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HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

2-Mercaptobenzothiazole (MBT) (sensitisation only)

Adopted by the SCCP
during the 4th plenary of 21 June 2005

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1. BACKGROUND

The Comité Européen de Normalisation (CEN – European Committee for Standardisation) has developed standards for MBT, some volatile compounds, and the antioxidants - Butylated hydroxyl toluene (BHT) and 6,6' di-t-butyl-2,2'-methylendi-p-cresol (A2246) for soothers and teats setting a migration limit of 8 mg/kg rubber for MBT, 30 µg/dm² for BHT and A2246 and 0.5% (m/m) for volatiles. The standard for soothers has been finalised (EN1400-3) whereas the one for teats and drinking equipment is in the final draft stages awaiting finalisation (Draft prEN 14350-2, ref. 1).

In its review of the CEN standards, the Scientific Committee on Food (SCF) agreed with the 8 mg/kg limit for MBT but restricted itself to the risks associated with the oral intake of MBT. The SCF identified the skin sensitisation risk as a relevant end point but did not comment on the pertinence of the CEN limit as this was considered to be outside the SCF remit. It did however recommend that the Commission seeks proper scientific advice to evaluate the CEN standard limit versus the risks for skin sensitisation of MBT.

In mid 2002, the Danish Environmental Protection Agency (DEPA) commissioned a study to measure the release of MBT (used as rubber vulcanising agent or accelerator) and three antioxidants (BHT, A2246, and A425) from natural rubber soothers and teats, and BHT, A2246 and A-245 and a number of volatile substances from silicone soothers and teats. A summary health assessment for MBT was also conducted. Separately in another study carried out in 2003, the DEPA also carried out a study to detect MBT and the antioxidants BHT and A2246 in consumer products (gloves, balloons, elastic rubber bands, etc) and measure their release.

In both studies, the investigators detected releases of MBT at levels both above and below the CEN standard limit value that in their view would constitute a risk for skin contact sensitisation for infants (from teats and soothers) and adults consumers (from rubber bands and rubber gloves). The investigators argue that it is impossible to fix a 'safe' migration limit for the skin sensitisation of MBT due to the lack of data on levels of induction of sensitisation and elicitation of skin sensitisation of pre-sensitised individuals. On that basis they argue that the CEN limit for MBT is too high and a limit as low as possible and below the detection limit should be set.

2. TERMS OF REFERENCE

The SCCP is asked to:

- Assess the potential skin sensitisation risks to infants and adults from exposure to 2-mercaptobenzothiazole (MBT) released from natural rubber products (soothers and teats, gloves, rubber bands etc). In doing so the Committee should, if possible, also identify a level or exposure below which the risk of skin sensitisation would not be expected to be manifested in real life use situations.
- In light of its response to question 1, comment on the appropriateness of the CEN standard limit for MBT of 8 mg/kg rubber for rubber soothers and teats as a means to minimise the skin sensitisation risk.

- Comment on any other possible health risks that may emerge from the findings of the submitted studies concerning the antioxidants BHT, A2246, A 245, and volatile compounds in particular where the levels released exceed the CEN standard values.
- Identify any additional investigative work that need to be done concerning the above chemicals or the others (e.g. certain phthalates) that were detected in the products tested in order to better assess their release and dimension the risks.

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2-Mercaptobenzothiazole

3.1.1.2. Chemical names

Benzothiazole-2-thiol
 2-Benzothiazolethiol
 2-Benzothiazolyl mercaptan
 1,3-benzothiazole-2-thiol(IUPAC)

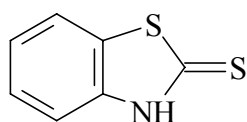
3.1.1.3. Trade names and abbreviations

Accelerator-M
 MBT

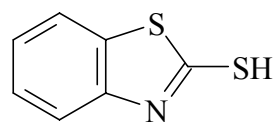
3.1.1.4. CAS / EINECS number

CAS : 149-30-4
 EINECS : 205-736-8

3.1.1.5. Structural formula



2-benzothiazolethione



2-benzothiazolethiole

3.1.1.6. Empirical formula

Formula : $C_7H_5NS_2$

3.1.2. Physical form

MBT is a pale yellow, crystalline substance with an unpleasant odour and a bitter taste. MBT can occur in two tautomeric forms, see above. Both in solution as well as in the crystal the equilibrium is almost completely on the side of 2-benzothiazolethione.

3.1.3. Molecular weight

Molecular weight : 167.25

3.1.4. Purity, composition, and substance codes

94 – 97 %

3.1.5. Impurities / accompanying contaminants

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3.1.6. Solubility

/

3.1.7. Partition coefficient (Log P_{ow})

/

3.1.8. Additional physical and chemical specifications

Organoleptic properties	:	/
Melting point	:	/
Boiling point	:	/
Flash point	:	/
Vapour pressure	:	/
Density	:	/
Viscosity	:	/
pKa	:	/
Refractive index	:	/

3.2. Function and uses

MBT is widely used in industry and in household articles. MBT is primarily used in the rubber industry as a non-volatile vulcanisation accelerator. MBT is also used as an intermediate in the production of other accelerators, some of which may decompose during vulcanisation and form MBT (BUA 1997).

The scope of this evaluation are teats and soothers for infants. For the purposes of the European Standard, the following terms and definitions apply:

Soother: article intended for satisfying the non-nutritive sucking needs of children (also known as pacifiers or babies' dummies).

Teat: flexible nipple which is the part of the soother designed to be placed in the mouth.

Feeding teat: substitute mother's nipple that when attached to a container permits a child to obtain fluid by sucking.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral / dermal / inhalation toxicity
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3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

MBT (500 mg) was applied to the intact or abraded skin of white New Zealand rabbits as a finely-milled powder in water and kept under occlusive cover for 24 hours. Skin reactions were evaluated at 24 and 72 hours. MBT did not cause any primary skin irritation (Monsanto 1985 - quoted from BUA 1997).

Ref.: 9

3.3.2.2. Mucous membrane irritation

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3.3.3. Skin sensitisation

Human Predictive (induction) Studies**Dermal contact**

In a human maximisation test, 9 of 24 test subjects showed a positive response (sensitisation). The induction concentration was 25% MBT, and sodium lauryl sulphate pre-treatment was used. Provocation was performed with 10% MBT. (Kligman 1966 - quoted from Nordic Council of Ministers 1991).

Human Elicitation Studies

MBT is an important allergen involved in rubber contact allergy. In patch tested populations, the occurrence of MBT sensitisation in different countries has been reported to be in the range of 0.9 to 7.8%. (Schweisfurth 1995).

The prevalence of patch test responses to 16 allergens, including MBT, was studied in 1200 patients (presumably dermatological patients, although this is not stated) from various locations in North America. Five percent of the study population exhibited sensitisation to MBT. (Marzuli & Maibach - quoted from Nordic Council of Ministers 1991).

In 4824 Scandinavian patients a 2% MBT response rate was found using a similar test method (Marzuli & Maibach - quoted from Nordic Council of Ministers 1991).

The yearly incidence of MBT sensitisation among dermatology clinic patients during the years 1971-76 was 1.2-2.8% (Cronin 1980 - quoted from Nordic Council of Ministers 1991).

Among 810 contact dermatitis patients, 6.8% showed sensitisation to “rubber additives”. Further testing of the rubber additives sensitised patients revealed that 18.1% reacted to mercaptomix. (*Mercaptomix is used in a standard battery for contact allergy. Mercaptomix contains 4 mercaptanes: MBT (mercaptobenzothiazole), MMBT (morpholinylmercapto-benzothiazole), MBTS (dibenzothiazyl disulfide), and CBS (cyclohexylbenzothiazyl sulphonamide).*) (Song et al. 1979 - quoted from Nordic Council of Ministers 1991).

During a 12-year period, a total of 106 patients with rubber-related contact dermatitis were tested with a patch test using several rubber additives including MBT. Twenty-four percent showed sensitisation to MBT. It was concluded that MBT and TMT (tetramethylthiuram) are the main sensitising agents involved in rubber allergy. (Wilson 1969 - quoted from Nordic Council of Ministers 1991).

Among 1088 dermatological patients tested in 1971 in a standard test battery, 1% showed sensitisation to MBT (Ziegler & Süss 1975 - quoted from Nordic Council of Ministers 1991).

Among 6621 patients from 8 different countries, the mean occurrence of responses to epicutaneous testing of MBT was 2.9% (Cronin 1980 - quoted from Nordic Council of Ministers 1991).

Among 18 workers exposed occupationally to MBT-containing dust, 6 experienced skin problems related to dust exposure. A patch test was not performed. (National Institute for Occupational Safety and Health 1979 - quoted from Nordic Council of Ministers 1991).

Among 11 surgeons suffering from contact dermatitis in relation to use of surgical gloves, 7 showed a positive patch test response to MBT (Fisher 1975 - quoted from Nordic Council of Ministers 1991).

Among 50 spinal injury patients using uridoms and experiencing penile skin problems, 22% showed contact allergy to the mercaptomix¹. Among 114 other patients using uridoms, a 13% occurrence of subjective symptoms possible related to rubber allergy was reported in a questionnaire survey. (Brandsbury 1975 - quoted from Nordic Council of Ministers 1991).

Among 12 cases of contact dermatitis related to occupational exposure to MBT-containing cutting oil, 7 subjects showed a positive response to MBT in a patch test (Fregert & Skand - quoted from Nordic Council of Ministers 1991).

In 3125 (presumably dermatological) North American patients, a 3.0% occurrence of contact allergy to mercaptomix was found (Bühler 1985 - quoted from Nordic Council of Ministers 1991).

In a study of elicitation threshold with MBT leaching from sensitising products (Foley catheter, rubber foot-wear, gloves, rubberised fabrics, bathing caps), Emmet et al showed that the least concentration of MBT that produced an observable reaction was 0.01% corresponding to a threshold exposure of 4.5 µg/cm² MBT. Wide variability occurred with a 100 fold difference among the 12 sensitised subjects.

Ref.: 7

No report of sensitization or contact dermatitis occurring by soothers or teats has been found.

Animal Studies

A maximisation test has been performed using 20 guinea pigs. During the induction phase, 0.1 ml of a 1% MBT solution was applied intradermally and 0.1 ml of a 25% solution was applied epicutaneously. Freund's complete adjuvant was used. In the provocation test, 15% MBT was applied epicutaneously and the response was evaluated at 48 and 72 hours. Eight guinea pigs showed a positive response. (Magnusson & Kligman 1969 - quoted from Nordic Council of Ministers 1991).

In a guinea pig maximisation test (24 guinea pigs) of MBT, 20% of the animals showed a positive response (Ziegler & Süss 1975 - quoted from Nordic Council of Ministers 1991).

In four groups of 10 guinea pigs, 2-7 animals per group were sensitized to MBT following epicutaneous application in a Buehler test. In this study, cross-sensitization to MMBT (morpholinylmercaptobenzothiazole, structurally related to MBT) was demonstrated in the majority of animals reacting to MBT. (Wang & Suskind 1987 - quoted from Nordic Council of Ministers 1991).

Groups of 10 guinea pigs were tested for sensitization to MBT by using the guinea pig maximisation test, the Siat procedure, and a modified Draize test. In the guinea pig maximisation test, 6 animals showed a positive response, whereas negative responses were observed using the other two test procedures. (Goodwin et al. 1980 - quoted from Nordic Council of Ministers 1991).

In a comparison of guinea pig maximisation test (GPMT) with local lymph node assay (LLNA), Basketter found MBT to be an inducer of sensitisation with a concentration of the induction patch of 10% in GPMT and the same concentration to be positive in LLNA (EC3)

Ref.: 3

Ikarashi found MBT to be positive in LLNA at a concentration of 4.2% but using a cell proliferation factor of 2 instead of 3 (EC3).

Ref.: 4

In a study of allergenic potential of rubber chemicals used in the production of medical gloves, De Jong used a modified LLNA test by pre-treating the ear of experimental animals with 1% sodium dodecyl sulfate (SDS). The 15 chemical tested were ranked according to increasing EC3 values – thus decreasing allergenic potential. MBT was ranked in the 9th position, i.e. a moderate sensitizer: effective concentration- 4:1 acetone olive oil - inducing a stimulation index of 3 (EC3) = 9.9%. It was weaker than MBTS (dibenzothiazyl sulfide); EC3 2.9%, but stronger than MBI (mercaptobenzimidazole) EC3 14.7, and stronger than Zinc MBT EC3 30.3%.

Ref.: 10

In another study of sensitising activity of rubber contact sensitizers, the same group found MBT to be negative in LLNA at concentrations up to 17%. The LLNA test became positive (EC3) at a concentration of 17% after pre treatment with 1% SDS. They considered MBT to be a “weak sensitizer”.

Ref.: 5

In the most recent comprehensive review of sensitising potency of chemicals, Basketter and co-workers ranked MBT as a “moderate sensitizer”; the sensitizers were classified as “extreme”, “strong”, “moderate”. So MBT was in the lowest category of sensitizers:

- EC3 concentration of 9.7% in LLNA
- 40% incidence of sensitisation after a 1% injection concentration in GPMT
- 55% incidence of sensitisation after a 75% topical induction concentration in Buehler test.

Ref.: 11

3.3.4. Dermal / percutaneous absorption

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3.3.5.	Repeated dose toxicity
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3.3.5.1.	Repeated Dose (28 days) oral / dermal / inhalation toxicity
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3.3.5.2.	Sub-chronic (90 days) oral / dermal / inhalation toxicity
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3.3.5.3.	Chronic (> 12 months) toxicity
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3.3.6.	Mutagenicity / Genotoxicity
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3.3.7.	Carcinogenicity
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3.3.8.	Reproductive toxicity
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3.3.9.	Toxicokinetics
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3.3.10.	Photo-induced toxicity
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3.3.11.	Human data
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3.3.12.	Special investigations
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3.3.13. Safety evaluation

• According to CEN when elastomeric components of soothers are tested, the migration of MBT should not exceed 8 mg/kg; BHT should not exceed 30 µg/100 ml or 60 µg/dm²; Antioxidant 2246 should not exceed 15 µg/100 ml or 30 µg/dm². When silicone rubber components of soothers are tested, the volatile compounds content should not exceed 0.5 % (m/m). The test methods are as follow: all samples are to be immersed in boiling water (conforming to EN ISO 3696, Grade 3) for 10 min to remove the surface coating arising from the manufacturing processes and ensure that the materials used are stable in boiling water. MBT and its metal- salts are quantitatively determined following extraction into an aqueous migration liquid (Acetic acid 3 % in water). MBT is identified and determined by HPLC and UV-detection; The method is also used for the qualitative and quantitative determination of the antioxidants BHT and 2246

Ref.: 6

• As for Drinking equipment i.e. reusable feeding teats and drinking accessories; reusable feeding bottles and drinking cups; single-use feeding bottles, feeding teats, drinking accessories and single-use feeding bags, the same methods and limits were defined

Ref.: 1

• The Danish department of food performed a study of MBT migration from teats and soothers on use by children. The migration of MBT from four teats and five soothers was investigated by measuring the amount of MBT that migrated after 24 hours at 40°C to food simulants for milk (water) and juice (3% acetic acid). Two soothers showed migration of MBT to the water and acidic food simulants at levels of 0.09 and 6.7 mg MBT/kg rubber and 0.19 and 12.0 mg MBT/kg rubber respectively. The limit of detection was 0.01 mg MBT/kg rubber.

Ref.: 2

• The Danish Environmental Protection Agency has performed a study of MBT liberation in natural rubber. The following products were selected:

Ref 8

- Balloons (two types)
- Space hopper (non-stop balls) (three types)
- Eraser (three types)
- Soft masks (one type)
- Cleaning gloves (three types)
- Erotic appliances (two kinds of dildos and two types of latex cloths)
- Elastic bandages (one type)

All samples were cut to a size of exactly 10cm². It was decided not to boil the samples before migration. Artificial sweat and spit were used as simulants. Sweat was used for elastic bandages, gloves, dildos, latex cloths, and mask. Spit was used for the balloons, space-balls, and erasers. The samples were incubated at 40°C for 24 hours. The migration test was carried out in duplicate. Following incubation the simulant was extracted with dichloromethane, dried over water free sulphate and evaporated to dry. The simulant was re-dissolved in acetonitrile, after which the extract is analysed at high-pressure liquid chromatography (HPLC). The extract was analysed for content of MBT, MBTS, BHT, and A2246. The limits of detection for the single components were:

MBT : 2 µg/dm²
BHT : 20 µg/dm²
A2246 : 6 µg/dm²

MBT and MBTS have been detected in one sample of cleaning gloves; MBT, MBTS, and A2246 in the sample of elastic bandage. The samples with positive MBT result were both tested with sweat as simulant; the artificial sweat is acid (pH = 3) and MBT is more soluble in acid than in water.

In the simulant for the cleaning gloves 160 µg/dm² (average) was detected and MBTS was detected qualitatively. 460 µg/dm² (average) MBT was detected in the simulant of the elastic bandage and MBTS was detected qualitatively and the level for A2246 was 15 µg/dm² (average). BHT could not be detected in either of the samples.

When the values for the migration of MBT are converted into mg/kg the result may be compared with the values that were found in soothers

Different simulants were used in the two investigations (soothers used water and 3% acetic acid). However, there may be a rough comparison as all simulants are aqueous, and two of which are acid (acetic acid and sweat). The value of cleaning gloves was 80 mg/kg (average) and 120 mg/kg (average) for elastic bandages. Migration of MBT to water and 3% acetic acid was detected from values of 0.16 to 11 mg/kg for soothers, meaning that migration of MBT for gloves and elastic bandage was 10 to 1000 times larger. The qualitative information may be underestimated due to generally low recoveries of the method and the possibility for reformation between MBT and MBTS.

- A round-robin study of an international standardization working group compared the efficacy of different sample preparation procedures for determining the sensitization potency of rubber medical devices for MBT. Extraction protocols included the ISO method that applies physiological saline and/or vegetable oil extraction media without concentration (designated here "simple extraction") and the Japanese method that applies organic solvent extract (in this study chloroform-acetone mixture) after concentration to the test systems (designated here "exhaustive extraction"). The exhaustive method was more sensitive than the simple extraction method for identifying sensitizing hazard but gave some false positive results in animal experiments

Ref.: 12

- In 2001, a retail market survey of 19 samples of teats and soothers was performed in the Netherlands. The migration of 2-mercaptobenzthiazole (MBT) was measured. MBT was detected in only one natural rubber sample, migration being well below the limit of 0.3 mg/teat.

Ref.: 13

- The residues of additives and other chemicals were investigated by GC/MS in natural rubber products for food contact, which included nipples, packing, gloves and a net for ham. The packings and gloves contained 980 to 6,570 mg/g of vulcanization accelerators, such as zinc dimethyldithiocarbamate, zinc diethyldithiocarbamate (EZ), zinc di-n-buthyldithiocarbamate (BZ) and 2-mercaptobenzothiazole. A migration test was carried out for some samples, No chemicals were released into water, 4% acetic acid or 20% ethanol at 60 °C for 30 min.

Ref.: 14

- An analytical method by which residuals of rubber accelerators in single-use medical gloves could be determined qualitatively and quantitatively was used. 19 different glove brands were analysed for content of accelerators, and the results were compared to manufacturers' ingredient claims of the identical gloves. No accelerators were detectable with the described chemical analysis in phosphate extracts, whereas acetone was demonstrated to be a technically suitable medium for extraction. ZMBT was found in 9/19 gloves with amounts of 0.15 to 1.5 $\mu\text{mol/g}$ glove material. However, more kinetic studies of the extraction procedure and studies of skin penetration were considered needed to document that the extraction procedure simulates the clinical situation.

Ref.: 15

MARGIN OF SAFETY

There is only one human induction study (human maximisation test): 9 of 24 test subjects showed a positive response (sensitisation) with an induction concentration of 25% MBT; sodium lauryl sulphate pre-treatment was used.

A real calculation of the margin of safety is not possible as the sensitising concentration of MBT established in animal experiments (LLNA, GMPT) cannot be extrapolated to human in real life situations. So the amount of MBT leaching out of rubber products (including soothers and teats) are not indicative of the real potential for sensitisation but may be useful to compare the risks encountered with various rubber articles. They are to be compared to clinical experience of sensitisation with rubber articles.

A concentration of 1% MBT in a patch test delivers 450 $\mu\text{g}/\text{cm}^2$ (ref. 7).

A concentration of 10% MBT (lower limit to induce sensitisation) will be $4500 \mu\text{g}/\text{cm}^2 = 450 \text{ mg}/\text{dm}^2$.

The upper limit admitted (CEN) of leaching for MBT out of rubber is 8 mg MBT/kg rubber.

The weight of a teat is 5g for a surface area between 0.18 and 0.8 dm^2 .

8 mg/kg rubber gives 40 μg MBT/5 g rubber teat.

The amount of MBT in contact with the skin and mucous membrane when using a rubber teat of 0.18 dm^2 is 222 $\mu\text{g}/\text{dm}^2$; with a teat of 0.8 dm^2 its 40 $\mu\text{g}/\text{dm}^2$.

So there is a factor 1000 between the maximum quantity of MBT released by a teat in 24 hours = 222 $\mu\text{g}/\text{dm}^2$ and the minimum quantity necessary for sensitisation in animal experiment = 450 mg/dm^2 . For gloves and elastic bandages, the amount of MBT released may reach the quantity necessary for sensitisation in animal experiment.

3.3.14. Discussion

MBT has been determined as a moderate skin sensitizer in animal experiments. However, clinical observation shows that MBT is a common and important contact allergen. Soothers and teats, when the migration limit of 8 mg/kg rubber (CEN standard) is observed will release MBT at a concentration 1000 times lower than animal experimental estimations of induction levels.

Of note is the fact that no report of contact sensitization to MBT from use of soother or feeding teats has been reported. As gastrointestinal absorption of antigens is known to induce tolerance

in animal experiments, it cannot be excluded that the use of rubber teats and soothers may have a protective role against skin sensitisation.

Ref.: 16, 17

Migration of MBT from gloves and elastic bandage was 10 to 1000 times greater than in teats, but several extraction methods with discordant results have been used. Their relevance to clinical situation is not known. Levels of MBT as well as amount of migration from rubber soles and heels of footwear are not known. As foot dermatitis from rubber contact allergy is not uncommon, an investigation of the migration and sensitising potential of allergenic substances used in their manufacture using standardized methods and realistic modes of extraction is recommended.

4. CONCLUSION

In response to the questions asked, the SCCP is of the opinion that:

- The risk of sensitisation from exposure to MBT released from natural rubber soothers and teats is negligible.
- The CEN standard limit of 8 mg/kg rubber for soothers and teats is appropriate to minimise sensitisation risks.
- No data on health hazards emerging from the release of BHT, A2246, A245 and volatile compounds were available.
- There may be a considerable greater migration from gloves and elastic bandages.

The SCCP would like to draw the Commission's attention to the need to investigate the release of MBT and similar allergic substances from other consumer products (e.g. foot wear) which have the potential to cause sensitisation (producing allergic contact dermatitis) in the consumer.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. European standard draft prEN 14350-2 December 2001 ICS Child use and care articles - Drinking equipment - Part 2: Chemical requirements and tests
2. Soothers and feeding bottles – chemistry and health. The Danish Institute for Food Inspection and Nutrition, The Danish department for food. December 1999
3. Basketter D.A. and Scholes E.W. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. *Fd Chem Toxic* 1992; 30:1 65-69.
4. Ikarashi Y, Tsuchiya T, Nakamura A. Evaluation of contact sensitivity of rubber chemicals using the murine local lymph node assay. *Contact Dermatitis* 1993; 28: 77-80.
5. De Jong W.H., Tentij M, Spiekstra S.W., Vanderbriel R.J., Loveren H.V. Determination of the sensitizing activity of the rubber contact sensitizers TMTD, ZDMC, MBT and DEA in

- a modified local lymph node assay and the effect of sodium dodecyl sulfate pre-treatment on local lymph node responses. *Toxicology* 2002; 176: 123-134.
6. European standard **EN 1400-3** September 2002 ICS 97.190 Child use and care articles - Soothers for babies and young children - Part 3: Chemical requirements and tests
 7. Emmett E.A, Risby T.H, Taylor J, Chen C.L., Feinman S.E. Skin elicitation threshold of ethylbutyl thiourea and mercaptobenzothiazole with relative leaching from sensitizing products. *Contact dermatitis* 1994; 30: 85-90.
 8. MBT liberation in natural rubber The Danish Department for Food. Danish Environmental Protection Agency. Survey of Chemical Substances in Consumer Products Survey No 22, 2003 Jane Pors, MSc ; René Fuhlendorff, MSc
 9. MBT Danish EPA study EN version
 10. De Jong WH, Van Och FMM, et al. Ranking of allergenic potency rubber chemicals in a modified local lymph node assay. *Toxicological sciences* 66, 226-232 (2002)
 11. Basketter DA, Andersen KE, Liden C, et al. Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations of the relevance for elicitation. *Contact dermatitis* 2005; 52: 39-43
 12. Nakamura, A., Kanazawa, Y., Sato, H., Tsuchiya, T., Ikarashi, Y., De Jong, W.H., Andersen, K.E. & Knudsen, B.B. 2003. Evaluation of allergic potential of rubber products: Comparison of sample preparation methods for the testing of polymeric medical devices. *Journal of Toxicology-Cutaneous and Ocular Toxicology* **22**, 169-185.
 13. Bouma, K., Nab, F.M. & Schothorst, R.C. 2003. Migration of N-nitrosamines, N-nitrosatable substances and 2-mercaptobenzothiazole from baby bottle teats and soothers: a Dutch retail survey. *Food Addit. Contam* **20**, 853-858.
 14. Kawamura, Y., Nakajima, A. & Yamada, T. 2001. Residual chemicals in natural rubber products for food contact use. *Journal of the Food Hygienic Society of Japan* **42**, 179-184.
 15. Knudsen BB, Hametner C, Seycek O, Heese A, Koch HU, Peters KP. 2000 Allergologically relevant rubber accelerators in single-use medical gloves. *Contact Dermatitis*. 2000 Jul;43(1):9-15.
 16. van Hoogstraten IM, Boden D, von Blomberg ME, Kraal G, Scheper RJ. Persistent immune tolerance to nickel and chromium by oral administration prior to cutaneous sensitization. *J Invest Dermatol*. 1992 Nov;99(5):608-16.
 17. van der Burg CK, Bruynzeel DP, Vreeburg KJ, von Blomberg BM, Scheper RJ. Hand eczema in hairdressers and nurses: a prospective study. I. Evaluation of atopy and nickel hypersensitivity at the start of apprenticeship. *Contact Dermatitis*. 1986 May;14(5):275-9.

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