

Ethylene Oxide REACH Consortium Comments on the Proposal for the Classification of Ethylene oxide as Acute Tox. 3 (H301), Skin Corr. 1B (H314), Eye Dam. 1 (H318), Skin Sens. 1 (H317), Repr. 2 (H361df) under the CLP Regulation

This document is submitted by the lead registrant, BASF SE on behalf of the Ethylene oxide REACH Consortium.

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Summary

In this document, we comment on the classification of ethylene oxide (EO) by the Environment Agency Austria (EAA) in the CLH dossier of August 2016. We compare the conclusion of the EAA with the data used in the CLH dossier for acute oral toxicity, skin irritation, eye irritation, skin sensitization, and reproductive toxicity, thereby checking the validity of the individual studies and conclude whether the studies can be used for classification at all, and if so, whether the classification as proposed by EEA is warranted.

Acute Tox. 3 (H301): It is questionable if EO should have a classification for acute toxicity via oral route at all. EO is a gas under normal conditions which is already classified as toxic by inhalation (R23) according to Annex I to Directive 67/548/EEC. Therefore, the main exposure route is covered and classified. Furthermore, EO only occurs in its gaseous form for the population at risk for accidental exposure (workers in plants producing or processing EO). In addition, a stricter classification or in general a classification for oral toxicity does not have a positive impact on human health as no applications and uses are intended where oral exposure could apply.

Skin Corr. 1B (H314): EO is a very volatile substance that may evaporate rapidly. When tests are performed under occlusive conditions, the results are worst-case, because under semi-occlusive conditions, exposure would be less. As a result, a classification as 1B is scientifically questionable at the moment and further investigations (preferably *in vitro* studies) are advisable to perform a reliable assessment and to finally conclude on a possible sub-classification. In the meantime, due to the current available data a classification as Skin Corr 1 or Skin Corr 1C instead of Skin Corr 1B as proposed by the EAA should rather be considered.

Eye Dam. 1 (H318): There are currently no reliable data available for this endpoint and the CLP demands that based on a classification for skin corrosion, a substance shall implicitly also be classified for Eye damage 1. Therefore, as the current data for the endpoints skin and eye are both not sufficient to draw a final conclusion on (sub) classification, it is advisable to introduce further *in vitro* tests to first clarify the skin corrosion concern and thereafter conclude on the respective hazard classification for the eye.

Skin Sens. 1 (H317): According to Annex I of Regulation (EC) No. 1272/2008, substances shall be classified as skin sensitizers (Category 1) in accordance with the following criteria: (a) if there is

evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test. Criteria (a) is not met as the substantial number only relates to people being exposed to medical equipment sterilized with EO via blood, which potentially could respond when in contact with the skin, although there is currently no data to support that claim. A literature search and internal investigations at BASF, Ineos and Sasol have shown that there was not a single case of skin sensitization reported in industrial plants in the last 20 years at these companies where EO is either produced or processed. Consumer uses are not intended for EO. Reports in the public literature on allergic symptoms in hospital workers using EO sterilized equipment, gowns, sutures etc. mostly describe IgE-mediated type I immediate reactions that are not relevant for IgG-dependent skin sensitization response. As a result, the requirement for “substantial number” is not met. Criteria (b) is not met, because no reliable animal data is available. Therefore, classification for skin sensitization is not warranted.

Repr. 2 (H361df): The EAA summarized 19 publications to draw a conclusion on the reproductive potential of EO which can be split in four categories: effects on the testis, effects on the female fertility, effects on development, and data obtained in humans. In general, the EAA was quite conservative regarding the effects observed. In several cases effects are classified as adverse by the EAA, while the authors of the publication consider the effects not relevant. Instead of combining all effects observed without judging the reliability of the individual studies in which the effects were reported, our approach was to evaluate every single paper and conclude whether the effects observed in each paper could be attributed to exposure to EO and not be secondary to other toxic effects and determine whether EAA’s conclusions are justified.

Regarding the endpoint male fertility, the effects on the testis reported in the discussed studies are limited to slight degenerations in the seminiferous tubules and reversible germ cell degeneration. The only adverse effect caused by EO exposure is the reduced number of implantations in one study out of three available on the endpoint female fertility. One of them does not show an effect on fertility and the second one of those showing effects has clear limitations in the method as no information is available on toxic effects on the dams besides fertility effects. Therefore, taking into account the overall weight of evidence on the endpoint fertility, a classification of EO as Repr. 2, H361f is not overall conclusive. In the developmental studies no relevant adverse effects were observed after exposure to EO. Thus, a classification for developmental toxicity (H361d) is not warranted.

Acute oral toxicity

Current Annex VI classification: None

Registrants classification: Acute Tox. 4 (H302: Harmful if swallowed)

Proposed classification by EAA: Acute Tox. 3 (H301: Toxic if swallowed)

Available data*

Rat (m)	LD50: 330 mg/kg bw	Smyth (1941)	Klimisch 2
Rat (?)	LD50: 100-200 mg/kg bw	Hollingsworth (1956)	Klimisch 3
Rat (?)	LD50: 72 mg/kg bw	RTECS (1996)	Klimisch 4 (secondary source)
Guinea pig (m/f)	LD50: 270 mg/kg bw	Smyth (1941)	Klimisch 2
Rabbit (?)	LD50: 631 mg/kg bw	Woodard (1971)	Klimisch 4 (secondary source)
Rat and mice	LD50: 250-350 mg/kg bw	Patty's (1971)	Klimisch 4 (secondary source)

*according to a literature search performed in September 2016

Data discussed by EAA

Rat (m)	Rat: LD50= 330 mg/kg bw Guinea pig: LD50 = 270mg/kg bw (m/f)	Smyth (1941)	Klimisch 2
Rat	LD50: 330 mg/kg bw	Bruhin (1961)	Study not available
Mice (m/f), Guinea pig	Mice: LD50: 280 mg/kg bw (female) Mice: LD50: 365 mg/kg bw (male) Guinea pig: LD50 = 270mg/kg bw (m/f)	Woodard (1971)	Study not available

Conclusion EAA:

The available data on this route of exposure is limited. For oral exposure an LD50 = 330 mg/kg bw for male rats can be derived (Smyth, 1941; Bruhin, 1961). Mice and Guinea pigs were the most sensitive species with an LD50 of 280mg/kg bw and 270mg/kg bw, respectively. LD50 values are all in the same order of magnitude.

Conclusion Consortium:

It can be concluded that the only usable data is the study by Smyth (1941) as for all other studies the original reports are not available. Smyth et al. (1941) reported an acute oral LD50 value of 330 mg/kg bw for male rats. In this study, a liquid EO preparation caused narcotic effects, however confined to the dose levels in the lethal range. Based on this study in rats classification should be Acute Tox. 4 (H302: Harmful if swallowed). However, in addition to the rat data, Smyth (1941) also reported a study in guinea pigs indicating that the substance should be Acute Tox. 3 (H301: Toxic if swallowed). As was common practice before 2010, only one study was submitted to ECHA (in this case the study with rats by Smyth). This explains the difference between the data of EAA and the registrants and the fact that in the REACH dossier the guinea pig study was not taken into consideration for the classification and labelling of EO.

According to section 3.1.2.2.1 of Annex I of Regulation (EC) No. 1272/2008; *“The preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat [...] When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD50 value [...]”*. The Guidance on the Application of the CLP Criteria (2015) states the following, *“In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by*

a robust justification. If there is information available to inform on species relevance, then the studies conducted in the species most relevant for humans should normally be given precedence over the studies in other species.” In 2010, the registrants expert judgement was that the rat is the most appropriate species and relevant for classification of a hazard for humans.

Nevertheless, the main question is, if a classification for acute toxicity via oral route is overall constructive and warranted due to results of animal tests. First, EO is a gas under normal conditions which is already classified as toxic by inhalation (R23) according to Annex I to Directive 67/548/EEC. Therefore, the main exposure route is covered and classified. Furthermore, EO only occurs in its gaseous form for the population at risk for accidental exposure (workers in plants producing or processing EO). A classification should therefore only apply to the gaseous form and due to the before mentioned is therefore not warranted for the oral exposure route. In addition, a stricter classification or in general a classification for oral toxicity does not have a positive impact on human health as no applications and uses are intended where oral exposure could apply. As a result, it is questionable if EO should have a classification for acute toxicity via oral route at all.

Skin corrosion

Current Annex VI classification: Skin Irrit 2

Registrants classification: Skin Irrit 2

Proposed classification by EAA: Skin Corr 1B, H314

Data discussed by EAA and available data*

Rabbit, occlusive, 1-60 minutes: 10% and 50% aq. EO	Irritating Scar formation	Hollingsworth (1956)
Rabbits, DOT method, occlusive , 4 hours, undiluted	Corrosive - Edema score 24-72 hours: 4 - Erytheme score 24-72 hours: 4 - Hemorrhages and chemical burns observed	Celanese Chemical Co (1972)
Several human data		Alomar et al. (1981) Biro et al. (1997) Cárdenas-Camarena L. (1998) Sexton (1950) Hanifin (1971)

*according to a literature search performed in September 2016

Conclusion EAA:

Liquid ethylene oxide causes severe skin lesions (chemicals burns, hemorrhages, scar formation) in *in vivo* animal testing. Case reports also demonstrate corrosive potential of the substance. Ethylene oxide or solutions of ethylene oxide are highly reactive alkylating agents that react with many constituents of tissue resulting in cellular and tissue dysfunction and destruction. Ethylene oxide is strongly corrosive with rapid evaporation from skin.

Three subcategories for classification as corrosive are provided: subcategory 1A, where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B, where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C, where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days. For ethylene oxide one positive study with exposure duration of 4h (showing chemicals burns, hemorrhages) and one positive study with exposure durations from 1-60 minutes (showing hyperemia, edema at 6min or longer) is available. Positive evidence from humans is available.

Conclusion Consortium:

Hollingsworth et al. (1956) reported a skin irritation study using rabbits. EO was tested as 10 and 50% aqueous solutions under occlusive conditions. Hyperemia and edema resulted when the duration of skin contact was 6 minutes or longer. The severer exposures resulted in scar formation. The intensity of response was roughly proportional to the length of exposure time and the concentration. Celanese Chemical Company (1972) established a corrosive skin reaction for rabbits. The unchanged test substance was tested in a patch test over a study period of 4 hours. Human information shows that EO does cause severe (irreversible) effects. These studies are not included in the REACH dossier yet. According to 3.2.2.4 of Annex I of Regulation (EC) No. 1272/2008; “[...] primary emphasis shall be placed upon existing human experience and data, followed by animal experience [...]”.

EO is a very volatile substance that may evaporate rapidly. When tests are performed under occlusive conditions, the results are worst-case, because under semi-occlusive conditions, exposure would be less. As a result, a classification as 1B is scientifically questionable at the moment and further investigations (preferably *in vitro* studies) are advisable to perform a reliable assessment and to finally conclude on a possible sub-classification. In the meantime, due to the current available data a classification as Skin Corr. 1 or Skin Corr. 1C instead of Skin Corr. 1B as proposed by the EAA should rather be considered.

Serious eye damage

Current Annex VI classification: Eye Irrit 2

Registrants classification: Eye Irrit 2 (H319: Causes serious eye irritation)

Proposed classification by EAA: Eye Dam 1, H318

Data discussed by EAA and available data*

Rabbit, 0.1 and 1% dilution	Irritating	McDonald (1977)	Klimisch 2
-	Severe irritation and corneal injury	Kodama (1961)	Klimisch 4 (secondary source)

*according to a literature search performed in September 2016

Conclusion EAA:

One study on eye irritation (McDonald, 1977) is available. The ethylene oxide concentration tested is very low (max 1%) and no information in the stability of the test preparation is given, therefore the value of this study is limited. However ethylene oxide is a corrosive substance and such substances are automatically considered to be severely damaging to the eyes.

Conclusion Consortium:

Only one study is available regarding eye irritating properties of EO. McDonald et al. (1977) performed a topical ocular instillation of 0.05 ml/dose at 10-minute intervals using rabbits. Normal and irritated eyes responded similarly, so that the maximum non-damaging concentration was 0.1% EO (dilution with a balanced salt solution). A unique finding was that irritating concentrations of EO produced delayed but more severe ocular changes for normal eyes when compared to irritated eyes. This study is not compliant with any official test guideline and should therefore not be used as the basis for classification. Nevertheless, there are currently no other reliable data available and the CLP demands that based on a classification for skin corrosion, a substance shall implicitly also be classified for Eye damage 1. Therefore, as the current data for the endpoints skin and eye are not sufficient to draw a final conclusion on classification, it is advisable to introduce further *in vitro* tests to first clarify the skin corrosion concern and thereafter conclude on the respective hazard classification for the eye.

Skin sensitisation

Current Annex VI classification: None

Registrants classification: None

Proposed classification by EAA: Skin Sens 1 (H317: May cause an allergic skin reaction)

Data discussed by EAA and available data

Guinea pig; too limited information	Non sensitizing (only result given)	Woodard (1971)	Klimisch 4 (secondary source)
Passive cutaneous anaphylaxis (PCA) assay rat and mice	Mice produced ethylene oxide specific antibodies, while rats did not respond.	Chapman (1986)	Klimisch 2
Nineteen publications with human data			

*according to a literature search performed in September 2016

Conclusion EAA:

Ethylene oxide is a sensitizing agent. Type I (anaphylaxis) and Type IV (contact dermatitis) hypersensitivity reactions have been observed in individuals exposed to ethylene oxide (WHO, 2003). No animal studies according to standard test protocols are available. Classification for this endpoint is based on human data (dermal and parenteral route of exposure) and the known mechanism (alkylating agent). Ethylene oxide should be classified as skin sensitizer (Category 1), H317 (May cause an allergic skin reaction). According to CLP guidance document classification into sub-categories can only be carried out if data are sufficient. The available human data (clinical surveillance and case reports) do not provide information on the size of the exposed population, or

on the extent (no information on release of ethylene oxide from sterilized material) and the frequency of exposure. Based on the available data no classification into a subcategory is proposed.

Conclusion Consortium:

Nineteen publications of alleged skin sensitizing properties have been reported by the EAA. Of these 19 studies, 15 were on the determination of EO specific antibodies in hemodialysis patients (14 articles: Bommer (1985), Caruana (1985), Dolovich (1978 and 1984), Grammer (1984 and 1985), Lemke (1987), Marshall (1984 and 1985*), Monbaliu (2010), Pearson (1987), Röckel (1988), Rumpf (1985), Wass (1988) and platelet donors (1 article: Leitman (1986)). These patients/donors are exposed to EO, because medical equipment is sterilized with EO and unintentionally EO was still present on the equipment. As a result, the patients/donors come in contact with EO via blood contact, experiencing immediate type I (most common) and not delayed type IV allergic reaction seen with dermal sensitizers. It has to be pointed out that based on the skin sensitization AOP published by OECD, which clearly describe the mode of action involved in this process, the proposed H317 only covers type IV allergic reaction and not the described type I reactions seen in these publications. In addition, the reactions did not primary occur via dermal exposure. As a consequence, the induction phase for sensitization is not via the dermal route. It is not specified in Regulation (EC) No. 1272/2008 that the induction phase should also be via the dermal route, but according to section 3.4.1.2 of Annex I of Regulation (EC) No. 1272/2008 a skin sensitizer means a substance that will lead to an allergic response following skin contact. This implicitly means that the elicitation phase of sensitization has to be via the skin.

These publications proved however the ability of EO to cause an IgE-mediated allergic type I reaction by means of a RAST or a skin prick test, the latter of which is not highly diagnostic for immediate type EO allergy and the former is predictive in ~60% of long-term patients on hemodialysis that experienced severe immediate type reactions to EO. Both tests are able to identify whether a person is sensitized to a certain substance, but not whether the dermal route of exposure can provoke the allergic reaction. Of the 15 publications no evidence of contact dermatitis is provided by means of a patch test. The study by Dolovich (1978) suggests that a patch test has been performed, but in the publication by Dolovich (1984) it is clear that the skin test was performed by intracutaneous injection. In table 25 of the CLH report, it is stated that in the publication by Dolovich (1984) a skin test was performed, but after reviewing this article it turns out to be a skin-prick test. Overall, all the publications do not prove that ethylene oxide is a skin sensitizer.

* The article by Marshall 1985 reports the same results as Dolovich in 1984.

This leaves four publications discussed by the EEA in which possible skin sensitizing effects are studied: three case-reports and one volunteer study. These studies will be discussed in more detail as these studies might be relevant for classification purposes.

The case report by **Jacson (1991)** is in French and only the abstract is in English. The following summarizes the translated content:

The study by Jacson (1991) describes the case of a 27 year old woman who went to see a doctor in April 1985, because she suffered from edema of the eye lid, rhinitis and asthma since a couple of months. She reported to have been a nurse in a surgery room. After suffering from an immediate-type reaction (hives hands and conjunctivitis) in contact with sterile surgical gloves she was assigned to work at the reception desk. However, she still suffered from constant rhinitis as a common cold or

asthma after this change of the job environment which also did not disappear even on days off. She had no allergic history and skin tests were negative to several inhalable allergens. A 1% prick test with formaldehyde and a positive prick test was performed using a piece of latex glove sterilized with EO. A prick test using a piece of latex glove sterilized with gamma rays was negative. The RAST for EO was negative. Shortly after the consult, the young woman was assigned to another department where the symptoms disappeared.

In August 1988, the asthma had returned at night. This occurred immediately after she started working at another hospital. Again, several allergens were tested negatively and therefore skin prick tests with several surgical gloves were performed. Positive effects were observed for EO sterilized latex gloves, EO sterilized latex gloves being washed, EO sterilized vinyl gloves, latex gloves sterilized with gamma rays, and unsterilized latex gloves unsterilized. No positive response was obtained from the skin prick test with formaldehyde. This time the RAST for EO, latex, and formaldehyde was positive.

In November 1988 the woman was hospitalized due to a miscarriage. Equipment for anesthesia was sterilized without EO, but the surgeon was wearing latex gloves. Upon awakening, about 20 minutes after the procedure, a tingling throat, cough, and finally asthma appeared. These phenomena declined one hour after the administration of corticosteroids. In October 1989, she gave birth in difficult conditions. Sterilization was performed and the obstetrician was wearing neoprene gloves. No allergic complication occurred.

This case report tells the disease history of a woman with several allergies, including latex allergy with discrepancies in the results of the tests performed to find the cause of the symptoms. The contradicting test results were not explained and the cause of the symptoms remains unclear. Overall, it is important to point out that this publication assessed the IgE-mediated, Type I anaphylactic shock mechanism which is not relevant for IgG-dependent skin sensitization response. Therefore, this study is not considered to be relevant in the scope of the evaluation of the sensitizing properties of EO.

In a case report by **Caroli (2005)**, a nurse showing eczema at both forearms after wearing new gowns was described. To determine whether an allergic response was present, a patch test was performed. A positive reaction was observed, no EO specific IgE was detected. However, IgE antibodies are not characteristic of delayed-type IV reaction of skin sensitizers. A check for IgG was not included. The authors concluded that although “standardized patch testing is not established for EO, and the differentiation between an irritant and an allergic contact dermatitis may therefore be difficult.”, the reaction observed in the one nurse should be a delayed-type hypersensitivity response because 20 colleagues exposed to the same gown did not develop skin reactions. This is of course not a scientifically justified argument. It is commonly known that also skin irritation is an individual-specific response and the absence of irritation in one person does not mean that someone else will also not show irritating effects when exposed to the same substance. Therefore, we consider the conclusion by the authors to be not justified and the skin reaction observed might well be a skin irritant effect. Therefore, this study does not indicate that EO is a skin sensitizer.

In the third case report (**Kerre, 2009**) a similar case as reported by Caroli (2009) has been described. In the patch test with EO a reaction was seen on day 2 and 4. The author suspected a delayed-type hypersensitivity reaction. To add further weight to their conclusion, they tested the patient also with epichlorohydrin, a chemically similar substance. For that substance a positive reaction was seen

after 2 and 4 days. The method of the patch test has not been described by the authors and effects seen at day 2 and 4 seem to imply that these effects are a delayed-type hypersensitivity reaction. However, in a normal patch test, the exposure time is 48 hours. As a result, the effect observed at day 2 (the first observation) can still be an irritating reaction instead of an allergic reaction. Furthermore, their substance which should add further weight to their conclusion is also a corrosive substance and the same comments apply to that substance. Therefore, the conclusion by the authors is not justified and this study does not indicate that EO is a skin sensitizer.

The fourth study is a volunteer study described by **Shupack (1981)**. In this test, 12 volunteers were exposed to EO. In Phase 1, patches with EO (1000 and 2000 ppm) were applied to the backs of the human volunteers and removed at intervals between 1, 2, 4 or 8 hours. In Phase 2 (time between phases not reported) patches with 10, 100 and 1000 ppm were applied to the same volunteers with the same protocol. In all volunteers, skin irritating effects were observed after Phase 1 that disappeared within 72 hours in eleven volunteers. One volunteer (No. 12) showed a skin reaction approximately three weeks after the initial exposure to EO. After Phase 2, no skin irritating effects occurred at 10 ppm, only in one subject at 100 ppm and in all subjects at 1000 ppm. However, all effects disappeared within 2-3 days. In No. 12, a skin reaction appeared again three weeks later. However, the authors concluded that “this long an interval between re-exposure to an antigen to which an individual has been previously sensitized and the occurrence of the reaction is inconsistent with the usual time course of delayed cutaneous hypersensitivity reactions”. Furthermore, histology was negative for delayed hypersensitivity reactions and therefore the authors concluded that the observation in this one patient “does not conform to the typical description of allergic hypersensitivity reactions”. Based on this study no conclusion can be drawn on the skin sensitizing potential of EO.

Overall conclusion

In the original submission of the EO REACH dossier to ECHA, the consortium concluded that due to missing valid data, classification concerning skin sensitization cannot be made. Experimentally, there was and still is only one negative animal study of insufficient validity available (secondary source; unclear and possibly multicomponental composition of test material). No further investigation was considered necessary nor needs to be carried out in regard that EO is a flammable gas in air at room temperature, fluid EO is corrosive and the test substance is classified as a mutagen and genotoxic carcinogen. Furthermore, due to the high volatility and corrosivity of liquid EO, experimental investigation did not appear to be appropriate.

EAA decided to completely review the available human data on allergic responses to ethylene oxide. As described above, most of the studies identified by the EAA are not investigating dermal exposure and can therefore not be used for classification purposes. The publications that appear to be investigating or describing reactions after dermal exposure, are 3 case reports and 1 volunteer study, not providing unequivocal support that EO has skin sensitizing potential. The majority of reports with EO allergies describe IgE-mediated type I immediate reactions that occur in patients with compromised health status with other reported allergies or and with advanced disease states, such as patients on haemodialysis, fatal kidney disease, asthma, spina bifida, thalassemia. The EAA concludes that EO should be classified as skin sensitizer (Category 1), H317 (May cause an allergic skin reaction). However, according to section 3.4.2.2.1.4 of Annex I of Regulation (EC) No. 1272/2008, substances shall be classified as skin sensitizers (Category 1) where data are not

sufficient for sub-categorization in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test. Criteria (a) is not met as the substantial number only relates to people being exposed to medical equipment sterilized with EO via blood, which potentially could respond when in contact with the skin, although there is currently no data to support that claim. A literature search and internal investigations at BASF, Ineos and Sasol have shown that there was not a single case of skin sensitization reported in industrial plants in the last 20 years at these companies where EO is either produced or processed. Consumer uses are not intended for EO. Reports in the public literature on allergic symptoms in hospital workers using EO sterilized equipment, gowns, sutures etc. mostly describe IgE-mediated type I immediate reactions that are not relevant for IgG-dependent skin sensitization response. As a result, the requirement for “substantial number” is not met. Criteria (b) is not met, because no reliable animal data is available. Therefore, we consider that classification for skin sensitization is not appropriate in this case.

Reprotoxicity

Current Annex VI classification: None

Registrants classification: not classified

Proposed classification by EAA: Repr. 2, H361fd

Data discussed by EAA and available data (fertility)*

One-generation reproductive toxicity in rats; 10, 33, 100 ppm	EO did not affect male fertility. Resorptions in dams were not different between groups, but there were reduced number of implantations, the number of pups born and litter sizes were significantly lower.	Snellings et al. (1982c)	Klimisch 2
Two-generation study in guinea pigs	Degeneration of testis tubules and reduction of testis weights. No further data available.	Hollingsworth et al. (1956)	Klimisch 4 (very poor documentation) - not in REACH dossier
Two-generation study in rats	Slight degeneration of testis tubules. Testes appeared small. No further data available.	Hollingsworth et al. (1956)	Klimisch 4 (very poor documentation) - not in REACH dossier
Spermatogenic function in cynomolgus monkeys after exposure to 50 and 100ppm	100 ppm: weight gain decreased significantly. No changes in the urinalysis, clinical chemistry, hematology, or electrocardiographic indices. Decreased nerve conduction velocity measurements in 2 of the 12 monkeys. Sperm counts and sperm motility significantly reduced in all exposed monkeys.	Lynch et al. (1984)	Klimisch 4 (no clear dose-response and no macroscopic or histopathological examination of testis) - not in REACH dossier
Testicular toxicity and alterations of glutathione metabolism after chronic inhalation in rats (0, 100, 250 ppm)	No effects on body weight and testicular weights. Epididymal weights decreased at highest dose and epididymal sperm counts were reduced in the same group. Slight degenerations in the seminiferous tubules of testis. LDH X	Mori et al. (1991)	not in REACH dossier

	activity in the testis was not affected. Number of immature heads and abnormal sperm heads significantly increased at 250 ppm, while teratic type sperm head increased at all dose levels, but dose independent.		
Effects of inhaled ethylene oxide on spermatogenesis in rats (0, 500 ppm)	Body weight gain not affected. Relative weights of the testes and the epididymides decreased in a time-dependent manner. Degeneration and exfoliation of germ cells observed at all exposure durations. Some seminiferous tubules showed germ cell recovery at 13 weeks compared with 6 weeks.	Mori et al. (1989)	not in REACH dossier
Mouse sperm morphology test in mice (0, 200, 400ppm)	Authors concluded that ethylene oxide increased the frequency of abnormal sperm cell in a dose-dependent matter.	Ribeiro et al. (1987)	not in REACH dossier
Subchronic inhalation study in mice (0, 10, 50, 100, 250 ppm)	Minimal changes in certain erythroid parameters, increased liver weight, decreased testicular weight and decreased spleen in highest dose group. Locomotor effects at all dose-levels. However, no clinical or histopathological findings on organ weight to support or explain any of these apparently treatment-related effects. Percentage of the decrease in testicular weight not reported.	Snellings et al. (1984)	not in REACH dossier
Exposure of female mice before mating (0, 1200 ppm) and within hours after mating (300 ppm)	Number of implants per female significantly reduced at 300 ppm but not at 1200 ppm. No information available on other toxic effects. Difficult to conclude whether the observed implantation loss is a primary result of EO exposure or a secondary effect – LC50 is 600 ppm).	Generoso et al. (1987)	not in REACH dossier
Reproductive-toxicologic assessment of EO by inhalation exposure of rats and rabbits (150 ppm)	Resorptions were significantly increased in rats (not rabbits), but the number of live fetuses was unchanged. Absolute numbers of resorptions not given but necessary to consider the relevance of the observed effect. Number of fetuses was decreased.	Hardin et al. (1983)	Klimisch 4 - not in REACH dossier

Data discussed by EAA and available data (developmental toxicity)*

Teratology study in rats exposed by inhalation (0.018, 0.059 and 0.18 mg/l) from GD 6 – 15)	A higher rate of malformations not discovered. Fetal weights were lower in the highest dose group (no mention of body weight gain by the dams). NOAECs for maternal toxicity and teratogenicity found to be 0.18 mg/l air.	Snellings et al. (1982b)	Klimisch 2
One-generation reproductive toxicity in rats	Significantly fewer pups born per litter in highest dose group. Number of implantation sites in the high dose group significantly lower. Longer gestation period, but considered not relevant, because the gestation length was within the normal range for this strain.	Snellings et al. (1982c)	Klimisch 2
Teratology study in rabbits by inhalation (0.27 mg/l) from GD 1-19 or 7-19.	No evidence of maternal toxicity, adverse effects on development, or structural malformations. The NOAECs for maternal toxicity and teratogenicity was found to be 0.27 mg/l.	Hackett et al. (1982)	Klimisch 2

Reproductive-toxicologic assessment of EO by inhalation exposure (150ppm) of rats and rabbits	No mortality in nonpregnant or pregnant females of either species, but maternal rats showed toxic signs at necropsy. Body weight reduced only in the rats exposed before and during gestation, but the spleen and kidney weights increased in all three exposed groups. Rabbits appeared to be unaffected.	Hardin et al. (1983)	Klimisch 4 - not in REACH dossier
EO administered intravenously to mice	<u>Not</u> a relevant route of exposure!	LaBorde et al. (1980)	not in REACH dossier
Short duration exposure (inhalation) of rats (0, 400, 800, 1200 ppm)	Only effect observed is reduced fetal weight in combination with a reduced body weight of the dams.	Saillenfait et al. (1996)	not in REACH dossier
Dose-rate effects of EO exposure on developmental tox in mice	Increased fetal death, reduced fetal weights, increased eye malformations and decreased crown-rump length in combination with reduced maternal body weights in all groups.	Weller et al. (1999)	not in REACH dossier
EO developmental tox study of maternally inhaled vapor in rats (0, 50, 125, 225 ppm)	Only mild developmental delay effects occurred secondary to maternal toxicity.	Neeper-Bradley et al. (1993)	not in REACH dossier
EO treatment of the murine zygote	Reduction in the number of live fetuses, and malformations observed after 1 and 6 hours post-mating exposures. No information on maternal toxicity available.	Rutledge et al. (1989)	not in REACH dossier

*according to a literature search performed in September 2016

In addition: Four epidemiology studies.

Conclusion EAA:

The available data show that ethylene oxide is toxic to male reproductive organs and affects pregnancy outcomes (reduced number of implantations) in female animals at concentrations of 100ppm and above. Developmental Toxicity (increased number of resorptions, reduced number of pups born, reduced fetal body weights, reduced length and variations in ossification, skeletal malformations (cervical/thoracic) and malformation of the eye) occurred in the same order of magnitude. Additionally some effects (increased spontaneous abortions) were also reported in humans, though these data in humans have some deficiencies (insufficient information on exact exposure). The available knowledge on differences in metabolism among different species, including man, is considered insufficient to conclude that the reproductive toxicity seen in several animal species is not relevant for humans.

Overall it can be concluded that EO has the potential to affect male reproductive organs and female fertility and a potential for developmental toxicity cannot be excluded. These effects are not considered attributable to secondary unspecific toxicity. However, as there are some uncertainties related to the data base, a classification in Category 1B appears not justified, but Category 2 (suspected human reproductive toxicant) is proposed. Ethylene oxide should be classified as Repr.2, H361fd.

Conclusion Consortium

The EAA summarized 19 publications to draw a conclusion on the reproductive potential of EO. These publications can be split in four categories: effects on the testis, effects on the female fertility,

effects on development, and data obtained in humans. In general, the EAA is quite conservative regarding the effects observed. In several cases effects are classified as adverse by the EAA, while the authors of the publication consider the effects not relevant. Therefore, we re-evaluated all data brought forward by the EAA and determine whether their conclusions are justified.

Male fertility

Six publications address the toxicity of EO to the male fertility by investigating sperm parameters and the testes: Mori (1989), Mori (1991), Ribeiro (1987), Snellings (1982c), Snellings (1984), Hollingsworth (1956) and Lynch (1984). A major limitation of all these studies is the amount of information available on the general toxicity. As according to section 3.7.2.1.1 of Annex I of Regulation (EC) No. 1272/2008, a substance can be classified when reprotoxic effects are observed in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effects on reproduction is not a secondary non-specific consequence of other toxic effects. When information on general toxicity is absent, according to this section no conclusion can be drawn whether the reprotoxic effects are sufficient for classification. The EAA concludes that EO has the potential to affect male reproductive organs and below we evaluate the articles on which effects the conclusions are based by discussing every publication.

The EAA states that several relevant effects were observed in **Hollingsworth et al. (1956)**. However, the study by Hollingsworth is so poorly described regarding methods and observed effects that any solid conclusion cannot be drawn based on this study and this study should be disregarded. The consortium rated this study with a Klimisch 4 (not assignable) and did therefore not include it in the REACH dossier.

Reported in a short communication only, **Ribeiro (1987)** exposed male Swiss Webster mice 6 hours per day for 5 days to 0, 200 or 400 ppm EO and 1, 3, and 5 weeks after the beginning of exposure and studied the cauda epididymal sperm morphology. It was concluded by the authors that EO increased the frequency of abnormal sperm cell in a dose-dependent matter at both EO dose levels and all time points examined which may correlate with the mutagenic potential of EO. This effect was considered relevant by the EAA. However, if EO was to affect spermatogenesis, according to the CLP criteria (2015) its effect should be observed (...) *in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effects on reproduction is considered not to be a secondary non-specific consequence of the other toxic effect*. It is not clear if the abnormal sperm morphology is the result of a mutagenic or fertility effect. Therefore, the toxicological relevance of the effects described in this study is ambiguous.

Snellings (1984) exposed B6C3F1 mice to EO vapor at concentrations of 0, 10, 48, 104, and 236 ppm, 6 hours per day, 5 days a week for 10 (males) or 11 (females) weeks. Several effects were observed in the highest dose group and included minimal changes in certain erythroid parameters, increased liver weight, decreased testicular weight and decreased spleen weight which was noted also in the 100 ppm group. In addition, locomotor effects were observed at all dose-levels. However, there are no clinical or histopathological findings on organ weight to support or explain any of these apparently treatment-related effects. Furthermore, as the percentage of the decrease in testicular

weight is not reported, the severity of this effect is unknown. In contrast to what EAA in table 54 of the CHL dossier states, the NOAEC for fertility should be set at the highest dose tested.

Lynch (1984) exposed *Cynomolgus* monkeys to 50 or 100 ppm EO 7 hours a day, 5 days a week for 24 months. In the 100 ppm exposed group weight gain decreased significantly in comparison to the control group. Exposure to EO did not induce changes in the urinalysis, clinical chemistry, hematology, or electrocardiographic indices of the monkeys. Decreased nerve conduction velocity measurements were obtained from 2 of the 12 monkeys. Several other effects on neurotoxic parameters were also found. Sperm counts and sperm motility were significantly reduced in all exposed monkeys. These effects are considered relevant by the EAA. However, no clear dose-response could be observed and no macroscopic or histopathological examination of the testis was performed. Therefore, the toxicological relevance of these effects is unknown.

Snellings (1982c) exposed Fischer344 rats to EO vapor at 10, 33, or 100 ppm during a 12 week pre-mating period and during mating in a one-gen range-finder study to the follow-up 2-gen reproductive toxicity study where more parameters were gathered. The percentages of males proven fertile are lower for the 100 ppm exposure group compared to control, but not statistically significant. Sperm parameters were not investigated and no histopathology was performed. This study indicates that EO does not affect male fertility, but also this study has limitations in the study of male fertility effects.

Mori (1991) exposed male Wistar rats to EO at concentrations of 50, 100, or 250 ppm for 6 hours a day, 5 days a week for 13 weeks. No effects on body weight were observed. The testicular weights did not change, but the epididymal weights decreased in the 250 ppm group and epididymal sperm counts were reduced in the same group. In the testis slight degenerations in the seminiferous tubules was observed. LDH X activity in the testis was not affected. The number of immature heads and abnormal sperm heads was significantly increased in the 250 ppm group, but the teratic type sperm head was increased at all dose levels, but dose independent. This makes the assessment of the sperm morphology questionable and no final conclusion can be drawn. Overall, several parameters regarding testicular toxicity were investigated in this study and the effects observed were limited in severity and subsequently toxicological relevance is unknown.

Mori (1989) exposed male Wistar rats to EO at a concentration of 500 ppm, 6 hours per day, 3 days a week, for 2, 4, 6, or 13 weeks. Testicular toxicity was investigated. Body weight gain was not affected. The relative weights of the testes and the epididymides decreased in a time-dependent manner. This is a consistent effect seen in both Mori studies. Degeneration and exfoliation of germ cells were observed at all exposure durations. Some seminiferous tubules showed germ cell recovery at 13 weeks compared with 6 weeks.

Concluding, there is overall limited information on the effect of EO on male fertility due to limitations in method and reporting of the data in most studies. The EAA reported all effects without considering the validity of the study and the severity of the effect. We are of the opinion that a lot of badly performed studies do not make one good one. When individually considering the validity of the studies, the amount of evidence on testis effects is limited. Especially as there is almost no information available on other toxic effects at the concentrations tested. The results of Rebeiro

(1987) are questionable. Effects were observed in the studies by Lynch (1984) and Snellings (1984), but without additional information the toxicological relevance of these effects is unknown. The only studies which do show an adverse effect on the testis are the studies by Mori (1989 and 1991). In the study by Mori (1991) slight degenerations in the seminiferous tubules of the testis was observed, but this was only a slight effect and the toxicological relevance is unknown. In the study by Mori (1989) degeneration and exfoliation of germ cells were observed, but with recovery after a longer exposure period. However, sperm and epididymides effects seem to be consistent in both studies and more sensitive parameters than the effects in the testis. Based on all available information no robust conclusion can be drawn whether EO affects male fertility.

Female fertility

Three relevant studies investigating female fertility are discussed below.

Generoso (1987) exposed female mice either once for 1.5 hours at 1200 ppm EO 1, 6, 9, or 25 hours post-mating, or to 1200 ppm EO for 1.5 hours per day for 4 consecutive days before mating or to 300 ppm EO for 6 hours per day for 10 exposures over a 14-day pre-mating period. Mice were killed on day 17 of gestation. The number of implants per female was significantly reduced at 300 ppm but not at 1200 ppm. No information is available on other toxic effects to the mice. It is therefore difficult to conclude whether the observed implantation loss is a primary result of EO exposure or a secondary effect. Especially as the LC50 in mice was determined to be 600 ppm, severe effects on the dams are to be expected.

Hardin B.D. (1983) exposed rats to 150 ppm EO 7 hours a day three weeks before mating and during gestation day 7 to 16. Body weight was reduced and spleen and kidney weights (absolute and relative) were increased. A statistically significant increase in the incidence of resorptions in the rats was observed (not in the rabbits), but not in the number of implants or the number of pregnant animals. The limitation in this study is that no absolute numbers are reported. The paper is much like a review paper with no actual data (i.e. group means, SD, etc) presented for the relevant endpoints. Considering the fact that resorptions are significantly increased, but the number of live fetuses has not changed, absolute numbers of resorptions are necessary to consider the relevance of the observed effect. As in the end the number of fetuses has not been decreased, the conclusion should be drawn that no effect on fertility was observed in this study.

* In table 54 of the CLH dossier, the EAA states that resorptions are increased in rats of group 3. This has to be group 4. This is essential, because group 3 is a developmental toxicity study while group 4 exposed animals pre-mating and during gestation.

Snellings (1982c) exposed Fischer344 rats to EO vapor at concentrations of 10, 33, or 100 ppm 6 hours per day for 5 to 7 days a week during a 12 week pre-mating, mating, gestation, and day 5 to 19 post-partum in a one-gen rangefinder study to the follow-up 2-gen reproductive toxicity study where more parameters were gathered. The percentages of females pregnant of the 100 ppm group were lower but not statistically significant compared to the control groups. Furthermore, the median number of implantation sites per pregnant female in the 100 ppm exposure group was 6, which is significantly lower than the other groups with a median between 10 and 11. Resorptions were not

different between groups, but there were reduced number of implantations, the number of pups born and litter sizes were significantly lower. This study suggests that EO affects fertility.

Concluding, three different studies are available on female fertility. One of them does not show an effect on fertility, while the two others do. One of these studies has limitation in the method as no information is available on toxic effects on the dams besides fertility effects. Nevertheless, the study by Snellings (1982c) shows that fertility is impaired in females due to ethylene oxide exposure.

Developmental toxicity

Eight studies are available investigating developmental toxicity. However, **LaBorde (1980)** exposed CD-1 mice intravenous to EO. This is not a relevant route of exposure and should not be used for classification purposes. As indicated in the CLH report of the EAA, 75-80% of EO is taken up by the body after inhalation in humans, but in mice and rats only 40 and 43% respectively is absorbed. Furthermore, metabolism at the first site of contact is via intravenous exposure not taken into account.

Hardin (1983) exposed rats and rabbits to 150 ppm EO 7 hours a day for three different time periods: (1): During gestation day 7 to 16 (rat) or 7 to 19 (rabbit). (2) During gestation day 1 to 16 (rat) or 1 to 19 (rabbit). (3) three weeks before mating and during gestation day 7 to 16 (rats only). In rats body weight was reduced in rats exposed before and during gestation. Spleen and kidney weights (absolute and relative) were increased in all three groups. A statistically significant increase in the incidence of resorptions was only observed in the rats exposed before and during gestation indicating that this is not a developmental effect. Fetal body weight and crown-rump length were reduced in all exposed groups (rats). All these effects observed were not quantified and therefore the severity of the effects cannot be determined. External, visceral, and skeletal examinations revealed no treatment-related effects other than an increased incidence of reduced skeletal ossification (primarily of the skull and sternbrae) in all exposed groups, but delayed ossification variations secondary to maternal toxicity (decreased body weight) does not warrant reproductive toxicity classification. Rabbits were unaffected.

Snellings (1982c) exposed Fischer344 rats to EO vapor at 10, 33, or 100 ppm for 5 to 7 days a week during a 12 week pre-mating, mating, gestation, and day 5 to 19 post-partum in a one-gen rangefinder study to the follow-up 2-gen reproductive toxicity study where more parameters were gathered. Males were no longer exposed after mating. According to the authors, the major treatment-related effect was significantly fewer pups born per litter in the 100 ppm dose group. In addition, the number of implantation sites in the high dose group was significantly lower compared to controls. A longer gestation period was observed, but this was considered not relevant, because the gestation length was within the normal range for this strain (although historical control data were not given in the report).

Snellings (1982b) exposed pregnant Fischer 344 rats 6 hours per day to either 10, 33, or 100 ppm EO on day 6 to 15 of gestation. No treatment-related effects of ethylene oxide were noted for maternal survival, litter size, number of implantations and resorption sites, and number of preimplantation losses. At 100 ppm, a significant depression of body weight in the fetuses was observed, but no

effects on malformations or other embryonic effects. The authors of the study conclude that the NOAEL is 100 ppm, while the EAA states that the NOAEL is 33 ppm. As the body weight of the dams is not reported it is unclear whether the body weight of the dams was reduced and partly explaining the reduction in fetal weight. As fetal weight reduction is the only parameter affected, the toxicological significance of this effect is questionable. However, other studies with EO did demonstrate maternal toxicity (reduced body weight and body weight gain and decreased feed consumption) suggesting that these fetal effects would be secondary to maternal toxicity.

Sailienfait (1996) exposed Sprague -Dawley rats via inhalation from gestation day 5 to 16 to 0, 400, 800, or 1200 ppm EO for 0.5 hours once a day or to 0, 200, 400, 800, or 1200 ppm EO for 0.5 hours thrice a day. Single exposure did not result in adverse effects. Repeated exposure resulted in reduced fetal weight at 800 and 1200 ppm and statistically reduced body weight in the dams at 1200 ppm. The dams of the 800 ppm also showed a reduced body weight gain of 19% and dams of the 400 ppm group showed a reduction of 23% compared to the respective controls. No effects on percentage pregnant females, number of implantations and resorption sites, live or dead fetuses were observed. In addition, no malformations were observed in any group. As the only effect observed is reduced fetal weight in combination with a reduced body weight of the dams it can be concluded that this study does not indicate that EO is a developmental toxicant.

Weller (1999) tried to determine whether Harber's Law is valid by exposing pregnant C57BL/6J mice on gestation day 7 via inhalation for 1.5, 3, or 6 hours resulting in exposure of 2100 or 2700 ppm-h EO. Maternal examinations were limited and only included behavioral changes 30 minutes and 24 hours after exposure and weighting being reported one day after exposure. Severe maternal effects were observed in all exposed groups. For instance mortality was observed in almost all dose groups. No mortality was observed in the 700 ppm x 3hour, 350 ppm x 6 hour and 450 x 6 hour group. Although limited data on maternal toxicity is available, mortality indicates that the other animals from the same group who survived experienced severe adverse effects occurred which quite likely affect the development of the fetuses and therefore these data cannot be used. In the four groups without mortality, increased resorption of the 700 ppm x 3 hour and 450 x 6 hour group, and increased incidences of eye defects in the 700 ppm x 3hour group was observed. Still, maternal toxicity is not known on these groups and therefore this study cannot be used for classification purposes.

Neeper-Bradley (1993) exposed pregnant CD rats to 0, 50, 125, 225 ppm EO for 6 hours per day on gestation day 6 to 15. Effects on the dams included reduced body weight gain in the 50, 125 and 225 ppm groups, reduced food consumption in the 250 ppm group, and increased relative maternal liver weights in the 225 and 125 ppm groups. Developmental toxicity included dose-dependent reduced fetal body weights of 4, 5 and 10% of control values in the 50, 125, and 225 ppm groups, respectively. In addition, skeletal variations were observed in the 125 and 250 ppm dose groups, but as these are mostly delayed ossifications variations in combination with a slight reduced fetal body weight these are not considered to be toxicological relevant. Based on the mild developmental delay effects observed it cannot be concluded that EO is a developmental toxicant as these occurred secondary to maternal toxicity. Thus, no reproductive classification is warranted.

Rutledge (1989) exposed female mice to 1200 ppm EO by inhalation 1, 6, 8, or 25 hours after mating or 1800 ppm 6 hours after mating for 1.5 hour. Effects observed were a reduction in the number of live fetuses, and malformations were observed after 1 and 6 hours post-mating exposures. No information on maternal toxicity is available and therefore it is not possible to conclude that the developmental effects observed are not secondary to maternal toxicity; however, other studies did note maternal toxicity at these extremely high doses. Especially because for mice the 4-hour LC50 is 660 ppm according to the CLH dossier of the EAA. Applying Harber's rule, 1200 ppm for 1.5 hours is equivalent to 450 ppm for 4 hours. The exposure in the study by Rutledge is close to the LC50 and most likely severe effects were observed in the dams. Due to limitations in reporting this study cannot be used for classification purposes.

Based on all evidence it can be concluded that EO does not cause structural abnormalities in the offspring. The effects on fetal body weight and delayed ossification were typically observed secondary to maternal toxicity. The only toxicological adverse effect observed is the reduction in implantation sites and resorptions observed by Hardin (1983) which was rather designed as a fertility study instead of a typical developmental toxicity study and Snellings (1982c). As this decrease in resorptions was absent when females were not exposed before gestation, this effect is not considered to be of developmental nature.

Human data

Four epidemiology studies are available investigating the relation between EO exposure and spontaneous abortions. In the study by **Hemminki (1982)**, female Finnish hospital staff was investigated. Information on several confounders was obtained by a questionnaire. Information about exposure to chemical sterilizing agents was obtained from supervising nurses. However, no information is available on the concentration. Information on spontaneous abortions was obtained from the questionnaires and from the hospital discharge register. For females exposed to EO during pregnancy an adjusted rate of 16.1% spontaneous abortions were reported compared to 7.8% in the females exposed to EO, but not during pregnancy. This effect was statistically significant. In the study by **Rowland (1996)**, 7000 female dental assistants were sent questionnaires and the parameters spontaneous abortions, pre-term births and post-term births were analyzed. Via several statistical analysis, the authors tried to find a statistical significant effect, but after correcting for all confounders no effect was observed 2.1 (95%CI= 0.7-5.7.). **Lindbohm (1991)** studied the relationship between paternal exposure to EO and increased risk of spontaneous abortion. The exposure status was based on occupational titles and industry without further specification of exposure levels. A total of ten pregnancies were identified and of these ten pregnancies, three spontaneous abortions were reported. The odds ratio was determined to be 4.7 (95% CI = 1.2-18.4). However, only correction for age was performed. Therefore these results are not considered to be reliable. In addition, the size of the cohort should have been too small to draw a final and reliable conclusion. **Gresie-Brusin (2007)** compared low exposed versus high exposed females in sterilizing units of hospitals and pregnancy outcomes of the last pregnancy in women currently employed in these units. Exposure was assessed by walk-through surveys, measurements of the concentrations of EO in sterilizing units at the time of the study and questionnaire-collected data. The difference between low and high exposure was based on type of work. Several relevant confounders were taken into account. There was a significant increase in risk of spontaneous abortion (POR = 20.8, 95% CI = 2.1–199) and pregnancy loss (POR = 8.6, 95% CI = 1.8–43.7) for pregnancies highly exposed to EO

compared to low exposed pregnancies. No associations were found between exposure to EO and stillbirth.

In general, human data is rarely usable for risk assessment due to limitations in study design. Nonetheless, the studies by Hemminki (1982) and Gresie-Brusin (2007) were quite well performed and they indicate that exposure to EO could result in an increase in spontaneous abortions. However, for both studies the exact exposure concentration is unknown.

Overall conclusion

Several studies show (indications for) reprotoxic effects of EO but if hazard assessment is solely based on publications in the public literature one should be careful interpreting these data. The approach of EAA is to combine all effects observed without judging the reliability of the individual studies in which the effects are reported. Our approach was to evaluate every single paper and conclude whether the effects observed in each paper could be attributed to exposure to EO and not be secondary to other toxic effects.

The EAA concludes that EO affects male reproductive organs and affects pregnancy outcomes (reduced number of implantations) in female animals at concentrations of 100 ppm and above and affects developmental toxicity (increased number of resorptions, reduced number of pups born, reduced fetal body weights, reduced length and variations in ossification, skeletal malformations and malformation of the eye). As the EAA does not take into account whether studies are performed adequately, several of effects they consider to be caused by EO are in fact not of toxicological relevance or the toxicological relevance is unknown.

Regarding the endpoint male fertility, the effects on the testis reported in the discussed studies are limited to slight degenerations in the seminiferous tubules and reversible germ cell degeneration. The only adverse effect caused by EO exposure is the reduced number of implantations in one study out of three available on the endpoint female fertility. One of them does not show an effect on fertility and the second one of those showing effects has clear limitations in the method as no information is available on toxic effects on the dams besides fertility effects. Therefore, taking into account the overall weight of evidence on the endpoint fertility, a classification of EO as Repr. 2, H361f is not overall conclusive.

In the developmental studies no relevant adverse effects were observed after exposure to EO. Thus, no classification for developmental toxicity is appropriate.

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