given appropriate vehicles only. No statistical difference in sensitisation reactions was seen between MDBGN or Euxyl K 400 treated animals. At 72 hours, 3, 1 and 0 animals showed positive results when tested with 1, 0.3 and 0.1% MDBGN in OO, respectively. One animal responded positive when tested with Euxyl K 400 (equivalent to 1% MDBGN in PE only).

A cumulative contact enhancement test (CCET) was used to examine the skin sensitisation potential of Euxyl K 400 and MDBGN (99.1% purity) alone (Wahlkvist et al. 1999). Guinea pigs were induced with a topical application (occlusive patch for 24 hours) of one of the following: 30%, 20%, 10%, 3%, 1% MDBGN (in acetone: AO (1:2)) or vehicle alone on days 0, 2, 7 and 9 of the study. Freund's complete adjuvant was administered on day 7. The animals were challenged topically with 3%, 1%, 0.3%, 0.1%, 0.01% and 0.003% MDBGN in OO and vehicle alone on day 22. In this study, MDBGN induced skin sensitisation in guinea pigs in a dose-dependent manner when read at 72 hours after challenge.

In conclusion, the adjuvant tests in animals showed no or only minimal evidence of skin sensitising potential for MDBGN, except for two tests (CCET and modified FCA procedure) similar to adjuvant method which showed positive skin sensitisation reactions.

### **Local Lymph Node Assays (LLNA)**

A LLNA reportedly carried out according to OECD guidelines was used to examine the skin sensitisation potential of Euxyl K 400 and MDBGN (99.1% purity) alone (Wahlkvist et al. 1999). Mice received topical ear applications of 20%, 5%, 1.25% or 0% MDBGN dissolved in either dimethyl formamide (DMF) or PE vehicle for 3

consecutive days. Three days after the final application, mice were injected with [<sup>3</sup>H]thymidine and left for 5 hours before sacrifice. Lymph nodes were harvested, dissociated to single cells and [<sup>3</sup>H]thymidine incorporation determined. When administered in a solution of 5% and above MDBGN showed sensitisation reactions in mice as evidenced by a LLNA stimulation index of greater than 3 and increased lymph node size.

In a study by Wright et al. (2001) the LLNA assay was used to examine the skin sensitising potential of MDBGN in a variety of vehicles and in two separate testing centres. OECD guidelines were followed in this study. At day 0, MDBGN was applied topically to the ears of mice at the following concentration and vehicle combinations, 1-25% in acetone: olive oil (4:1), 0.25-50% in methylethylketone (MEK), 1-50% in DMF, 0.25-50% in dimethyl sulphoxide (DMSO), and 0.5%-7.5% in ethanol: water (90:10). Mice treated with the vehicles alone served as negative controls. On day 5, the animals were injected with [<sup>3</sup>H]thymidine and sacrificed 5 hours later for the determination of [<sup>3</sup>H]thymidine incorporation. Regardless of the vehicle, MDBGN induced skin sensitisation in a dose dependent manner. The highest stimulation index observed using each of the different vehicles was 7.1 for acetone: olive oil, 7.9 for MEK, 5.9 for DMF, 3.6 for DMSO and 13.6 for ethanol: water. The choice of vehicle did affect the skin sensitisation response of MDBGN. Using MEK, a lower concentration of MDBGN (0.5%) was required to return a positive reaction than any of the other vehicles ( $\geq 1\%$ ). Using DMSO provoked a toxic reaction on the dorsum of mouse ears, which made it impossible to increase the application concentrations to the dose levels necessary to derive an accurate EC3 value.

In a LLNA study carried out in accordance with OECD guidelines, MDBGN in acetone/OO was used as a positive control while testing the sensitisation potential of iodopropynyl butylcarbamate (Siebert 2004). A stimulation index of 6.1 and increased weight of lymph nodes were seen with an application concentration of 10% MDBGN. The result was within the range of historical data. This study was published in a section of the journal not subject to peer review.

**Table 1: Summary of skin sensitisation studies**

Type of test	Experimental Animals	Test substance	Doses	No of positive result	Reference
Non-adjuvant	Guinea pigs	MDBGN	<u>Induction:</u> 5% w/v in ethanol/water (80:20) (topical) <u>Challenge:</u> 5% w/v in acetone <u>Re-challenge:</u> 5, 2.5 and 1% w/v in acetone	MDBGN (w/v) in acetone <u>Challenge:</u> 5% = 1/19 <u>Re-challenge:</u> 5, 2.5 and 1% = 0/19	Hill Top Research, 1981d*
Non-adjuvant	Guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 5% in w/v ethanol/water (80:20) <u>Challenge:</u> 5% w/v in acetone <u>Re-challenge:</u> 2% w/v in water <u>Cross-challenge:</u> 2% w/v in water or 5% w/v in acetone	<u>Challenge:</u> 5% (w/v) in acetone = 1/20 <u>Re-challenge:</u> 2% (w/v) in water = 0/20 <u>Cross-challenge:</u> 2% (w/v) in water = 0/20 5% (w/v) in acetone = 0/20	Hill Top Research, 1982a*

Non- adjuvant	Guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 0.2% in w/v water <u>Challenge:</u> 0.2% w/v in water or 5% w/v in acetone <u>Re- challenge<sup>#</sup>:</u> 0.2% w/v in water or 5% w/v in acetone	<u>Induction:</u> 0.2% in w/v water <u>Challenge:</u> 0.2% w/v in water = 2/20 (48 hrs) 5% w/v in acetone = 4/20 (48 hrs) <u>Re-challenge</u> 0.2% w/v in water = 0/6 5% w/v in acetone = 0/6	Hill Top Research, 1982b*
Non- adjuvant	Guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 0.2% in w/v water <u>Challenge:</u> 0.2% w/v in water	<u>Challenge:</u> 0.2% w/v in water = 0/20	Hill Top Research, 1982c*
Non- adjuvant	Guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 5% in w/v ethanol/water <u>Challenge:</u> 5% in w/v acetone <u>Rechallenge:</u> 5% in w/v acetone	<u>Challenge:</u> 5% in w/v acetone = equivocal <u>Rechallenge:</u> 5% in w/v acetone = 0/20	Hill Top Research, 1982c*
Non- adjuvant	Guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 5% w/v in aqueous solution <u>Challenge:</u> 5% w/v in aqueous solution	<u>Induction:</u> 5% w/v in aqueous solution = 0/10 <u>Challenge:</u> 5% w/v in aqueous solution = 0/10	Biosearch Incorporat ed, 1982c*)
Non- adjuvant	Female guinea pigs/ Dunkin Hartley	Euxyl K400	<u>Induction:</u> 0.5% MDBGN w/v (topical and intradermal) <u>Challenge:</u> 0.1% MDBGN w/v	<u>Challenge:</u> 0.1% w/v MDBGN Test 1 = 3/24 Test 2 = 2/24	Bruze et al, 1988
Adjuvant	Male guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 5% in 80:20 ethanol/water or 5% in 80:20 ethanol/water with FCA (intradermal) <u>Challenge:</u> 5% in 80:20 ethanol/water or 5% in 80:20 ethanol/water with FCA	<u>Challenge:</u> 5% in 80:20 ethanol/water = 0/10 5% in 80:20 ethanol/water with FCA = 0/10	Biosearch Incorporat ed, 1982da*
Adjuvant	Guinea pigs /Pirbright	MDBGN	<u>Induction<sup>1</sup>:</u> 10% dilution in water, 10% dilution in FCA (1:1) and FCA in water (1:1) (intradermal) and 20% w/v MDBGN (topical) <u>Challenge:</u> 20% w/v MDBGN (topical)	<u>Challenge:</u> 20% w/v (topical) = 0/20	IBR, 1988b*
Adjuvant	Guinea pigs /Pirbright	MDBGN (UV irradiated)	<u>Induction<sup>1</sup>:</u> 10% dilution in AO, 10% dilution in FCA and	<u>Challenge:</u> 20% w/v (topical) = 0/20	IBR, 1988a*

			FCA (intradermal) and 20% w/v MDBGN (topical) <u>Challenge:</u> 20% w/v (topical)		
Adjuvant - Modified FCA procedure	Female guinea pigs/ Dunkin Hartley	MDBGN or Euxyl K400	<u>Induction:</u> MDBGN in FCA:saline (50:50) or Euxyl k400 in FCA:saline (50:50) (intradermal) <u>Challenge:</u> 0.3% and 0.1% MDBGN or 3% and 1% Euxyl K400 (topical)	MDBGN 0.3 % = 7/10 (72 hrs) 0.1% = 5/10 (72 hours)  Euxyl 400 3% = 7/20 (72 hours) 1% = 4/20 (72 hours)	Hausen, 1993
Adjuvant	Female guinea pigs/ Dunkin Hartley	MDBGN or Euxyl K400	<u>Induction:</u> 0.1% in AO (intradermal) and 10% in AO (topical) <u>Challenge:</u> MDBGN in OO: 1%, 0.3%, 0.1% or Euxyl K400 (MDBGN in PE: 1% and 0.3%)	MDBGN in OO 1% = 3/15 0.3 % = 1/15 0.1% = 0/15 Euxyl K400 (MDBGN in PE) 1% = 1/15 0.3% = 0/15	Wahlkvist et al, 1999
CCET	Female guinea pigs/ Dunkin Hartley	MDBGN	<u>Induction:</u> 30, 20, 10, 3, 1% in acetone:AO (1:2) (topical) <u>Challenge:</u> MDBGN: 3, 1, 0.3, 0.1, 0.03, 0.01 and 0.003% in OO	MDBGN in OO 3% = 26/40 1% = 18/40 0.3% = 9/40 0.1% = 8/40 0.03% = 2/40 0.01% = 5/40 0.003% = 5/40	Wahlkvist et al, 1999
LLNA	Mice	MDBGN or Euxyl K400	MDBGN in PE or DMF = 0, 1.25, 5.0 and 20% w/v	MDBGN in PE 1.25%; SI = 2.3 – 3.1 5.0%; SI = 6.3 – 7.6 20%; SI = 8.4 - 12  MDBGN in DMF 1.25%; SI = 2.3 – 2.4 5.0%; SI = 5.7 - 6.3 20%; SI = 7.4 - 7.9	Wahlkvist et al, 1999
LLNA <sup>##</sup>	Mice	MDBGN	1-25% in acetone: olive oil (4:1), 0.25- 50% in methyl ethylketone (MEK), 1-50% in DMF, 0.25- 50% in dimethyl sulphoxide (DMSO), 0.5%-7.5% in ethanol: water (90:10)	acetone: OO; SI = 7.1 MEK; SI= 7.9 DMF; SI = 5.9 DMSO; SI = 3.6 ethanol: water; SI = 13.6	Wright et al, 2001
LLNA	Mice	MDBGN	10% MDBGN in OO	SI = 6.1	Siebert, 2004

Methyldibromo glutaronitrile (MDBGN), Olive oil (OO); Arachis oil (AO); dimethyl sulphoxide (DMSO), Freund's Complete Adjuvant (FCA)

<sup>#</sup>Only 6 animals with slightly patchy erythema were treated for re-challenge.

<sup>##</sup>Only the highest SI observed for the vehicles are reported on the table. For LLNA, the results are recorded as stimulation index (SI). SI greater than 3 indicates a positive result.

<sup>1</sup>The effective concentration of MDBGN used for induction was 2%.

\*Source: CIR (1996)

### 2.5.2 Human volunteer studies (patch test surveys)

Human patch test studies on MDBGN or Euxyl K 400 carried out in Australia and in other countries are reported below. The studies are presented according to the country where the testing has been conducted. These studies are also summarised in Table 2.

A number of studies have been performed in Italy. In a two-year period from September 1988 to September 1990, 2057 patients suspected of having contact dermatitis were patch tested with Euxyl K 400 (Tosti et al. 1991). Euxyl K 400 was used at a concentration of 2.5% in either petrolatum (pet.) or ethanol (eth.). Twenty-four (1.2%) of the patients had a positive reaction to Euxyl K 400 (in pet. and eth.). Eleven of the positive patients were then additionally patch tested with 0.5% Euxyl K 400 in pet. and 5% PE in pet.; there was 1 positive reaction to PE and 3 mild reactions to Euxyl K 400. This subset of patients was then challenged with two skin care lotions, one containing 0.1% Euxyl K 400 while the other was preserved with 0.2% methyl and propyl paraben mix and 0.2% diazolidinylurea. The lotion containing Euxyl K 400 was applied twice daily to the right forearm for two weeks, and the other lotion applied in an identical fashion to the left forearm. Five of the 11 patients (45%) developed itching dermatitis on the forearms treated with lotion containing Euxyl K 400 within 5 to 7 days.

In a follow up study, Tosti et al. (1995) performed another series of patch tests at the same institute. From January 1991 to October 1994, 3455 patients with suspected contact dermatitis were patch tested with 2.5% Euxyl K 400 in petrolatum. Additional test conditions were administered to the above patients as follows: Euxyl K 400 (0.5% aqueous (aq.)) in 3022 patients, Euxyl K 400 (1.5% aq./propylene glycol) in 729 patients and MDBGN (0.5% pet.) in 919 patients. The percentage of positive patch results for the various conditions were 2.8% for Euxyl K 400 (2.5% pet.), 1.78% for Euxyl K 400 (0.5% aq.), 0.68% for Euxyl K 400 (1.5% aq./propylene glycol) and 2.3% for MDBGN (0.5% pet.). Fourteen of the patients positive to Euxyl K 400 (2.5% pet.) participated in a study in which they applied a generic skin care lotion preserved with 0.1% Euxyl K 400 to the inner forearm, twice a day for two weeks. Eight of the 14 patients (57%) showed itchy dermatitis on the treated area after 5 to 7 days.

Motolese et al. (1991) tested 1033 patients suspected of having contact dermatitis using a series of patch tests that included 0.15% and 2% Euxyl K 400 in propylene glycol. At the lower concentration, 1.7% of the subjects were positive for Euxyl K 400, with 2% showing positive reactions at the higher concentration. At another institute, 889 patients suspected of allergic contact dermatitis were patch tested with 0.2% Euxyl K 400 in a hydro-alcoholic vehicle (Corazza et al. 1993). The percentage of patients positive to Euxyl K 400 was 0.56%.

In the Netherlands, the incidence of MDBGN sensitivity has also been studied extensively. From January to April 1991, 1142 patients suspected of allergic contact dermatitis were tested with 0.05% MDBGN in petrolatum (De Groot et al. 1991b). A positive reaction to MDBGN was observed in 0.5% of the subjects tested.

In a report by Van Ginkel and Rundervoort (1995), between January 1993 and July 1994, 814 patients were patch tested with MDBGN (0.1% pet.). Of the subjects tested 16 (2.0%) were positive to MDBGN.

In a follow up study by De Groot et al (1996a), the prevalence of contact allergy to MDBGN was investigated in a multi-centre study. All patients suspected of having contact dermatitis were patch tested with MDBGN at concentrations of 0.05%, 0.1% and 0.3% w/w dissolved in pet.: soy lecithin. Euxyl K 400 (0.5% pet.) and MDBGN (0.1% pet.) were additionally tested in some centres. A total of 2943 patients were patch tested of which 119 (4.0%) were positive to 1 or more concentrations of MDBGN.

In a study by Okkerse et al. (1996), 1019 patients with suspected contact dermatitis were patch tested with Euxyl K 400 (0.5% pet.) and MDBGN (0.1% pet.). In 2.4% of the patients there was a positive reaction to both Euxyl K 400 and MDBGN. No patient was positive to one but not the other patch.

There are two reports of MDBGN patch testing from the United States. Several clinics located across the USA patch tested 3549 patients between July 1992 and June 1994 (Marks et al. 1995). A standard panel of 52 allergens including 1% Euxyl K 400 in pet. was screened in each patient, with 1.5% of the test population positive for MDBGN. Researchers from a university in the USA obtained retrospective patch test results from 163 patients (Jackson and Fowler 1998). Among the test panel was Euxyl K 400 at 1% and 2.5% in petrolatum. Patch testing was conducted according to standard techniques and evaluated at 48 and 96 hours. Of the patients, 11.7% had positive reaction to Euxyl K 400.

Between January 2002 and September 2002, Danish researchers patch tested 807 patients with suspected contact dermatitis (Zachariae et al. 2003). MDBGN (0.3% in pet.) returned a positive response in 2.9% of the test subjects. In another report from Zachariae et al. (2005) conducted a year later, 766 patients suspected of having contact dermatitis were tested in a patch test series including MDBGN (0.3% in pet.). A positive patch result to MDBGN was observed in 4.9% of the subjects tested. In 2003, it was noted that MDBGN was the third most frequent cause of contact allergy in that clinic. In another report from Denmark, 2146 patients from 4 clinics were patch tested with 0.3% MDBGN in petrolatum or 1.5% Euxyl K 400 in petrolatum (equivalent to 0.3% MDBGN and 1.2% PE) (Johansen et al. 2005). One hundred and ten (5%) of the patients were positive to MDBGN or Euxyl K 400.

From January 1990 to December 1994, the Information Network of Departments of Dermatology (IVDK), a network of 24 German dermatology departments conducted patch tests on various preservatives, antimicrobials and biocides, including Euxyl K 400 and MDBGN (Schnuch et al. 1998). There were 3 groups containing the following number of patients: 25584 for Euxyl K 400 (0.5% pet.) in the standard series, 11422 for Euxyl K 400 (0.5% pet.) in the preservative series and 1726 for MDBGN (0.1% pet.) in the industrial biocide series. In the standard group 1.8% were positive while in the preservative group 2.3% returned positive results, and for the industrial biocide group 2.1% were positive to MDBGN.

In another study by the IDVK, 4615 patients were patch tested with Euxyl K 400 (0.5 and 1.0% in pet.) between May 1997 and April 1998 (Geier et al. 2000). MDBGN (0.3% in pet.) was also tested in 4343 of the above patients. Positive patch results were observed in 3.5% (1.0% Euxyl K 400 in pet.), 2.3% (0.5% Euxyl K 400 in pet.) and 5.0% (0.3% MDBGN in pet.) of the patients. Additionally, MDBGN at 0.1% in pet. and 0.3% in pet. were tested in 988 selected patients. For 0.1% MDBGN, 1.4% were positive and for 0.3% MDBGN 3.3% were positive.

The IVDK, which had expanded to 40 clinics in Germany, Austria and Switzerland, conducted a series of patch tests using patient's own cosmetics between 1998 and 2002 (Uter et al. 2005). As Euxyl K 400 and MDBGN are common ingredients in cosmetics these were included in a common allergen panel run concomitantly with the individual's cosmetics. Euxyl K 400 was used at 1% in pet. and MDBGN at 0.3% in pet. for patch tests. For Euxyl K 400, 13.2% of patients were positive when sensitised to own cosmetics, which dropped to 5.3% when not sensitised to own cosmetics. For MDBGN, 19.6% of patients returned a positive result if sensitised to their own cosmetics, while 7.8% of patients not sensitised to their own cosmetics were positive.

In Finland, 736 patients were patch tested with Euxyl K 400 (0.5% pet.) between January 1989 and April 1996 (Aalto-Korte et al. 1996). Positive patch tests to Euxyl K 400 were observed in 1.5% of the patients. In another study, Hasan et al (2005) retrospectively analysed patch test results from 7 dermatological clinics collected between 1995-1997 and 2000-2002. The panel of cosmetic allergens tested included 0.1% MDBGN. The prevalence of MDBGN sensitivity increased from 1.0% in 1995-1997 to 1.5% in 2000-2002, although this increase was not statistically significant.

In a brief report from the United Kingdom (UK), two patients with dermatitis tested positive to Euxyl K 400 and 25 controls were negative. In addition, 1800 patients were patch tested with 1% Euxyl K 400 (aq.) in 1989, with no positive reaction observed in any of the patients (Ross et al. 1992). McFadden et al (2000) conducted a retrospective analysis of 11739 patients who were patch tested over a period of 10 years at the same institute as reported by Ross et al (1992). From January 1989 patients were patch tested with Euxyl K 400 (0.5% pet.) until July 1997 when it was substituted with MDBGN (0.3% pet.) up until the end of the study in 1999. There was a significant increase in MDBGN sensitivity during the period between 1994-1999 (0.6%) when compared to 1989-1993 (0.4%). During the year 2000, 3062 patients were patched tested at seven centres located in the UK (Britton et al. 2003). Among the allergen panel was MDBGN (0.1% pet.), which induced an allergic reaction in 2.4% of the test subjects.

In 1990, Portuguese researchers patch tested 400 patients with 1% Euxyl K 400 (aq.) and 30 patients with 0.1% MDBGN (pet.) (Torres and Soares 1992). There were no positive patch test reactions.

A French study that took place between 1988 and 1994 conducted patch tests on 1217 patients with contact dermatitis (Vigan et al. 1996). Euxyl K 400 was tested at 2% in petrolatum. The incidence of contact allergy to MDBGN increased from approximately 1% in 1990 to more than 3% in 1994.

Guimaraens et al. (2000) reported on 528 patients from Spain who were patch tested with Euxyl K 400 (0.5% pet.) between September 1998 and June 1999. The observed rate of skin sensitisation was 0.9%.

Investigators from Belgium retrospectively analysed 819 patients with suspected contact dermatitis who were patch tested between January 1998 and December 1999 (Kohl et al. 2002). A range of allergens present in cosmetics were screened, including Euxyl K 400 at 1.5% in petrolatum. Among the preservative/antioxidant category of cosmetic additives, sensitivity to Euxyl K 400 was the most prevalent and 2.8% of the subjects tested were allergic to this additive.

Wilkinson et al. (2002) reported on data collated from 16 clinics in 11 European countries over a 10-year period from 1991-2000. Seven common preservatives including 0.2% MDBGN in petrolatum were patch tested on 48485 patients. MDBGN

showed the greatest change in positive incidence of all the allergens tested, increasing from 0.7% in 1991 to 3.5% in 2000.

Eleven test clinics across nine European countries conducted a study examining the optimal conditions for patch testing MDBGN (Gruvberger et al. 2005a). In the period between January 2002 and June 2002, 2661 patients with suspected contact dermatitis were patch tested with 0.1%, 0.3%, 0.5% and 1.0% w/w MDBGN in petrolatum. The percentage of positive subjects varied with test concentration, with 4.4% of the patient population testing positive to the highest MDBGN patch concentration (1.0%) while only 1.1% returned a positive result at the lowest concentration tested (0.1%). For each test concentration the percentage of positive results was significantly lower than for the next highest concentration tested.

In the period between 2000 and 2004, 308 patients diagnosed with contact dermatitis were patch tested in Turkey (Boyvat et al. 2005). Among the allergens tested was Euxyl K 400 (1.5% pet.) to which 0.9% of the subjects returned a positive patch test result.

Zoller et al. (2006) retrospectively analysed patch test data of 2285 patients from Israel, screened between January 1995 and December 2004. Sensitivity to Euxyl K 400 increased steadily from 0.9 to 1.7% during the course of the study, while the MDBGN sensitivity rate remained stable at approximately 2%.

Euxyl K 400 (1.5% and 0.1% in pet.) and MDBGN (0.3% and 0.1% in pet.) patch test results collected over a 12-year period were analysed from two Australian clinics (Williams et al. 2007). Twenty of 2837 (0.7%) patients tested positive for Euxyl K 400 or MDBGN. The number of patients tested positive for either chemicals have been reported recently to have increased to 70 patients (Nixon 2008).

Overall, the sensitisation reactions from the patch test surveys varied between countries. Across all available patch test surveys (concentration range of 0.03-0.5% MDBGN), the prevalence rate of positive reactions ranged from 0-11.7% with a median prevalence rate of 2.0%. The prevalence rate increased to 19.6% in studies where 0.3% MDBGN was tested in patients sensitised to their own cosmetics.

**Table 2: Summary of human volunteer studies (patch test surveys). Studies are grouped according to country/region**

# Patients	Test Substance	% Positive	Year(s) Study Performed, Country	Reference
2057	2.5% Euxyl K 400 in pet. and eth.	1.2	1988-1990, Italy	Tosti et al. 1991
3455	2.5% Euxyl K 400 in pet.	2.8	1991-1994, Italy	Tosti et al. 1995
3022	0.5% Euxyl K 400 in aq.	1.8		
729	1.5% Euxyl K 400 in aq./propylene glycol	0.7		
919	0.5% MDBGN in pet	2.3		
1033	0.15% Euxyl K 400 in propylene glycol	1.7	Italy	Motolese et al. 1991
	2% Euxyl K 400 in propylene glycol	2.0		
889	0.2% Euxyl K 400 in aq./eth.	0.6	Italy	Corazza et al. 1993
1142	0.05% MDBGN in pet.	0.5	1991, Netherlands	De Groot et al. 1991

2943	0.05, 0.1 & 0.3% MDBGN in pet./soy lecithin	4.0*	1994, Netherlands,	De Groot et al. 1996
1019	0.5% Euxyl K 400 in pet. and 0.1% MDBGN in pet.	2.4	Netherlands	Okkerse et al. 1996
814	0.1% MDBGN in pet.	2.0	1993-1994, Netherlands	Ginkel and Rundervoort 1995
3549	1% Euxyl K 400 in pet.	1.5	1992-1994, USA	Marks et al. 1995
163	1% and 2.5% Euxyl K 400 in pet.	11.7**	USA	Jackson and Fowler 1998
807	0.3% MDBGN in pet.	2.9	2002, Denmark	Zachariae et al. 2003
766	0.3% MDBGN in pet.	4.9	2003, Denmark	Zachariae et al. 2005
2146	0.3% MDBGN in pet. or 1.5% Euxyl K 400 in pet.	5.1	Denmark	Johansen et al. 2005
25584	0.5% Euxyl K 400 in pet.	1.8	1990-1994, IVDK	Schnuch et al. 1998
11422	0.5% Euxyl K 400 in pet.	2.3		
1726	0.1% MDBGN in pet.	2.1		
4615	0.5% Euxyl K 400 in pet.	2.3	1997-1998, IVDK	Geier et al. 2000
4615	1.0% Euxyl K 400 in pet.	3.5		
4343	0.3% MDBGN in pet.	5.0		
988	0.3% MDBGN in pet.	3.3		
988	0.1% MDBGN in pet.	1.4		
71	1% Euxyl K 400 in pet. 0.3% MDBGN in pet.	13.2 <sup>#</sup> 19.6 <sup>#</sup>	1998-2002, IVDK	Uter et al. 2005
1262	1% Euxyl K 400 in pet. 1% Euxyl K 400 in pet.	5.3 7.8		
736	0.5% Euxyl K 400 in pet.	1.5	1989-1996, Finland	Aalto-Korte et al. 1996
5218	0.1% MDBGN	1.0	1995-1997, Finland	Hasan et al. 2005
11786		1.5	2000-2002	
1800	1% Euxyl K 400 in aq.	0	1989, UK	Ross et al. 1992
11739	0.5% Euxyl K 400 in pet. or 0.3% MDBGN in pet.	0.4	1989-1997, UK	McFadden et al. 2000
3062	0.1% MDBGN in pet.	2.4	2000, UK	Britton et al. 2003
400	1% Euxyl K 400 in aq.	0	1990	Torres and Soares 1992
1217	2% Euxyl K 400 in pet.	2.1	1988-1994, France	Vigan et al. 1996
528	0.5% Euxyl K 400 in pet.	0.9	1998-1999, Spain	Guimareaens et al. 2000
819	1.5% Euxyl K 400 in pet.	2.8	1998-1999, Belgium	Kohl et al. 2002
48485	0.2% MDBGN in pet.	0.7 (1991)	1991-2000, 9 European countries	Wilkinson et al. 2002
		3.5 (2000)		
2661	0.1% MDBGN in pet. 0.3% MDBGN in pet. 0.5% MDBGN in pet. 1.0% MDBGN in pet.	1.1 1.9 3.0 4.4	2002, 9 European countries	Gruvberger et al. 2005
308	1.5% Euxyl K 400 in pet.	0.9	2000-2004, Turkey	Boyyat et al. 2005
2285	Euxyl K 400 MDBGN	0.9-1.7 ~2.0	1995-2004, Israel	Zoller et al. 2006

2837	Euxyl K 400 or MDBGN	0.7	1993-2004, Australia	Williams et al. 2007
*	Positive to one or more concentration			
**	Positive to one or both concentrations			
#	Patients were sensitised to their own cosmetics			

### 2.5.3 Human volunteer studies (studies on MDBGN pre-sensitised individuals)

Patients identified as sensitised to MDBGN were enrolled in a study examining whether inclusion of MDBGN in rinse-off products would elicit an allergic response (Tosti et al. 2000). The subjects were 12 adults previously found to have positive reactions to Euxyl K 400 and MDBGN but who were free from any cutaneous lesions for at least the previous 3 months. Individuals were supplied with a shampoo containing 0.02% MDBGN. All subjects were initially patch tested with a shampoo identical to the test formula except it did not contain MDBGN to confirm none of the subjects was sensitised to any component of the shampoo matrix. Following a negative patch test, patients were instructed to use the shampoo with MDBGN in a normal manner at least 3 times a week for a period of 9-13 weeks. Of the 11 subjects who completed the study there was no skin reaction, dermatitis or reports of itching from using the shampoo with MDBGN.

In a study by Jensen et al. (2004), 19 patients pre-sensitised to MDBGN (positive reaction to 0.3% MDBGN in pet. between 1998-2002) participated in a repeated open application test (ROAT). This study was performed blinded and randomised so that during the study neither the patient nor investigators knew which soap contained the MDBGN. In this test the subjects were asked to wash a 5-10 cm<sup>2</sup> area on the inside of their forearms twice daily for up to 4 weeks, with two of the subjects continuing for a fifth week. Each patient was given two bottles of liquid soap, one for each forearm, which were identical except for the addition of 0.1% MDBGN to one of the bottles. Seven of the 19 (37%) developed dermatitis on the test area after 6-34 days. No adverse skin reaction was observed in a separate control group, which was comprised of 9 subjects who had previously tested negative to MDBGN.

Eighteen patients with contact allergy to MDBGN (positive patch test to MDBGN within the last 4 years) were enrolled in a double-blinded study investigating whether pre-sensitised individuals could tolerate MDBGN in leave-on cosmetics at a concentration of 0.005-0.01% (Kynemund Pederson et al. 2004a). As a control, 10 volunteers without MDBGN sensitivity were also included in the study. Patients and controls were patch tested on the upper back with MDBGN (0.3% in pet.), PE (1% in pet.), low lipid content (20%) moisturiser without MDBGN and high lipid content (70%) moisturiser without MDBGN. The patches were removed after 2 days and readings were done on day 3 and day 7. Thirteen (72%) of 18 patients and none of the controls had positive patch tests to 0.3% MDBGN. All patients and controls returned negative patch tests to phenoxyethanol and the two lipid moisturisers. In a ROAT, each participant were initially given a tube of high lipid moisturiser and a tube of low lipid moisturiser, each containing 0.005% MDBGN. Both moisturisers were applied twice a day to areas on the left and right side of the neck. If no reaction was observed after 14 days, the moisturisers were substituted for ones containing 0.01% MDBGN. As soon as a skin reaction was observed in an individual the test was stopped. For the overall ROAT results, 11 (61%) patients developed dermatitis to the low and/or high lipid moisturisers, between 2 and 19 days. Ten of the subjects reacted to the 0.005% MDBGN low-lipid moisturiser and of this 10, three were also positive to the 0.005%

MDBGN high-lipid moisturiser. One patient did not react to moisturiser containing 0.005% MDBGN but did return a positive ROAT result to both types of moisturiser containing 0.01% MDBGN at day 19. All of the control subjects returned negative ROAT results.

In a study examining the effect of detergent on MDBGN allergic reactions, 20 patients with a pre-existing MDBGN sensitivity were subjected to a series of patch tests (Kynemund Pederson et al. 2004b). MDBGN was tested at the following concentrations 0.001, 0.005, 0.01, 0.05 and 0.1%, with and without 0.25% sodium lauryl sulfate (SLS). Patches were removed after 24 hours and skin evaluation was performed at Days 1, 3 and 7. In addition, evaluation of skin reactions was performed by measurement of transepidermal water loss (TEWL) and skin colour. Between 47% and 79% of subjects at the various doses gave a positive response to MDBGN. In most subjects the patches containing SLS induced a positive response at a lower MDBGN concentration when compared to patches without detergent. Expressed as an odds ratio, responses were augmented by a factor of 6.4.

Patients from 10 European and 1 American clinic were recruited for a ROAT study (Gruvberger et al. 2005b). There were 51 subjects who had either positive or doubtful reactions to at least one of the 4 following patch test conditions: 0.1, 0.3, 0.5 and 1.0% MDBGN in petrolatum. For the ROAT, patients were given 2 moisturising lotions that were identical except one contained 0.03% MDBGN and the other was preserved with 0.1% methyl paraben and 0.2% propyl paraben. Each bottle of moisturiser was applied consistently to a 5cm<sup>2</sup> area of either the left or right upper ventral aspect of the arm. Lotion was applied twice daily for two weeks, unless terminated because of a positive ROAT result or at the patient's request. Observation was done on day 1 (before ROAT), then after 1 week, 2 weeks or at the request of the patient. The identity of the lotions was blinded from the subjects and investigators until the end of the study. Eighteen (35.3%) of the patients developed a positive ROAT response to MDBGN-containing lotion application alone.

In a ROAT study by Schnuch et al. (2005), 39 patients with a previous positive patch test to Euxyl K 400 were provided with two ointments, one with Euxyl K 400 (0.025%) and one without Euxyl K 400 (vehicle control). Patients applied one ointment to the same area on the inside of one forearm and the other ointment to the opposing forearm, twice daily for up to 2 weeks or until a positive ROAT developed. If there was no reaction the ointment containing 0.025% Euxyl K 400 was substituted for the next higher concentration (0.05% Euxyl K 400) to be used for the next 2 weeks. The ointment was then substituted again if there was no reaction, to a higher concentration of 0.1% Euxyl K 400, and application continued for a final 2-week period. The majority of patients developed a positive ROAT result during the course of the study - 33.3% reacted to the ointment with the lowest Euxyl K 400 concentration, 20.5% separately reacted to the middle concentration and 7.7% separately reacted to the highest concentration. No skin reactions were observed in response to the lotion without preservative. At the end of the ROAT, confirmatory patch testing was performed in 24 patients, each tested with 0.1, 0.2, 0.3 and 0.5% MDBGN in petrolatum. Positive reactions were observed in 62%, 67%, 71% and 92%, respectively.

Individuals sensitised to MDBGN (n=19) participated in a study comparing single versus repeated daily exposure to MDBGN (Jensen et al. 2005). Twelve control individuals who had tested negative to MDBGN also participated in the double-blind, randomised test. All subjects were patch tested with solutions ranging from  $1.3 \times 10^{-5}$  to 0.2% MDBGN in eth.: aq. (1:4). Patch test solutions were applied onto filter paper

discs and mounted on the subject's back. The patches were removed after 2 days and readings taken on day 3 and day 7 to determine the patch threshold value. Following the patch test, subjects were instructed to apply drops of a test solution to an area on the inside of the forearm, with at least 3 hours between each application. The test solutions were 0.04, 0.01 and 0% MDBGN in eth.: aq. (1: 4). On one arm patients applied the 0.04% MDBGN once a day and the 0% solution three times a day, while for the other arm the patients applied the 0.01% solution four times a day. After 3 weeks the study was terminated and 73.7% (14) of the subjects had developed dermatitis on both arms. Both methods of exposure (1 or 4 exposures/day) were equally likely to induce an allergic reaction in MDBGN sensitised individuals. None of the controls showed any skin reactions.

In another study by Jensen et al. (2006), seventeen patients already sensitised to MDBGN were recruited for a study examining the effect of allergen re-exposure to previous sites of MDBGN induced contact dermatitis. Ten control individuals, based on negative reactions to a patch test with MDBGN (0.2% in eth.: aq.), also participated in the study. Areas of dermatitis were induced with MDBGN by a) patch testing with serial dilutions of MDBGN ranging from 0.0001% to 0.2% in eth.: aq. (1:4) on the lower back of each patient and b) applications of 4 solutions on the inside of the forearm: 0.0004-0.05% MDBGN (low dose); 0.0016-0.2% MDBGN (high dose); 1% sodium lauryl sulphate (SLS) in water and 20% ethanol in water. The dermatitis was then allowed to heal completely, which took approximately one month. After healing, the treated areas were challenged by a) patch testing of the test site with six consecutive dilutions of MDBGN (concentration ranges from 0.0002-0.1% MDBGN) within a range according to the sensitivity of the patient determined from the previous patch test and b) washing test areas on the arm with liquid soap containing 0.1% MDBGN twice a day for up to 3 weeks or until dermatitis appeared. A statistically significant increased response to patch tests was seen on the areas with previous dermatitis on the back as compared to normal skin. Eight of the 9 subjects who developed dermatitis on the arms from the soap containing MDBGN had an augmented response on areas with previous dermatitis. All controls gave a negative response.

Excepting results from a single negative study, the prevalence rate of positive reactions when MDBGN pre-sensitised individuals were patch tested with MDBGN, or MDBGN in lotions or ointments at concentrations of 0.0001%-1%, ranged from 7.7%-92%.

#### **2.5.4 Human repeat insult patch testing**

Hill Top Research conducted seven separate human repeat insult patch tests (HRIPT) between 1981 and 1984 (Hill Top Research 1981b\*; Hill Top Research 1981c\*; Hill Top Research 1983\*; Hill Top Research 1984a\*; Hill Top Research 1984b\*). In the induction phase, subjects applied a series of 9 occlusive patches containing MDBGN for 24 hours during a 3-week period. The test sites were graded at 24 hours after removing the first patch and 48 hours after removal of the last (9th) patch. There was no treatment for two weeks after which a challenge patch containing MDBGN was applied. Skin reactions were scored at 48 and 96 hours after challenge. The same concentration of MDBGN used for induction was also used during the challenge phase and varied from 0.0012-0.0396% depending on the particular study. Skin reactions were classed as either an irritant or sensitisation response, based on the time course of the reaction and the results of further testing of the subject. Only 1 subject of the 731 tested was classed as sensitised to MDBGN.

In a HRIPT study, 160 human volunteers were patch tested using a solution of 0.3% MDBGN in corn oil. No results are presented in the article, however the authors concluded from this study was that MDBGN was not a skin sensitiser (Lederer et al. 1982). In another study using 0.3% MDBGN (Tektamer 38) in corn oil, a negative result was obtained in 52 volunteers following 12 daily applications over a 3-week period (Mathias 1983).

Overall, the available HRIPT study showed no or only slight evidence of skin sensitising potential for MDBGN.

### **2.5.5 Single case studies**

There have been numerous case studies described since the first report of contact sensitivity to MDBGN in 1983 (Mathias 1983; Senff et al. 1989; Keilig 1990; De Groot et al. 1991a; Pigatto et al. 1991; Torres and Soares 1992; Ross et al. 1992; Gebhart et al. 1993; O'Donnell and Foulds 1993; Fernandez et al. 1995; Silvestre et al. 1996; De Groot 1997; Armstrong et al. 1999; Chang et al. 2000; Erdmann et al. 2001; Wong and Beck 2001; Kelterer et al. 2002; Diba et al. 2003; Young and Beck 2004; Sanchez-Perez et al. 2005; Bruze et al. 2005; Lujan-Rodriguez et al. 2006; Jong and Statham 2006). The majority of reported case studies result from leave-on skin products containing MDBGN. These include various creams, lotions, ointments and gels. Other common sources of sensitisation also include liquid soaps and moist toilet paper (baby wipes). Of the reported occupational cases, most result from the use of hand washing liquids or barrier creams, although MDBGN sensitivity has also been triggered by contact with glue used to stick labels to bottles in the workplace. A recent Australian report noted a case allergic contact dermatitis from MDBGN in the adhesive used in sanitary pads (Williams et al., 2007).

## **2.6 Repeated dose studies**

### **2.6.1 Oral toxicity**

MDBGN (98%) at 0, 167, 1000 or 4000 ppm (males: 0, 4.8, 28.9 and 101.5 mg/kg/day; females: 0, 5.3, 37.3 and 109.8 mg/kg/day) was added to the feed of four male and four female beagle dogs for 13 weeks (Hazleton Laboratories America 1980c\*, USEPA 1996). Another eight dogs (equal numbers of each sex) were fed an unmodified diet and served as the control. Animals were sacrificed for necropsy at the end of the study. The highest dose was originally 6000 ppm, however after observing decreased food consumption, constipation and vomiting the dose of MDBGN was reduced to 4000 ppm at 1 week into the study and maintained for the duration of the study. At the highest dose of 4000 ppm follicular cell hypertrophy and hyperplasia of the thyroid gland was noted. There was also increased pigmentation of the liver and spleen as well as increased extramedullary hematopoiesis. A NOAEL was not determined in this study. A LOAEL was considered to be 167 ppm (the lowest dose tested) based on a dose-related increase in absolute and relative thyroid weights in males.

In a follow up study to further investigate the effects on the thyroid, eight more beagles (four of each sex) were fed a diet containing 0 and 167 ppm MDBGN for 13 weeks (Hazleton Laboratories America 1982\*, USEPA 1996). No difference was found in circulating levels of the thyroid hormones triiodothyronine (T<sub>3</sub>) or thyroxine (T<sub>4</sub>). Female dogs receiving the diet containing MDBGN had larger thyroid glands than control dogs. No differences were observed between treated and control animals when examined histopathologically.

In another study, beagle dogs (4-6 dogs/sex/group) were diet fed with 0, 10, 100 or 400 ppm MDBGN (males: 0, 0.288, 3.1 or 102 mg/kg/day; females 0, 0.331, 3.11 or 119 mg/kg/day) for 3 months (USEPA 1996). In addition, a 3-month recovery period using 2 dogs/sex at 0, 100 or 400 ppm was conducted. Treatment-related effects observed included decreases in food consumption and body weights. Anaemia, hypertrophy or hyperplasia in the thyroid gland, axonal degeneration in the brain and spinal cord, and seminiferous tubule degeneration in the testis were reported. Animals in the recovery group showed a less severe form of axonal degeneration. The NOAEL was determined to be 10 ppm for females based on diarrhoea and emesis observed in females at 100 ppm. For males, the NOEL was determined to be 100 ppm based on the effects seen at the highest dose (400 ppm).

### **2.6.2 Dermal toxicity**

Repeated dermal application of MDBGN (98%) at doses of 0, 1000, 2000 and 4000 mg/kg bw to rats (5/sex, strain not specified) for 21 days (6 hours/day, 5 days/week) caused severe dermal irritation (MB Research Laboratories, 1992a\*). Although no raw data were provided, moderate to severe eschar was reported in all dosed animals by week 2 and none was observed in controls. Feed consumption was decreased in mid- and high dose males at day 8. There was no evidence of systemic toxicity. Slight but consistent decreases in haematocrit values, haemoglobin concentrations and erythrocyte counts were observed in all treated groups, particularly at the high dose.

MDBGN at 0.025% in formulation or 0.3% in an aqueous dilution of the trade ingredient was applied to the shaved and abraded skin of New Zealand Albino rabbits (5/sex/group), 5 days/week for 28 days (CTFA 1982\*). A control group was dosed with distilled water. Animals treated with 0.025% MDBGN showed moderate to severe erythema, and slight to moderate oedema, which persisted until the end of the study. Histopathological examination of the treatment sites showed slight to moderate acanthosis, mild to slight hyperkeratosis, and minimal to moderate inflammatory infiltration, and in one animal, slight focal necrosis and slight focal abscesses at the test site was noted. In animals treated with 0.3% MDBGN, slight erythema and yellowish discolouration, which continued until the end of the study were observed. Histopathological examination of the treated sites revealed slight focal or diffuse acanthosis and minimal focal inflammatory infiltration. Two animals from each exposure group developed slight reactive submandibular lymph node hypertrophy, which was considered as an indirect response to cutaneous irritation. Differential leukocyte counts for females dosed with 0.025% MDBGN revealed a relative neutrophilia, which was interpreted as indicative of a mild inflammatory response to treatment. All other lesions/abnormalities were randomly distributed. The authors did not address the findings that the higher dosed animals exhibited less severe responses.

MDBGN in acetone at doses of 0, 37.5, 75, 150 300 or 600 mg/kg bw were applied to shaved skin of F344/N rats (5/sex/dose) for 5 days/week for 16 days (NTP 2008). No mortality was reported and mean body weights were comparable between the test and control groups. At the application site, irritation, thickened skin and ulcers were observed in treated animals. At 600 mg/kg bw, thyroid gland weights were significantly lower in males than the control group. Liver and kidney weights were significantly increased in females dosed with 300 and 600 mg/kg. Nonneoplastic lesions such as epidermal hyperplasia and hyperkeratosis, sebaceous gland hyperplasia and dermal chronic active inflammation were observed at the test site in all treated animals. Necrosis, ulcer and parakeratosis of the epidermis also occurred in most of the treated animals.

In B6C3F1 mice (5/sex/dose), MDBGN in acetone was dermally administered at 0, 75, 150, 300, 600 or 1200 mg/kg bw for 5 days/week for 16 days (NTP 2008). No mortality was reported. In males, the final mean body weight and the thymus weights were significantly reduced at 300 mg/kg. The liver and heart weights were significantly increased at 600 and 1200 mg/kg, and the kidney weights were significantly increased at 150 and 600 mg/kg bw. For females, the liver weights were significantly increased at 1200 mg/kg bw. Thymus weights were significantly reduced in all female dosed animals. Skin lesions at the application sites such as epidermal hyperplasia, hyperkeratosis, parakeratosis, necrosis, and ulcers; dermal chronic active inflammation; and sebaceous gland hyperplasia occurred in all treated animals.

In a 3-month study, MDBGN in acetone was administered dermally on the shaved dorsal area in F344/N rats and B6C3F1 mice (10/sex/dose) at 0, 0.2, 0.6, 2, 6, or 18 mg/kg bw, 5 days/week for 14 weeks (NTP 2008). Additional rats (10/sex/dose) designated for clinical pathology testing were treated with the same doses for 23 days. In rats, all animals survived except for a female rat that died on day 91. Mean body weights of treated animals were comparable with controls. Clinical findings of toxicity included thin hair coat in both sexes and irritation at the application sites in males. At the application sites, epidermal hyperkeratosis was significantly increased in all treated animals. Epidermal hyperplasia was significantly increased in males at  $\geq 0.6$  mg/kg bw/day. Chronic active inflammation and sebaceous gland hyperplasia were significantly increased in males at doses 6 and 18 mg/kg/bw/day. In females, epidermal hyperplasia and chronic active inflammation were observed at  $\geq 2$  mg/kg bw/day. All mice survived to the end of the study. Mean body weights were similar between dosed and control groups. The liver and lung weight of dosed females were significantly lower than the control group. In both sexes, minimal epidermal hyperplasia and hyperkeratosis at  $\geq 2$  mg/kg bw/day, and sebaceous gland hyperplasia at 6 or 18 mg/kg bw/day were significantly increased at the application site. Epidermal necrosis and parakeratosis were also significantly increased in males at 18 mg/kg bw/day. In the dermis, fibrosis was significantly increased in both sexes. Chronic active inflammation was also significantly increased in males at 18 mg/kg bw/day and in females at  $\geq 2$  mg/kg bw/day.

## 2.7 Mutagenicity

Several in vitro and in vivo mutagenicity studies on MDBGN have been reviewed by the CIR (1996), USEPA (1996) and NTP (2008).

### 2.7.1 In vitro

At  $\leq 30\mu\text{g}$  per plate, MDBGN was negative in Ames tests conducted using *Salmonella typhimurium* (Merck Sharp & Dohme Research Laboratories 1978\*).

MDBGN was tested in *Salmonella typhimurium* in two independent assays (NTP 2008). In the first assay, 0.1 to 333  $\mu\text{g}$  per plate MDBGN was tested in TA97, TA98, TA100 and TA1535 with or without metabolic activation (S9). There were no increases in mutant colonies observed in this test. In the second assay, MDBGN was tested in TA 98 and TA100, and *Escherichia coli* WP2, with and without S9. No mutagenic activity was observed at the test concentration range of 25 to 100  $\mu\text{g}$  per plate.

No increase in mutation frequency was observed when MDBGN was screened in two separate mutation assays utilising mouse lymphoma cells ( $\leq 300$   $\mu\text{g}/\text{mL}$  with S9 and  $\leq 7.1$   $\mu\text{g}/\text{mL}$  without S9) and another assay that used V-79 Chinese hamster lung cells

(up to 50 µg/mL with S9 and 0.3 – 1.0 µg/mL without S9) (EG & G Mason Research Institute 1981\*; EG & G Mason Research Institute 1982\*; Merck Sharp & Dohme Research Laboratories 1985\*, USEPA 1996).

MDBGN significantly increased the frequency of chromosome aberrations observed in a mammalian chromosome aberration test using Chinese hamster ovary (CHO) cells (6.20 – 11.03 µg/mL with S9 and 106.79 – 189.84 µg/mL without S9). A dose dependent response was also noted in this study (Microbiological Associates 1982a\*, USEPA 1996).

MDBGN did not induce unscheduled DNA synthesis when run in an Unscheduled DNA Synthesis (UDS) assay ( $\leq 100$  µg/mL with S9 and  $\leq 10$  µg/mL without S9) utilising IMR-90 fibroblasts (Merck Sharp & Dohme Research Laboratories 1983\*, USEPA 1996). MDBGN was also screened in two separate transformation assays (17.6 – 82.5 µg/mL with S9) that both utilised BALB/C-3T3 cells and did not affect the transformation rate in either of the assays (Litton Bionetics 1984\*; Microbiological Associates 1990\*).

### 2.7.2 In vivo

Male mice were fed diets containing 83.5, 500 and 3000 mg/kg bw MDBGN and then mated with untreated females (Hazleton Laboratories America 1980\*a). Positive (triethylenemelamine) and negative (non dosed) controls were also included. No dominant lethal mutations were produced in male mice. The incidence of malformation, resorption, foetal death, dead implants and foetal viability was comparable between the MDBGN and control groups.

Following the intubation of MDBGN (100 mg/kg bw) into Sprague-Dawley rats, the animals were sacrificed and the bone marrow collected, and the incidence of chromosomal aberrations noted (Microbiological Associates 1991\*). No significant increase in the percentage of chromosomal abnormalities was noted in MDBGN treated animals. In another rat study, there was also no change in the percentage of chromosomal aberrations observed in response to intubation of 5, 17, or 50 mg/kg/day MDBGN over 5 days (Microbiological Associates 1982b\*).

The larvae of *Drosophila melanogaster* were fed diets to which 500 or 1000 mg/kg MDBGN had been added (University of Wisconsin Zoology Department 1992\*). Mutagenicity was then assessed by the induction of aberrant wing spots. There was no difference in mutation rates between untreated and MDBGN fed animals, while the positive control (dimethylnitrosamine) did result in an increase in the frequency of wing mutations.

In an in vivo micronucleus test, male and female mice were treated with dermal application of 0.2 to 18 mg/kg MDBGN in acetone for 3 months (NTP 2008). There were no increases in the frequencies of micronucleated normochromatic erythrocytes reported from the bone marrow smears. No significant changes in the percentages of polychromatic erythrocytes were observed.

Overall, MDBGN did not show evidence of mutagenic activity in a variety of in vitro and in vivo assays, except for one assay where increased frequencies of chromosomal aberrations in CHO cells were observed in an in vitro chromosomal aberration test.

## 2.8 Carcinogenicity

Groups of 50 F344/N rats/sex were dermally administered 0, 2, 6 or 18 mg/kg bw/day with MDBGN in 95% ethanol, 5 days/week for 105 weeks (NTP 2008). In both sexes at the 18 mg/kg bw dose, body weights were 7% less than the control group after 1 year. There were no increases in the incidences of neoplasm at the site of application in dosed rats. Irritation and significant increase in incidences of epidermal hyperplasia at the site of application were reported in all dosed animals administered with 6 or 18 mg/kg bw/day. The occurrence of minimal to mild inflammation of the dermis was significantly increased in males at the high dose and in all dosed females. The incidence of epidermal necrosis was also significantly increased in high dosed females. The occurrence of inflammation of the nose was significantly increased in all dosed males. The incidences of alveolar bronchiolar adenoma and pituitary gland adenoma in all groups were within the historical control range. The combined incidence of mammary gland fibroadenoma, adenoma or adenocarcinoma occurred with a negative dose response, and the incidence was significantly decreased in females at 6 mg/kg bw dose.

In another study, B6C3F1 mice (50/sex/dose) were dermally administered with 0, 0.6, 2 or 6 mg/kg MDBGN in 95% ethanol, 5 days/week for 105 weeks (NTP 2008). Body weights of the treated groups were similar to that of the control group. No treatment-related clinical findings were reported. There were no increases in the incidences of neoplasm at the site of application in dosed mice. At the site of application, incidences of hyperplasia of the epidermis were significantly increased in all dosed females and in males dosed at 2 and 6 mg/kg bw/day. The occurrence of chronic active inflammation in the dermis was also significantly increased in all dosed females. There was a significant negative dose related trend in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). In addition, the incidences were within the historical control range, thus the changes were not regarded as treatment related.

## 2.9 Reproductive and developmental toxicity

MDBGN was administered via diet to Sprague-Dawley rats (10/sex/group) at dose levels of 0, 83.5, 500 and 3000 ppm (6.3, 37, and 275 mg/kg/day for males and 7.5, 43 and 360 mg/kg/day for females) commencing seven days before mating and throughout the 14-day mating, gestation and lactation periods (Hazleton Laboratories America 1980b\*, USEPA 1996). No treatment related effects were seen in the parental generation. After weaning, forty (20/sex/group) of the offspring (F<sub>1</sub> rats) were fed diets containing the same concentration as the parental generation for 90 days. After 13 weeks the F<sub>1</sub> rats were necropsied and tissues were analysed. A dose related increase in the severity of splenic extramedullary haematopoiesis was reported. Other effects seen at the high dose group in both sexes of the F<sub>1</sub> animals included a significant decrease in body weight, increase in pituitary weight, decrease in brain weight, and increases in relative pituitary and relative spleen weight. In addition, high dose males showed a significant increase in relative liver weight and high dose females showed increase relative thyroid weight. The NOAEL was determined to be 6.3 mg/kg/day for males and 7.5 mg/kg/day for females, based on effects on the spleen and changes in various organ weights seen at higher doses.

Between days 6 and 15 of gestation, pregnant female rats were administered 0, 25, 100 or 175 mg/kg/day MDBGN (98%) orally (Birnbaum et al. 1983\*). The dams were sacrificed at day 20 and the foetuses recovered for examination. Apart from a

significantly higher resorption rate in the 175 mg/kg treatment group (10.0%) compared to the non-treated control (2.7%), no significant differences were observed between any of the groups. It should be noted that the 10.0% resorption rate was in the range of historical controls, and in the absence of other signs of embryotoxicity (malformations and foetal weight reductions), the incidence of resorptions was not considered biologically significant. The USEPA (1996) reviewed the same study and noted that because there was treatment-related toxicity in dams (maternal weight gain decrements) in the 100 and 175 mg/kg groups, the resorptions observed in these groups may not be clearly associated with potential developmental toxicity of the test material. The NOAEL for developmental toxicity was determined to be 175 mg/kg/day (the highest dose tested).

In another study, MDBGN (100%) was administered to New Zealand White rabbits (20/dose) by oral gavage at doses 0, 10, 30 or 60 mg/kg/day on gestational days (GDs) 6 through 18 (USEPA 1996). There was no treatment-related toxicity in offspring taken by caesarean section and examined from external and internal morphological changes. The NOEL for maternal and developmental toxicity was 60 mg/kg/day (the highest dose tested).

### 3. Conclusions

Worldwide, MDBGN is reported to be used as a preservative and biocide in a wide range of products, including paints, emulsions, dispersed pigments, adhesives, joint cements, metalworking fluids, cosmetics, paper, inks, waxes and household detergents. In Australia, MDBGN is imported as mixtures and as a raw chemical for local formulation. MDBGN was reported in products that varied from adhesives and coatings to personal care products, including sunscreens, shampoos, shower gels and wet wipe hand towels. MDBGN was reported to be present in shower gels and shampoos at between 0.003 and 0.004% and in sunscreens at 0.04%.

MDBGN is readily absorbed following oral and dermal administration. Once inside the body, MDBGN is rapidly metabolised to 2-MGN before eventually being eliminated from the body, mostly via urine. Debromination of MDBGN occurs prior to systemic distribution; therefore, tissue exposure to parent chemical is expected to be low.

MDBGN is moderately toxic by oral route (LD50 770 mg/kg for males and 515 mg/kg for females), and is of low toxicity by dermal ((LD50 >5g/kg) and inhalation (LC50>13 mg/L) exposures. In pure form (98%) MDBGN is a severe eye irritant. Equivocal results were obtained from skin irritation tests in animal studies. However, repeat dose dermal toxicity tests reported moderate to severe erythema, and slight to moderate oedema. Non-neoplastic skin lesions were also reported.

The skin sensitising potential of MDBGN has been extensively investigated in numerous animal and human studies. Results obtained from animal studies vary according to the type of animal study undertaken. Overall, based on in vivo and in vitro animal data, weight of evidence suggests that MDBGN is a skin sensitiser.

In humans, the prevalence of MDBGN sensitivity has been monitored in numerous countries and over an extended period by the routine patch testing of contact dermatitis patients. A number of patch test studies have also been carried out on normal individuals and on individuals pre-sensitised to MDBGN. Most case reports of contact dermatitis from MDBGN were attributed to cosmetics or toiletries. Overall, available human data indicate that MDBGN is a human skin sensitiser.

In long-term repeat feeding studies in animals, the observed effects of MDBGN were thyroid follicular cell hypertrophy, thyroid hyperplasia, increased pigmentation of the liver and spleen and increased extramedullary haematopoiesis when administered at high doses (4000 ppm) in dogs. Follow-up studies found no significant changes in levels of thyroid hormones.

Repeated dermal application of MDBGN was associated with moderate to severe erythema, and slight to moderate oedema. Non-neoplastic lesions at the application site were also reported. These consisted of epidermal hyperplasia, hyperkeratosis, parakeratosis, necrosis, and ulcers; dermal chronic active inflammation and sebaceous gland hyperplasia.

Available studies suggests that MDBGN is not mutagenic, carcinogenic or a reproductive or developmental toxin.

In the EU, MDBGN was permitted in rinse-off products at a maximum concentration of 0.1% and was prohibited in cosmetic sunscreen products at a concentration of >0.025%. However, based on the latest SCCP opinion (2006) that MDBGN is a skin

sensitiser and that no safe use-levels in cosmetic leave-on and rinse-off products have been established, MDBGN is no longer permitted to be used in any cosmetic products within the EU.

In Australia, allergy clinics have reported cases of allergy associated with the use of MDBGN as a preservative, most commonly in hand cleaner. In 2008, NICNAS made a submission to the NDPSC recommending the scheduling of MDBGN in the SUSDP. As a result, MDBGN is to be listed in the SUSDP in Schedule C for cosmetic use and products intended to be in contact with the skin, in Schedule 6 for other uses and in Appendix F, Part 3 with specific warning statements and safety directions. The implementation date for these new scheduling requirements is 1 January 2010.

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