TOXICOLOGICAL EVALUATION OF CARCINOGENICITY INDUCED BY ETHYL ACRYLATE

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1 INTRODUCTION

One of the main ingredients of the odorant for domestic gas called Gasodor® S-Free is ethyl acrylate (> 50 %). In 1986 the International Agency for Research on Cancer (IARC) re-evaluated the substance specific data and later on classified ethyl acrylate as "*possibly carcinogenic to humans*" (Group 2B), with the last update performed in 1999. Basis for this evaluation is the evidence taken from oral gavage studies in rats and mice. In these studies for both species a dose-related increase in incidence of squamous-cell papilloma and carcinoma of the forestomach was observed. No treatment-related tumours were observed after dermal exposure or when ethyl acrylate was tested by inhalation in the same species. IARC classified on conclusion of sufficient evidence of carcinogenicity in experimental animals and no relevant epidemiologic data (IARC, 1986; 1987; 1999).

Since the original evaluation more substance specific data became available as well as a general understanding of the correlation of gavage studies with the occurrence of the mentioned tumour incidences was developed within the toxicological community. This led to the fact that in today's registration for ethyl acrylate under the European chemicals legislation (REACH; EC, 2006) no classification for mutagenicity and carcinogenicity is included according to the rules laid down in Regulation (EC) no 1272/2008 (EC, 2008), which is in agreement with the opinion of several expert panels and the harmonized classification according to Annex VI of the Regulation (EC) no. 1272/2008 (EC, 2008) and is also presented by the European chemical Substances Information System (EC, 2012).

Aim of this survey is to give a short summary of the available data concerning genotoxicity/mutagenicity and carcinogenicity of ethyl acrylate, describe the mode of action leading to tumour development in gavage studies and outline existing opinions of expert panels. Final conclusions will help to integrate the out-dated IARC classification into the context of the current knowledge.

This overview on ethyl acrylate was based mostly on data coming from secondary sources (ECHA, 2012b; Environment Canada, 2011; OECD, 2004; WHO, 2006). In order to address the most current data a literature search was performed in PubMed (NLM, 2012) covering the publication period of the last two years. Relevant data were cited at the respective paragraphs.

2 SUMMARY ON DATA CONCERNING CARCINOGENICITY

The information on epidemiological studies or on experimental animal data are taken from various secondary sources (Environment Canada, 2011; OECD, 2004; WHO, 2006). For citations on the original sources please go to the specific reviews.

2.1 OBSERVATIONS IN HUMANS

In a publication from 1991 (Walker et al., as presented in Environment Canada, 2011) results of three cohorts were presented. In an early cohort study (Bristol 1933-1945; 3942 men) excess mortality from colon and rectal cancer was identified, when local rates of 20 years were compared to three years of working in a job with high exposure. However, the exposure was not quantified and exposure to clearly carcinogenic substances such as ethylene dichloride and acrylonitrile could not be ruled out. In the late Bristol study (1946-1986; 6548 men) even though exposure to other substances (i.e. lead, methyl methacrylate, ethylene dichloride, methylene chloride and acrylonitrile) could not be ruled out, no excess mortality from any cause was observed. Exposure levels were semi-quantitative. The unchanged mortality rate was also true for another cohort study which was conducted in a Knoxville plant (1943-1982; 3381 men). In this case exposure also occurred to various substances used in acrylic sheet manufacturing. Exposure levels were only semi-quantitative and it was not distinguished between methyl methacrylate and ethyl acrylate.

Summary:

Overall, no indication for a carcinogenic potential of ethyl acrylate can be derived from the epidemiological studies available.

2.2 ANIMAL DATA

Oral exposure

At first, the conditions and results of the study on rats and mice which led to the IARC classification are summarised.

These studies were conducted by the National Toxicology Program in 1986. Either male and female F344/N rats or B6C3F_1 mice were used as experimental animals. Dose range finding studies were conducted first. Groups of 10 animals/sex/dose were treated with ethyl acrylate 5 days/week for 13 weeks. Rats received up to 100 mg ethyl acrylate/kg body weight/day. The first study in mice was conducted using doses of up to 25 mg/kg bw/day, whereas in the second study the mice were treated with doses up to 100 mg/kg bw/day. In each study the animals received the test material in corn oil by gavage. Because of the absence of effects observed in these 13 week studies a 14-day study was conducted using higher dose levels of ethyl acrylate. Groups of five rats or mice/sex/dose were treated via gavage with doses of 0, 100, 200, 400, 600 and 800 mg ethyl acrylate/kg bw/day for 14 consecutive days. Histopathological examinations were limited to the stomach (only dose groups up to 400 mg/kg for rats and up to 600 mg/kg for mice were examined histopathologically; animals of higher dose groups revealed the same gross lesions as

animals in 400 mg/kg dose group). Incidence of ulcerative and non-ulcerative inflammation was elevated starting from the 400 mg/kg bw/day dose group.

After the dose range finding studies, the two year studies were conducted using a dosing regimen of 0, 100 or 200 mg/kg bw/day administered via oral gavage in corn oil 5 days/week for 103 weeks (substance purity 99 to 99.5 %; groups of 50 animals/sex/dose). The results from this two year carcinogenicity study are summarised in Table 1.

Under the conditions of this study, ethyl acrylate was found to be carcinogenic to F344/N rats or $B6C3F_1$ mice as squamous cell papilloma or carcinoma of the forestomach were induced. It is noted that male animals were affected more than female animals. The irritative nature of ethyl acrylate is reflected also as hyperkeratosis, endothelial hyperplasia and acute or chronic inflammation were obvious in the forestomach.

Various short-term or subchronic repeated dose toxicity studies (either 14, 28 or up to 90 days) in male rats supported the adverse effects found after bolus dosing via gavage of ethyl acrylate. Dose-related increases of hyperplasia and hyperkeratosis of squamous epithelium of forestomach and/or stomach were found. Additionally in some studies submucosal edema and ulceration as sign of acute and chronic inflammation was reported. In studies in which recovery periods were included a significant decline of the irritative effects was observed (Rohm and Haas, 1987; 1986b; Frederick et al., 1990; Ghanayem et al., 1991 as presented in Environment Canada, 2011).

In another chronic toxicity study, male Fischer 344 rats (5-16/group) were treated with 0 or 200 mg of ethyl acrylate (purity: 99%) per kg bw per day via gavage, 5 days per week for either 6 or 12 months followed by a recovery period up to 15 months. Only rats treated for 12 months developed forestomach tumours (squamous cell papilloma and/or carcinoma) during 2 to 9 months of recovery. None of the other animals (either 12 months of treatment with immediate sacrifice or animals only treated for 6 months with recovery period) revealed carcinogenic effects of ethyl acrylate. Again forestomach hyperplasia was observed in all treatment groups in different stages of severity (Ghanayem et al., 1993, 1994 as presented in Environment Canada, 2011).

In spite of the tumour development observed in chronic gavage studies, no carcinogenic effects were obvious in studies with rats and dogs when other oral administration methods were used. For example, dogs were dosed using gelatine capsules dissolved in corn oil whereas rats were treated with ethyl acrylate via drinking water (Borzelleca et al., 1964 as presented in Environment Canada, 2011).

Table 1 **Incidence of lesions of the forestomach taken from the carcinogenicity studies in F344/N rats and B6C3F$_1$ mice (NTP, 1986)**

	Neoplasm or non-neoplastic lesions	Dose (mg/kg per day)		
		0 (vehicle control)	100	200
Male rats	Hyperkeratosis	0/50*	37/50	46/50
	Epithelial hyperplasia	1/50	41/50	46/50
	Acute and/or chronic inflam.	1/50	8/50	28/50
	Squamous cell papilloma	1/50	15/50	29/50
	Squamous cell carcinoma	0/50	5/50	12/50
	Squamous cell papilloma or carcinoma	1/50	18/50	36/50
Female rats	Hyperkeratosis	0/50	24/50	46/50
	Epithelial hyperplasia	0/50	34/50	49/50
	Acute and/or chronic inflam.	1/50	3/50	20/50
	Squamous cell papilloma	1/50	6/50	9/50
	Squamous cell carcinoma	0/50	0/50	2/50
	Squamous cell papilloma or carcinoma	1/50	6/50	11/50
Male mice	Hyperkeratosis	0/48	19/47	28/50
	Epithelial hyperplasia	0/48	17/47	26/50
	Acute and/or chronic inflam.	0/48	3/47	8/50
	Squamous cell papilloma	0/48	4/47	9/50
	Squamous cell carcinoma	0/48	2/47	5/50
	Squamous cell papilloma or carcinoma	0/48	5/47	12/50
Female mice	Hyperkeratosis	2/50	14/49	32/48
	Epithelial hyperplasia	3/50	12/49	30/48
	Acute and/or chronic inflam.	1/50	4/49	12/48
	Squamous cell papilloma	1/50	4/49	5/48
	Squamous cell carcinoma	0/50	1/49	2/48
	Squamous cell papilloma or carcinoma	1/50	5/49	7/48

*Incidence/No. of animals necropsied; inflam.: inflammation

Inhalation exposure

When Fischer 344 rats or B6C3F_1 mice were exposed to ethyl acrylate via inhalation for up to 27 months, no treatment-related neoplastic lesions were observed (substance purity > 99.5 %; experiment 1: 0, 100, 310 mg/m³; experiment II; 0 or 20 mg/m³; 6 hours/day, 5 days/week). The lowest observed adverse effect concentration (LOAEC) of experiment I was established at 100 mg/m³ based on the non-neoplastic lesions observed of the olfactory mucosa due to the irritation potential of ethyl acrylate. In experiment II no adverse effects were observed at all (Miller et al., 1985 as presented in Environmental Canada, 2011).

Dermal exposure

In the study of DePass et al. (1984 as presented in Environmental Canada, 2011) male C3H/HeJ mice were treated with either acetone or 25 µl of undiluted ethyl acrylate (purity 99 %; i.e. approx. 23 mg/application thus 800 mg/kg bw/day) on the dorsal skin 3 times per week for their lifetime. Mean survival time of ethyl acrylate treated animals was 408 days. Due to irritation the animals developed dermatitis, dermal fibrosis and hyperkeratosis, but no tumour development was observed.

Another experiment used a transgenic mouse model (TG.AC (v-Ha-ras)). These animals are specifically designed to exhibit tumourigenesis of the skin, with a predisposition for papilloma. The skin of these mice is genetically initiated and is therefore useful for “rapid screening of tumour promoters, non-genotoxic carcinogens and for assessing anti-tumour and anti-proliferative agents”. In the test conducted by Nylander-French and French in 1998 ethyl acrylate was dissolved in acetone and 200 µl were painted on the back of female mice three times per week for 20 weeks under open conditions. No tumour development was observed in this assay (Carcinogenicity endpoint study record (ESR) 005; ECHA, 2012b).

Summary:

A two year study gavage application (i.e. delivery of the total dosage at one time via stomach tube, once per day) resulted in forestomach tumours (papilloma and carcinoma), accompanied by clear signs of irritation (hyperplasia, hyperkeratosis, acute and chronic inflammation, ulceration) in rats and mice. In the same and other species, no tumours were observed when ethyl acrylate was given in comparable doses using other types of oral applications (capsules, drinking water) or via other routes of administration (inhalation, dermal).

3 SUMMARY ON DATA CONCERNING GENOTOXICITY AND MUTAGENICITY

A large set of available data is given in Environment Canada (2011). This data were supplemented, when appropriate with information available from other reviews (OECD, 2004; WHO, 2006) or the REACH registration dossier for ethyl acrylate (ECHA, 2012b).

3.1 IN VITRO

In numerous reverse bacterial gene mutation tests (AMES test) with or without metabolic activation ethyl acrylate never gave a positive test result. Negative test results were also found in an *umu* test in *Salmonella typhimurium* (tests for induction of DNA repair, thus being an indirect test for DNA damage), but induction of mitotic recombination in *Sacharomyces cerevisiae* D61.M revealed positive results. Using another *Sacharomyces cerevisiae* strain (D4), negative results were obtained. When mammalian cells were used, increased gene mutation frequencies were found in mouse lymphoma cells (L5178Y $TK^{-/-}$), but not in Chinese hamster ovary (CHO) cells at the *hprt* locus. Within the mouse lymphoma test almost exclusively small colonies were found, which are indicative of chromosomal aberrations rather than gene mutations (Williams and Iatropoulos, 2009). Besides gene mutation also clastogenetic studies were performed. When treated with ethyl acrylate, several cell types (i.e. mouse lymphoma, mouse splenocytes, CHO and Chinese hamster lung) showed increased numbers of cells with chromosomal aberrations. Negative results were obtained in the absence of metabolic activation for CHO cells and mouse splenocytes in G_0-phase. Fowler et al., (2012) reported mostly positive results in *in vitro* micronucleus tests. Thereby various cell lines which lack p53 activity were found to be more prone to clastogenic effects, than those cells being p53-competent. The authors indicated that the positive results might be “misleading (false)”. Diverse results were obtained when sister chromatid exchange was investigated in CHO cells and mouse splenocytes.

Summary:

Ethyl acrylate showed negative test results in gene mutation assays with bacteria and mammalian cells at the *hprt* locus, but was positive in mammalian cells at the the *tk* locus (mostly small colonies indicative of clastogenic effects rather than gene mutations). Induction of chromosomal aberrations was observed in various strains. These data revealed that ethyl acrylate has no potential to induce gene mutations, but might have a potential to induce cytogenic effects (i.e. chromosomal aberrations; based on cellular toxicity via energy depletion secondarily leading to DNA double strand breaks, for further mechanistic explanations see 4.1), therefore posing a limited clastogenic potential *in vitro*.

3.2 IN VIVO

Various authors reported that ethyl acrylate was not mutagenic in *in vivo* micronucleus tests conducted with different strains of mice and examining bone marrow cells. Ethyl acrylate was administered intraperitoneally in doses up to 1500 mg/kg bw either once or on two consecutive days. Positive (i.e., mutagenic) or conflicting results for induction of micronucleus were only found when either male Balb/c or C57B16J mice were used under similar test conditions as described above. At least in one of these studies the concentration ranges used induced a significant reduction of the relation of polychromatic to normochromatic erythrocytes, indicating cytotoxicity (Henschler, 1986). No induction of micronucleated peripheral blood cells (erythrocytes) was found in the transgenic mice treated under the conditions already presented in section 2.2 “dermal exposure”. Further negative results were obtained in a variety of tests:

- no chromosomal aberrations in male C57BL/6 mice,
- no sister chromatid exchange observed in male C57BL/6 mice,
- no DNA binding occurred in male Fischer 344 rats, and
- negative results in sex-linked recessive lethal tests with *Drosophila melanogaster*.
- No DNA damage induced in leukocytes of transgenic mice in carcinogenicity study (TG.AC (v-Ha-ras)) investigated via COMET assay (Genotoxicity in vivo ESR 005; ECHA, 2012b).
- No DNA damage induced in forestomach 3 hours after gavage administration (0.1 to 4 grams per rat in concentrations of 0.1 to 4 % in corn oil; Morimoto et al., 1990 as presented by Williams and Iatropoulos, 2009).

Summary:

In vivo consistently negative results were found in most of the assays for gene mutation and chromosomal mutation. Based on the results of the *in vivo* assays no genotoxic potential was attributed to ethyl acrylate.

4 MODE OF ACTION

Even though the mechanistic background of the induction of forestomach tumours is still not fully elucidated, hereafter we present the mechanistic steps thought to contribute to the tumourigenic activity of irritant chemicals after bolus gavage dosage and especially focus on the knowledge of ethyl acrylate.

4.1 GENOTOXIC MODE OF ACTION

Ethyl acrylate does not induce gene mutations in mammalian (CHO) and non-mammalian cells taking together the results from various *in vitro* and *in vivo* tests. However, it has a certain clastogenic activity *in vitro* as chromosomal aberrations were seen in various assays. This activity was not confirmed *in vivo*. This effect pattern was shown to be true not only for ethyl acrylate but also for a variety of other esters of acrylic or methacrylic acid (Johannsen et al., 2008). These authors discuss that positive test results especially in mouse lymphoma cells might correlate with cellular toxicity (such as apoptosis and necrosis) mediated via energy depletion of cells (GSH or non-protein sulfhydryl group containing cofactors) and mitochondrial impairment. Due to fast metabolism of ethyl acrylate to non-toxic metabolites (i.e. acrylic acid and ethanol which are both further metabolized and excreted as CO_2 in the end) under physiological conditions, no dose levels would be reached *in vivo* that could induce this high dose mediated depletion and cellular disruption. The authors conclude that this chemical class of acrylates and methacrylates “does not pose an *in vivo* mutagenic risk”.

Others confirmed that the increased mutant frequency in mouse lymphoma cells (L5178Y $TK^{-/-}$) can more likely be attributed to cellular toxicity than to real gene-mutation effects and, therefore, can be considered as clastogenic effects (Ciaccio et al., 1998; Williams and Iatropoulos, 2009).

Summary:

No genotoxic mode of action is thought to be responsible for tumourigenesis observed in the bolus gavage studies in mice and rats.

4.2 NON-GENOTOXIC MODE OF ACTION

It is generally accepted that the carcinogenic effects found with ethyl acrylate are linked to its irritating effect caused at the site of entry (i.e. forestomach) in the rat and mouse gavage studies.

Mechanistically it is assumed that regenerative/compensatory cell proliferation following the irritation resulted in the tumour formation of the forestomach (e.g. Smit and van Raaij, 2004). The following steps occur until tumour formation is observed. First, signs of local irritation like inflammation, hyperkeratosis and hyperplasia are obvious. These events then lead to site-specific neoplastic lesions (Smit and van Raaij, 2004; Williams and Iatropoulos, 2009). Overall, the time and dose-dependent induction of forestomach tumours in rodents is considered the result of a non-genotoxic mode of action (Butterworth, 1989; Environment Canada, 2011; OECD, 2004; Smit and van Raaij, 2004; WHO, 2006).

The following observations support this conclusion:

A **time-dependency** was observed. Tumours developed only after chronic (> 6 months) bolus administration of ethyl acrylate via gavage. If administration was ceased before the critical exposure length, proliferative and inflammatory effects already induced regressed and tumours failed to develop (see test results of Ghanayem et al., 1993 and 1994).

Furthermore, the chronic studies are indicative of the **organ specificity** of the local irritant effect. Cell proliferative and inflammatory effects are seen in studies with oral, inhalation or dermal exposure always and only at the site of contact (oral: forestomach; inhalation: nasal cavity; dermal: skin). However, the forestomach of rodents presents a specific target due to some anatomical and physiological characteristics.

The forestomach in rodents (no comparable organ exists in humans) is a non-glandular organ of the gastro-intestinal tract in which the oesophagus empties and which is connected to the glandular stomach. The forestomach is lined by keratinized, stratified squamous epithelium; the stomach however is lined by a specialised glandular epithelium. In humans, only the oral cavity and the upper two thirds of the oesophagus have a comparable squamous epithelium whereas the entire stomach is lined with glandular mucosa (Environment Canada, 2011). In rats, the forestomach accounts for 60 % of the entire stomach, in mice up to 70 %. The pH value in the forestomach is approximately 6, i.e. less acidic than in the stomach (which allows a certain microflora to populate this organ) and pH changes may occur with respective compounds present. Most importantly, the forestomach in rodents is a food storage organ. The retention time is ranging from half day to two or three days (Williams and Iatropoulos, 2009). In humans, food storage in the pre-stomach compartments with similar cell linage is not relevant, thus no concordant organs in humans exist. In the gavage studies performed the transit time was even more prolonged as the bolus administration was before noon, i.e. during the sleeping cycle of nocturnal rodents, thus meaning the parasympathic nervous system is in charge (Williams and Iatropoulos, 2009). Additionally, the vehicle used in the NTP study – corn oil – itself is a known mild irritant and mitogen, which further extends the retention time of the compound in the forestomach compared to other vehicles, e.g. water (Smit and van Raaij, 2004). Due to the prolonged retention time in this storage compartment of rodents this specific part of the gastro-intestinal tract is very vulnerable to local irritant effects. Besides the prolonged retention time, another physiological feature of

the forestomach contributes to this very specific susceptibility. The huge regenerative potency of this organ makes it prone for adverse effects caused by cytotoxic/irritant chemicals that induce sustained regenerative proliferation and thus provide the stimulus for neoplastic progression (IARC, 2003). Moreover, due to the specific anatomy of the entire stomach of rodents they are not able to vomit. In contrast humans have a vomiting reflex in order to get rid of irritating substances (Smit and van Raaij, 2004). Taken together, the squamous epithelial papilloma and carcinoma of the forestomach in rodents are a **species-specific effect**, not relevant for human health hazard assessment.

Besides the species specificity, the tumour formation also is a **high dose effect (bolus-specific)**. When lower doses (e.g. < 100 mg/kg bw/day) were administered no tumour formation was observed. Moreover, rapid systemic detoxification (see 4.1) is responsible for the fact that, besides the site of contact, no tumours were observed at any other site even in animals treated with high dosages. In addition, it has been clearly shown that administration of comparable dose levels via drinking water or parenteral administration does not result in tumour formation.

Summary:

Tumourigenesis induced by the irritant ethyl acrylate in the forestomach of rodents is considered to be caused by the irritating properties of the substance and not based on a genotoxic mode of action. As this effect is organ specific and depending on a special form of administration (gavage) the carcinogenic effects observed are not relevant for humans.

5 OPINIONS AND EVALUATION BY VARIOUS EXPERT PANELS

5.1 OECD SIDS

Within their summary conclusion the authors of the SIDS Initial Assessment Report stated that “ethyl acrylate is considered to pose no mutagenic hazard based on the available data.” In their assessment report they verified the fast metabolic degradation of ethyl acrylate and its strong irritating potential. Moreover, it was indicated that the respective studies, which led to the IARC classification, were considered to be inadequate to judge on the carcinogenic potential already by the study performing laboratory itself. According to them the NTP itself marked this basis for classification as “scientifically unjustifiable” in 2000, due to the known fact that the forestomach tumours in rodents are irrelevant to humans and were only seen after long-term gavage administration at doses which induce local irritation and cellular proliferation (OECD, 2004).

5.2 HARMONIZED CLASSIFICATION ACCORDING TO ANNEX VI OF REGULATION (EC) NO. 1272/2008

The harmonized classification according to Annex VI of the Regulation (EC) no. 1272/2008 (EC, 2008) can be retrieved from the Classification and Labelling Inventory presented by the European Chemicals Agency (ECHA, 2012a).

For ethyl acrylate the following classification is presented:

- Physical hazards
  - Flam. Liquid 2 — H225: Highly flammable liquid and vapour.
- Health hazards
  - oral Acute Tox. 4 — H302: Harmful if swallowed.
  - dermal Acute Tox. 4 — H312: Harmful in contact with skin.
  - inhalation Acute Tox. 4 — H332: Harmful if inhaled.
  - Skin Irrit. 2 — H315: Causes skin irritation.
  - Eye Irrit. 2 — H319: Causes serious eye irritation.
  - Skin Sens. 1 — H317: May cause an allergic skin reaction.
  - STOT Single Exp. 3 — H335: May cause respiratory irritation.

This classification reflects the current toxicological assessment of ethyl acrylate. In terms of health hazards, the classification accounts for the slight acute toxicity (oral, dermal, inhalation) as well as the obvious local effects (irritation of skin, eyes and respiratory tract, skin sensitisation) emerging after ethyl acrylate treatment. This classification is also presented by the European chemical Substances Information System (EC, 2012).

5.3 REGISTRATION DOSSIER ACCORDING TO REGULATION (EC) NO. 1907/2006

In the registration dossier according to Regulation (EC) No. 1907/2006 (EC, 2006) (REACH) the harmonized classification, as it was reported above (5.2), was repeated. No additional data were presented which would point to a genotoxic or carcinogenic activity of the substance.

5.4 OTHER COMMITTEES

Based on more or less the same experimental data as presented herein, the WHO (2006) and Environment Canada (2011) both concluded that the appearance of forestomach tumours in the two year studies has no relevance to humans. Ethyl acrylate, a known irritant, was administered in high doses. Thus, the effects were attributed to the irritating effects from ethyl acrylate bolus dosing which was directly delivered to the site where the adverse effect occurred “and not to effects of systemic concentrations in the whole animals” (see also 5.5).

Moreover, Environment Canada (2011) indicates that the tissue dose in the affected forestomach after the substance administration via oral gavage is not representative of the human exposure expected for ethyl acrylate.

5.5 GENERAL CONSIDERATIONS ON HUMAN RELEVANCE OF FORESTOMACH TUMOURS IN RODENTS

In more general terms, the mechanistic assumptions leading to forestomach tumours in rodents after repeated bolus gavage administration of irritant chemicals, which possess no direct genotoxic potential, leave this effect irrelevant for human health assessment according to various sources (Environment Canada, 2011; IARC, 2003; Smit and van Raaij, 2004; WHO, 2006). This is further endorsed by the fact that humans lack a forestomach. Despite that respective histopathological similar organs (see 0) exist, the additionally needed longer retention time is not given in these organs, further supporting the lack of relevance to humans of the observed effects in rats and mice after oral exposure via gavage (Environment Canada, 2011; IARC, 2003; Smit and van Raaij, 2004; WHO, 2006).

6 GENERAL CONCLUSION

The initial classification by IARC regarding carcinogenicity of ethyl acrylate was based on the observation that forestomach tumours were induced in male and female rats and mice during a two year gavage study.

Based on existing evidence and today's scientific knowledge and evaluation, these experimental observations are considered to be not relevant for humans:

- The data presented in this document clearly show that the tumour development is based on a non-genotoxic mode of action. As ethyl acrylate is a known irritant, the prolonged and repeated bolus administration by oral gavage is considered pivotal to induce local irritation and hyperplasia, which act as precursors for the tumour development in the forestomach of rats and mice. Moreover, anatomical and physiological characteristics only present in rats and mice (e.g. non-glandular forestomach in rodents used as food storage – with no similar organ in humans) promote tumour formation in this organ by irritant chemicals.
- This mechanistic conclusion is supported by the observation that carcinogenic effects were only seen after oral gavage administration, but neither when other forms of oral administration were used (capsules, drinking water) nor via other administration pathways (inhalation, dermal).
- In addition, the experimental data on genotoxicty indicate that ethyl acrylate is not mutagenic but may exert some clastogenic activity which is probably secondary to extensive cytotoxicity. Taken together, the available evidence does not indicate a mutagenic hazard arising from ethyl acrylate.

The collective evidence shows that the carcinogenic effects observed in gavage studies with rats and mice are not relevant for humans and that no classification for mutagenicity or carcinogenicity is required according to Regulation (EC) no. 1272/2008 (EC, 2008).

This conclusion is in agreement with the evaluations presented by various other committees (Environment Canada, 2011; OECD, 2004; WHO, 2006). Moreover, the European harmonised classification of ethyl acrylate does not classify ethyl acrylate for carcinogenicity (EC, 2012).

In conclusion, the IARC classification of ethyl acrylate is not considered correct based on today's scientific knowledge and does not need to be taken into account in the safety assessment of Gasodor® S-Free when used as a domestic gas odorant. The tumourigenic effects observed after oral bolus administration in rats and mice, which were the basis for the IARC classification, are not considered relevant for humans and ethyl acrylate showed no tumourigenic effects in rats and mice in chronic inhalation studies.

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