Committee for Risk Assessment

RAC

Opinion
on gallium arsenide in relation to toxicity to reproduction

ECHA/RAC/XXXXXXXXXX

May 29, 2013 revised draft

This document is a working document from the Committee for Risk Assessment of the European Chemicals Agency (ECHA) intended for internal use only. It has not been agreed and/or adopted by the Committee. In addition to Committee participants the document may only be disclosed to experts and advisors for the purpose of facilitating the work of the Committee.

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

Xx xx 2013
ECHA/RAC/AXX

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT

ON GALLIUM ARSENIDE IN RELATION TO TOXICITY TO REPRODUCTION

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on whether information on toxicity to reproduction submitted during the public consultation on carcinogenicity and the additional information submitted by Eurometaux in December 2011 would change the RAC opinion adopted on 25 May 2010 (Annex 1) recommending harmonised classification and labelling of gallium arsenide (CAS No. 1303-00-0) as toxic to reproduction Cat. 1B according to Regulation (EC) 1272/2008 on harmonised classification, labelling and packaging of substances and mixtures[1].

I. PROCESS FOR ADOPTION OF THE OPINION

Following a request from the European Commission, in the mandate of 21 December 2011 and the revised mandate of 17 April 2012 attached as Annex 2, the Executive Director of ECHA asked the RAC to evaluate the information on toxicity to reproduction submitted during public consultation on carcinogenicity and take into account also information submitted by Eurometaux in December 2011 in order to decide whether the previous opinion on the proposed harmonised classification and labelling for reproductive toxicity of gallium arsenide should be revised and to draw up an opinion accordingly.

II. ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Helmut Greim**

Co-rapporteur, appointed by the RAC: **Lina Dunauskiene**

The RAC opinion was adopted on [*to be added*].

The RAC opinion was adopted by a [*to be added*].

III. OPINION OF THE RAC

The RAC has formulated its opinion on whether the information on toxicity to reproduction submitted during the public consultation on carcinogenicity (11 March 2011 – 27 April 2011) and the information provided in the Additional Information Report (AIR) attached as Annex 4 would lead to the revision of the previous opinion adopted on 25 May 2010 which supported the proposal to classify gallium arsenide as toxic to reproduction in category 1B (according to the CLP Regulation).

The draft opinion was subject for a public consultation starting at [*to be added*]. Parties concerned and MSCAs were invited to provide comments until [*to be added*].

After examination of the information on reproductive toxicity submitted during the public consultation on carcinogenicity and in the Additional Information Report (AIR), the Committee reviewed its conclusion regarding the classification of gallium arsenide (GaAs) for reproductive toxicity in its opinion of 25 May 2010. The Committee maintains its view to classify

[1] CLP Regulation

gallium arsenide (CAS No 1303-00-0) as toxic to reproduction, Cat. 1B, H360F, according to the CLP Regulation.

This opinion is based on the following information:

Studies in mice, rats, and hamsters have all shown testicular toxicity after exposure to GaAs via inhalation and/or intra-tracheal installation. The testicular effects include decrease in testis weights, epididymis weights, spermatid counts, spermatozoa motility and increased testicular atrophy (mice and rats). These effects are clear-cut, clearly adverse and observed in two or more animal species. The effects are likely to cause reduced male fertility, although functional fertility has not been studied.

Testicular toxicity is observed also in animals which show no general clinical symptoms or reduced body weights. However, the lungs are a more sensitive target organ, and signs of lung toxicity are noted at lower GaAs concentrations than those causing testicular toxicity. In mice and hamsters, the signs of lung toxicity are not very severe at the concentrations where signs of testis toxicity appear (e.g., mild alveolar proteinosis in mice and mild and/or moderate inflammatory changes in the lungs of hamsters and mice). At the highest concentrations in mice where moderate testicular atrophy occurs, only a moderate alveolar proteinosis and a slight reduction in Hb (4-6%) are reported. Also, marked alveolar proteinosis is observed in rats at concentrations which do not produce any significant testicular toxicity (such as testis atrophy), reduced Hb, body weight gain or any clinical symptoms. Thus, no correlation between occurrence and severity of the lung toxicity and testis toxicity is shown. There is therefore no evidence for the testicular toxicity being secondary to other toxic effects in these studies.

The RAC is of the opinion that there is not sufficient evidence to support the mode of action (MoA) for the testicular toxicity proposed in the AIR (i.e. "lung effects being the primary effects of GaAs exposure leading to haematological changes and hypoxaemia triggering the testicular effects").

Other potential modes of action (MoAs) for the testicular toxicity have also been discussed by the RAC. One included a potential direct toxic action of Ga and/or As in the testes based on data on distribution of gallium or arsenic ions to the testes. The RAC has concluded that the available data base does not allow conclusions on which single MoA or possible combination of MoAs is responsible for the testicular toxicity.

IV. SCIENTIFIC GROUNDS FOR THE OPINION

Conclusions of the RAC opinion of 25 May 2010

No multi-generation studies investigating potential effects of gallium arsenide on fertility are available but repeated dose toxicity studies have reported data on reproductive organs. There are two 8-week tracheal instillation studies in rats and hamsters, and two 14-week inhalation studies in rats and mice. Several concentration-related testicular effects, such as testis atrophy, decreased testis and epididymis weights, spermatids counts and spermatozoa motility, have been observed in the whole-body inhalation studies on gallium arsenide in rats and mice. Similar testicular effects have also been reported in rats and hamsters following intratracheal instillations. Histopathological examinations of the testes in rats and hamsters revealed a spermiation failure as spermatid retention was observed at post-spermiation stages for both species.

The RAC noted that the minimal microcytic responsive anaemia (erythrocytosis and increased zinc protoporphyrin levels) in rats and mice in the 14-week inhalation studies would be consistent with iron deficiency or iron deficiency-like disorders.

Clear effects observed in the testes were considered by the RAC to be primary, as effects such as reduced epididymal spermatozoal concentration in mice exposed to 10 mg/m^3 were seen in the absence of a clinically significant reduction in haemoglobin concentration or reduced body weight. Also at higher concentrations, the effects on the testes were not considered to be the result of other toxic effects. This was supported by the potential of gallium to accumulate in the rat testes following inhalation exposure. Thus, due to the clear evidence of testicular toxicity in two species, the original proposal to classify gallium arsenide as Repr. 1B - H360F (CLP) was supported.

Effects on development of the offspring and effects on or via lactation were not evaluated.

Summary of information submitted during the public consultation on carcinogenicity and in the Additional Information Report (AIR)

During the public consultation on carcinogenicity, a significant quantity of information was submitted by parties concerned. Despite the clear scope of the consultation, some contributions also covered toxicity to reproduction. Assessment of the information provided during the PC on carcinogenicity is described in RCOM (Annex 3).

Furthermore, additional information related to reproductive toxicity of gallium arsenide was submitted in the AIR.

The additional information contained one toxicity study on GaAs *per se*. This study was a repeated dose toxicity study by Tanaka et al (2000) in which additional results of the hamster study by Omura 1996a were reported. These results showed lung toxicity in hamsters after 8-week intratracheal instillation of GaAs.

There were also two toxicokinetic studies on GaAs, in which Ga and As concentrations in the testes were measured in rats and mice following 12-day whole body inhalation exposure of GaAs (Mast studies in rats and mice, 1990).

The other studies were not on GaAs *per se*, but concerned: a correlation between hypobaric hypoxia and testis effects, human chronic obstructive lung diseases and testis effects, association of arsenic in blood and semen parameters in men visiting infertility clinics, some toxicokinetic data on other gallium compounds and finally, testis toxicity of gallium compounds following other routes of exposure than inhalation.

Outcome of this RAC assessment

Three main issues were identified by the Committee for consideration:

1) **Potential retention/accumulation of gallium and/or arsenic in the testes**
2) **Testis toxicity as a secondary non-specific consequence of other toxicity?**
3) **Potential modes of action (MoAs) of GaAs**

1) Potential retention/accumulation of gallium and/or arsenic in the testes

In the RAC opinion on CLH of gallium arsenide of May 2010, a potential of Ga to accumulate in the rat testes based on the data from a 2-year inhalation study (NTP 2000) was considered as supporting evidence for classification.

Industry provided additional studies related to gallium and/or arsenic distribution and/or retention in the body/testes (Mast 1990 (a, b); Ando, 1986; Engelstad et al., 1982; Ishii et al., 2011; Jonkhoff et al., 1995) and argued against any remarkable retention/accumulation of gallium in the testes and suggested that neither gallium nor arsenic are directly contributing to the testicular effects reported in mice and rats

after sub-chronic inhalation exposure. The RAC has evaluated these studies in relation to the potential distribution of Ga and/or As to the testes.

In the NTP-studies, analysis of Ga and As concentrations in the testes were only performed in the 2-year rat inhalation study. After 18 months, 1.5 µg Ga/g of testis, was found at the highest concentration studied (1 mg GaAs/m^3). This corresponded to a 30-fold increase in Ga concentration in the testes versus the whole blood. The As concentrations varied highly and did not seem to clearly accumulate during the exposure period. GaAs exposure up to the highest dose of 1 mg GaAs/m^3, did not cause any statistically significant testis toxicity in the rat (limited testis parameters were investigated in this 2-year carcinogenicity study).

No chemical analysis were performed in the 14-week NTP studies in which testicular toxicity were observed in rats and mice at higher exposure levels of GaAs than in the 2-year study. However, Mast (1990a) studied the accumulation of Ga and As in rat testes after 12 days of inhalation exposure to 0-75 mg/m^3 of GaAs, and found a dose related increase in concentrations of both Ga and As in the testes (17-fold and 5.5-fold relative to the detection level, respectively). In the mouse testes As was not found above detection levels after 12-day inhalation exposure of GaAs (0-37 mg/m^3) (Mast 1990b). However, inconsistent results were obtained for Ga at the highest exposure concentration (0.88 and <0.15 ug Ga/g testes in the 2 analysed samples). At lower exposure concentrations Ga concentrations in the testes were below detection levels.

A single intravenous injection of 0.4 ml Ga^{67} citrate per animal (10 – 100 µCi; weight of animals not specified) into Wistar rats resulted in the highest testis concentration at 3 hours, when 0.7% of the dose/g of tissue was found in the testes (Ando et al. 1986). In the testes the retention values remained at about 0.4% after 24 hours until the end of the observation period at 10 days. In the bone, spleen, liver, kidney and the adrenal glands, the retention values were generally higher (0.8-4% of the dose/g of tissue). The testes are clearly exposed to Ga, but Ga is retained in the testes to a lower extent than in some other organs.

Chiou et al. (2008) studied the effects of 15 subcutaneous administrations (during a period of 3 weeks) of As_2O_3 in mice, and observed a dose-dependent increase in As concentrations in the testes (40, 64 and 182 ppb As at 0, 0.3 and 3 mg As_2O_3/kg/day) as well as in testicular toxicity (inhibition of spermatogenesis).

Pant et al. have studied testicular toxicity and accumulation of As after administration of soluble sodium arsenite ($NaAsO_2$) to mice via the drinking water for 35 days (0-534 µmol $NaAsO_2$/l) (Pant et al., 2001) or for 365 days (53 µmol $NaAsO_2$/l) (Pant et al., 2004). After 35 days the concentration of As in the testes increased to 5.3 mg/kg in the highest dose group. In the 1-year study the concentration of As in the testes was 6.5 mg/kg. In both studies, evidence of testicular toxicity was observed at these concentrations of As in the testes.

The studies by Engelstad et al. (1982), Ishii et al. (2011) and Jonkhoff et al. (1995) describe scintigraphic results from humans injected intravenously with a single dose of radiolabelled gallium citrate. However, the testes are not mentioned in these studies, and the RAC is of the opinion that these scintigraphs are not helpful in determining potential (lack of) distribution to, and accumulation of gallium in the testes.

The overall view of these studies is that both gallium and arsenic ions are distributed to the testes, but that neither ion is retained or accumulated in the testes to a higher extent than to some other organs. The RAC concludes that these ions are distributed to the testes, where they potentially could directly contribute to testis toxicity.

2) Testis toxicity as a secondary non-specific consequence of other toxicity?

In the RAC opinion of 25 May 2010 it was concluded: "*The effect on testis is considered to be primary, as it is seen as reduced epididymal spermatozoal concentration in mice exposed to 10 mg/m^3 without clinically significant reduction in haemoglobin concentration or reduced body weight.....Also at higher doses the effects were considered to be primary and not resulting from other toxic effects"*

The comments from the submitter of the AIR suggested that the testicular effects may rather be a secondary effect to other toxic effects, i.e. a consequence of lung damage. Industry has provided the view, that there were rather severe effects on the lungs at and below the concentrations affecting sperms and the testes in the NTP (2000) inhalation studies on rats and mice.

It has been further recommended by Industry to consider some specific references (Webb et al., 1984, 1986, & 1987, and Goering et al. 1988) where lung effects of GaAs and/or gallium and arsenic retention in the lung were studied. However, since the testicular toxicity was not evaluated in these studies, they are not helpful in the further assessment of the mechanism behind the testicular toxicity, and will not be discussed in this opinion.

The communications by Omura et al. (1996a, 1996b) on the studies in hamsters and rats, respectively, which have been evaluated by the RAC in its previous opinion (March 2010), describe adverse effects of GaAs on the testes in both species, but without presenting information on other organs. During the public consultation on carcinogenicity the study by Tanaka et al. (2000) was identified, which reports effects seen in other organs of the Omura (1996b) study in hamsters. The authors describe that at a GaAs dose of 7.7 mg/kg given intra-tracheally, twice a week for 8 weeks, an almost twofold increase in lung weights was observed with moderate pneumonitis, mild exudation, mild thickening of pleura, mild fibrotic proliferation and mild alveolar or bronchiolar cell hyperplasia. Findings in other organs were slight to mild lesions in the convoluted tubules of the kidneys and slight centrilobular cytoplasmic vacuolar degeneration and decreased weight of the liver in the GaAs group in comparison to the control group. The RAC concludes that although several organs were affected by intratracheal exposure to GaAs, only mild to moderate lung effects were reported and hypoxaemia was not measured after GaAs exposure in hamsters by Tanaka et al. (2000). Accordingly, the new information from this study does not support the notion that the animals suffer from severe lung toxicity and hypoxia at exposure levels causing testis toxicity.

It was proposed that data from a study with indium arsenide could be helpful in clarifying the relationship between lung and testis toxicity. Thus, in the same studies in hamsters as mentioned above (Omura et al., 1996a and Tanaka et al., 2000), intratracheal administration of 7.7 mg/kg InAs, twice a week for 7 weeks, induced severe pneumonitis, exudation, thickening of pleura, fibrotic proliferation and alveolar or bronchiolar cell hyperplasia and from none to severe alveolar or bronchiolar cell hyperplasia with squamous cell metaplasia. Despite these severe lung effects, no changes were observed in the absolute testis and epididymis weight and in sperm counts which would have been expected if the testis effects were secondary to lung toxicity. Because no testis toxicity, but mortality (3/8), was observed in the Omura et al. (1996a) study following the InAs exposure, the authors performed the same experiment with a lower InAs concentration (4.0 mg/kg bw/day) twice a week for 8 weeks, and studied effects at end of the exposure as well as at 8, 16, 40, 64 and 88 weeks after the exposure period. Pulmonary effects were reported by Yamazaki et al (2000), and testis effects by Omura et al. (2000). At the end of the exposure period, this new study showed severe inflammatory responses in the lung with diffuse hyperplasia of bronchiolo-alveolar epithelium and mild interstitial fibrosis. Findings of 'localised hyperplasia of bronchiolo-alveolar epithelium with Periodic Acid-Schiff (PAS) positive mucinous exudation' were characterised as mild, whereas there were no findings of 'alveolar proteinosis-like lesions with PAS positive mucinous exudation'. At the end of the exposure period, the evidence of testis toxicity included

statistically significant decreases in testis weight, epididymis weight, and caudal sperm number (approximately 30%). During the 88 weeks follow up period, both lung and testis toxicity increased. The RAC concludes that also for indium arsenide, the animals suffered from both lung and testis toxicity; whether the testis toxicity is primary or secondary to lung toxicity cannot be assessed based on this study. It has been proposed by the submitter of the AIR and by Bomhard and Gelbke (2013) that the testis toxicity of GaAs is secondary to alveolar proteinosis. For InAs, testis toxicity occurs before any signs of alveolar proteinosis are observed. Later (e.g. week 16), the testis toxicity has become very severe while alveolar proteinosis is characterised as mild. The involvement of alveolar proteinosis in the testis toxicity of InAs is therefore not immediately apparent.

In the 14-week study by NTP, mice and rats were exposed by inhalation to 0.1, 1, 10, 37, 75 mg GaAs/m^3 for 14 weeks.

In male mice, mild hypospermia and testis atrophy started to occur at 10 mg/m^3, an exposure level that also caused lung effects. However, effects observed in the lungs were characterised as mild (e.g., with respect to alveolar proteinosis, hyperplasia, and inflammation). Relative lung weight was increased by 64%. In the AIR, alveolar proteinosis was proposed as the key effect mediating hypoxia and secondarily leading to testis toxicity. The RAC notes that at the dose where the testis toxicity was characterised as moderate testicular atrophy (37 mg/m^3), the alveolar proteinosis was described as only moderate (not marked). The RAC concludes that although lung and testis toxicity occur at similar dose levels in mice, there is no clear evidence in this study for the testis toxicity being secondary to the lung toxicity.

In male rats, decreased epididymis weights, hypospermia and testis atrophy were observed from 37 mg/m^3. Epididymal spermatozoal measurements revealed an about 7 and 19% reduction in motility at 10 and 37 mg/m^3, respectively, and an almost complete loss of mobility at 75 mg/m^3. Lung toxicity was described from concentrations of 0.1 mg/m^3, with all animals affected by alveolar proteinosis from 0.1 mg/m^3 and by alveolar histiocytic cellular infiltration from 1 mg/m^3. The alveolar proteinosis was reported as marked from 10 mg/m^3. Thus, at the dose where testis toxicity started to appear (10 mg/m^3), the relative lung weight was doubled and the lung pathology (alveolar proteinosis) marked. These results suggest that testis atrophy and hypospermia occur independently of alveolar proteinosis since at 10 mg/m^3 marked alveolar proteinosis was observed in the absence of testis atrophy and hypospermia. In the rat, the lungs are clearly more sensitive than the testes.

Erythrocytes and reticulocytes increased and mean red blood cell volume decreased dose dependently from 10 mg/m^3, followed by a decrease in haemoglobin and haematocrit values at 37 mg/m^3 and above. The effects were similar in mice (except that reticulocytes increased from 37 mg/m^3). There was an indication of haemolysis as shown by the occurrence of schistocytes at levels with increased erythrocyte counts and haemosiderosis in the liver at 37 and 75 mg/m^3 in rats and at 10 mg/m^3 and above in mice, in which it also occurred in the spleen. The reduction in haemoglobin concentration is considered by the RAC to have clinical relevance only in male rats at the highest dose (-13% Hb), and not in mice (only 5.6% reduction of Hb at the highest dose).

The haematological data could indicate a microcytic responsive anaemia, caused by iron deficiency or an iron deficiency-like disorder, leading to an increased haematopoiesis with insufficient haemoglobin synthesis.

The RAC notes that although there is moderate (in mice) or severe (in rat) lung damage at the highest exposure levels, the reduction in haemoglobin concentration is not very serious as the relatively small degree of anaemia would not result in hypoxia in the testes, and there are no signs in these studies of a clinically relevant hypoxaemia (e.g. pallor of mucous membranes, poor general health status, weakness, laboured breathing) or signs of hypoxia in the testes (e.g. no increase in

number of blood vessels or decrease in vessel diameter in the testis) or in other vulnerable organs. However, it is acknowledged that the oxygen tension of the blood has not been measured in any of the GaAs studies.

The US NTP (2000) conducted 16-day dose-finding studies and full 2-year inhalation studies on rats and mice. However, although rather extensive lung toxicity was observed in these studies (e.g., moderate alveolar proteinosis in all animals at 75 mg/m^3 in the 16-day study and moderate to marked alveolar proteinosis in almost all animals (49/50) at 1 mg/m^3 in the 2-year study), no clear and statistically significant testis toxicity was observed after the short or long term exposure to low concentrations of GaAs ($\leq$1 mg/m^3 GaAs).

Based on the NTP studies, the RAC concludes that the lung is a more sensitive target organ than the testes after inhalation exposure to low concentrations of GaAs, with signs of pulmonary effect occurring at lower concentrations than where signs of testis toxicity are apparent.

In summary, the evaluation of all available studies provides clear evidence that in hamsters, rats and mice the effects on testis and epididymal weights and spermatid counts, morphology and motility occurred in presence of other effects, especially in the lung. However, in mice and hamsters, the testis toxicity starts to occur at exposure levels where the signs of lung toxicity are not very severe. Unfortunately, the degree of hypoxaemia was not measured in the GaAs studies, so one can only speculate on the degree of hypoxia in the testes, if any, and its potential relation to testis toxicity. It is, however, quite clear to the RAC that there are very limited clinical signs of anaemia in the animals. Therefore, these studies, including the Tanaka study (2000), do not increase the understanding of the mechanism of action behind the testicular toxicity observed in mice and rats of the 90-day NTP-studies (NTP, 2000) or in hamsters (Omura, 1996b).

According to the criteria, substances with adverse effects on sexual function and fertility shall not be classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects (3.7.2.2.1. Annex I of the CLP Regulation). Therefore, adverse fertility effects should be evaluated against signs of marked systemic toxicity (CLP guidance 3.7.2.2.1.1). It is noted that in mice and hamsters the testis toxicity occurs in the absence of effects on body weights of the animals. There are signs of lung toxicity, but they are not very severe at the concentrations where signs of testis toxicity start to appear (e.g., mild alveolar proteinosis in mice and mild to moderate inflammatory changes in the lungs of hamsters). Also, marked alveolar proteinosis has been observed in rats at concentrations which did not produce testis atrophy or hypospermia, or reduced Hb or body weight gain or any general clinical symptoms. Although the design of the available studies does not permit a firm conclusion on the possible relationship between lung toxicity and testicular toxicity, the RAC is of the opinion that the data does not support that the testis toxicity is a secondary effect of lung toxicity.

When evaluating other toxic effects, the criteria also distinguish between reproductive toxic effects which are a specific or non-specific consequence of other toxic effects.

The submitter of the AIR speculates that it is a combination of alveolar proteinosis and microcytic anaemia that results in testis toxicity of GaAs (Bomhard and Gelbke, 2013). Lung toxicity does not normally result in testis toxicity in experimental animals. The RAC is of the opinion that there is not sufficient evidence to support such a mode of action (MoA), and should it be proven, it would qualify as a combination of rather specific effects and not a secondary, non-specific consequence of the other toxic effects.

3) Summary of potential modes of action (MoAs)

The testis is a very complex tissue, and it is therefore often difficult to establish modes of action (MoA) for testicular toxicants. For classification purposes, there is no need to know the MoA, as classification is based on the toxic effects. However, in some cases, there is sufficient information to demonstrate that the MoA is not relevant for humans, and in those cases the substance should not be classified. Information on MoA can also assist in deciding whether it is a direct effect on the organ or a "secondary non-specific consequence of other toxic effects" (3.7.2.1, Annex I, CLP). If the testis toxicity is solely produced as a non-specific secondary consequence of other toxic effect, classification is not warranted.

For GaAs, different MoAs have been discussed. Based on the available information, the RAC concludes that one or more of the proposed MoA(s), or even an unknown MoA might contribute to the testis toxicity. MoAs that have been proposed include hypoxia as a consequence of lung toxicity and direct toxic effects of GaAs or its ion(s) on the testes. The microcytic responsive anaemia could be an effect of an inhibition of delta-aminolevulinic acid dehydratase (ALAD), an enzyme involved in the heme biosynthesis pathway and shown to be inhibited by GaAs (Goering et al., 1988), and not a consequence of lung toxicity. Microcytic anaemia would be consistent with an iron deficiency or iron deficiency-like disorders in which iron was unavailable for the production of haeme. As gallium binds to transferrin and it is known that microcytic anaemia may develop in patients treated with gallium nitrate (Chitambar, 2010), the RAC considers the occurrence of a mild microcytic anaemia at the 10 mg/m^3 dose to be indicative of systemic toxicity.

These MoAs are discussed more in detail below. The RAC concludes that the available data indicates that the MoA for testis toxicity is relevant for humans and that there is not sufficient evidence to support that the testis toxicity could be a secondary non-specific consequence of other toxic effects, although some contribution of the lung toxicity to the testis toxicity cannot be fully excluded.

Potential impact of impaired lung function on the testes

One possible explanation for the testicular atrophy, reduced sperm counts and abnormal spermatids is GaAs-induced hypoxia. The comments provided by the submitter of the AIR propose a MoA involving impaired lung function, i.e., alveolar proteinosis resulting in less oxygen tension of the blood, and microcytic anaemia, which together would result in hypoxia of a sufficient degree to result in testis atrophy. According to the submitter of the AIR, the proposed MoA would indicate that the testis toxicity is a secondary effect caused by hypoxia and not a direct effect by GaAs, and therefore would not warrant classification according to them. This proposed MoA assumes that the exposure to GaAs causes sufficient alveolar proteinosis and anaemia to result in sufficiently severe hypoxia to affect the testes. However, there are no measurements of the oxygen tension in the experimental animals. The studies provided in the AIR only concern the second step of this proposed MoA, and show that severe hypoxia will indeed result in testis toxicity in humans (Semple et al. 1984; Gosney 1987; Aasebø et al. 1993; Verratti et al. 2008) and experimental animals (Shevantaeva and Kosyuga 2006; Gasco et al. 2003; Liao et al. 2010; Farias et al. 2005). The RAC acknowledges that hypoxia can affect the testicular function and even cause testicular toxicity, but notes that these studies do not offer quantitative dose response relationships. That is, they do not show which degree of hypoxia results in which extent of testis toxicity, which would be helpful if information on oxygen tension would have been available from the GaAs studies.

The RAC is of the opinion that there is no evidence for hypoxaemia in the animal studies on GaAs (e.g., no clinical signs such as pallor of mucous membranes, poor general health status, weakness, laboured breathing, or signs of hypoxia in other vulnerable organs). No increased vascularisation of the testis tissue has been reported in the histopathological study of the testes in the NTP study (no increase in number of blood vessels or decrease in vessel diameter in the testes) at the exposure levels causing testis toxicity. This proposed MoA therefore seems rather

speculative for GaAs. Unfortunately, there are no measurements of oxygen tension in the animals that could prove or disprove a hypoxia of a sufficient degree to explain testis toxicity.

The RAC has also evaluated the information available for the two proposed key effects involved in the suggested mode of action of lung toxicity causing hypoxaemia leading to testis toxicity: i.e. alveolar proteinosis and microcytic anaemia. It is acknowledged that alveolar proteinosis may cause hypoxaemia. However, the expected physiological response of the hypoxaemia would be increased haematocrit and haemoglobin levels, and not the microcytic anaemia that was observed. Thus, if alveolar proteinosis were considered to be the dominant effect of systemic GaAs toxicity, one would expect a different haematological response pattern than actually observed. The RAC further notes that at concentrations where testis effects starts to appear in mice and hamsters, the effects on the lungs are described in mice as mild with respect to alveolar proteinosis, hyperplasia, and inflammation (NTP, 2000), and in hamsters as mild to moderate (Tanaka, 2000). In contrast, the alveolar proteinosis in rats was described as marked when the first signs of testis toxicity appeared (NTP 2000). Regarding the microcytic anaemia, haemoglobin were generally only slightly reduced at the highest exposure levels in the mice and rat studies, and not at all at the concentration levels where the first signs of testis toxicity started to appear. Taken together, the RAC is of the opinion that the available data do not seem to support sufficiently severe effects on these two parameters to support the proposed MoA.

Potential direct toxic effects of gallium and arsenic on the testes

During public consultation the submitter of the AIR provided experimental studies, which indicate that application of relatively high doses of Ga-salts by other routes than inhalation does not result in toxic effects in the testes (Dudley and Levine 1949 as cited in Venugopal and Luckey 1978, Colomina et al. 1993).

The Dudley and Levine study (1949) is referred to with reliability 4 (not assignable) and from the AIR it seems that the testes was not studied (assuming that N.A. = not analysed). The RAC therefore concludes that this study is not helpful in assessing potential direct effects of gallium in the testes.

Subcutaneous doses of gallium nitrate to male mice up to 96 mg/kg bw per day, every other day for 14 days revealed no indications of an impairment of fertility (Colomina et al. 1993). Male mice were injected subcutaneously with gallium nitrate dissolved in physiological saline at doses of 0 (controls), 24, 48 and 96 mg/kg/day every other day for 14 days before mating with untreated females. There were detailed analysis of the male reproductive tract (sperms and histopathology), reproductive success, and male body weights. No effects on any parameter were noted. However, the RAC notes that 'free' Ga^{3+} has a low solubility in most aqueous solutions, and readily hydrolyses to various hydroxide species (e.g., $Ga(OH)_4^-$) limiting the absorption. Since gallium has been used therapeutically there is quite some experience on how to present it under physiological conditions to avoid problems with precipitation and to improve absorption. A review by Bernstein (2005) describes how gallium in saline will hydrolyse into $Ga(OH)_4^-$, which with a pH-dependent rate is transformed into insoluble $Ga(OH)_3$, that will precipitate, and then form crystalline GaO(OH). The review concludes that there is low oral absorption of gallium salts, "likely due in large part to the formation of poorly soluble gallium hydroxides in the gastrointestinal tract". The review also states that gallium used for therapeutic reasons should be chelated with citrate to prevent hydrolysis and formation of insoluble forms of gallium. The Colomina study uses subcutaneous injections of gallium nitrate dissolved in saline, so based on the medical experience described by Bernstein (2005), it is likely that there was a poor absorption of gallium ions in the Colomina study. Accordingly, the actual exposure is likely to have been much lower than 24, 48 and 96 mg/kg. Thus, the absence of effects on any

parameter in the Colomina study may just reflect a very low absorption, and this short term study is therefore not helpful in assessing potential direct effects of gallium ions in the testes.

Chiou et al. (2008) studied the effects of 15 subcutaneous administrations (for 3 weeks) of soluble arsenic trioxide As_2O_3 in mice, and observed a dose-dependent testicular toxicity (inhibition of spermatogenesis) as well as increasing concentrations of arsenic in the testes (40, 64 and 182 ppb As at 0, 0.3 and 3 mg/kg/day, respectively).

The testicular toxicity of As_2O_3 has also been studied after exposure of mice to the compound via the drinking water for 60 days (Li et al., 2012). The testicular toxicity of $NaAsO_2$ provided in the drinking water for 35 (Pant et al., 2001) or 365 (Pant et al., 2004) days was studied in mice. All tested arsenic compounds caused decreased testis weights, decreased sperm numbers, and sperm abnormalities. The concentration of As in the testes was 5.3 µg/g after 35 days of drinking water containing 534 umol $NaAsO_2$/l, and 6.5 µg/g after 1 year of drinking water containing 53 umol $NaAsO_2$/l. Based on the Pant studies, it seems that a concentration of about 5 µg/g arsenic in the testes may cause testis toxicity, in the absence of any lung toxicity.

The RAC notes that arsenic concentrations in the rat testes in the 2-year study varied between 0.3 and 1 µg/g between 2 and 18 months of inhalation exposure to 1 mg/m^3 GaAs (NTP 2000) and did not cause any statistically significant testis toxicity. Mast (1990a) found an at least 5-fold increase in concentrations of arsenic in the rat testes as the inhalation exposure to GaAs increased from 0 to 75 mg/m^3 in a 12-day study. When extrapolating from the 12-day Mast 1990 study to the 14-week NTP study on rats, in which identical daily doses of GaAs were tested, arsenic concentrations in the testes would be expected to be clearly higher than 1 µg/g at the 37 and 75 mg/m^3 GaAs concentrations, i.e. which caused testis atrophy and hypospermia in all tested rats.

Thus, based on the measured and estimated concentrations of As in the testes in these studies, it cannot be excluded that the testis effects observed after exposure to GaAs via the inhalation route, could be caused by a direct action of As ions in the testes.

Other potentially contributing factors

Regarding the haematological effects, one can speculate that instead of being caused by lung damage they could be caused by the demonstrated inhibition by GaAs of delta-aminolevulinic acid dehydratase (ALAD), an enzyme needed for heme biosynthesis (Goering et al. 1988) and/or by gallium forming complexes with iron binding molecules including transferrin, ferritin and lactoferrin leading to depletion of intracellular iron and apoptosis (Engelstad et al. 1982, Bernstein 1998).

V. OVERALL CONCLUSION ON THE CLASSIFICATION OF GALLIUM ARSENIDE AS TOXIC TO REPRODUCTION

Comparison with the criteria for classification for reproductive toxicity in the CLP Regulation

'*Presumed human reproductive toxicant*

The classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other effects or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effect. However, when there is mechanistic information which raises doubt about the relevance of the effect

for humans, classification in category 2 may be more appropriate.'

RAC conclusions

Studies in mice, rats, and hamsters have all shown testicular toxicity in animals after exposure to GaAs by inhalation and/or via intra-tracheal instillation. The testicular effects include decreased testis weights, epididymis weights, spermatid counts, spermatozoa motility and testis atrophy (rats and mice). These effects are clear-cut, clearly adverse and observed in two or more animal species. The effects are likely to cause reduced male fertility, although functional fertility has not been studied.

Testicular toxicity is observed also in animals which show no general clinical symptoms or reduced body weights. However, the lungs are a more sensitive target organ, and signs of lung toxicity are noted at lower GaAs concentrations than those causing testicular toxicity. In mice and hamsters, the signs of lung toxicity are not very severe at the concentrations where signs of testis toxicity appear (e.g., mild alveolar proteinosis in mice and mild and/or moderate inflammatory changes in the lungs of hamsters and mice). At the highest concentrations in mice where moderate testicular atrophy occurs, only a moderate (not marked or severe) alveolar proteinosis, and a slight reduction in Hb (4-6%), is reported. Also, marked alveolar proteinosis has been observed in rats at concentrations which do not produce any statistically significant testicular toxicity (such as testis atrophy), reduced Hb, body weight gain or any clinical symptoms. Thus, no correlation between occurrence and severity of the lung toxicity and testis toxicity is shown. There is therefore no evidence for the testicular toxicity being secondary to other toxic effects in these studies. In addition, as far as the RAC is aware, other recognised lung-toxicants are not known to exert secondary testicular toxicity mediated via such lung toxicity.

When evaluating other toxic effects, the criteria also distinguish between reproductive toxic effects which are a specific or non-specific consequence of other toxic effects. The RAC considers that if some aspects of the lung toxicity would be involved in the GaAs-induced testis toxicity, the testis toxicity is likely to be a specific consequence of the pulmonary toxicity in that case.

The submitter of the AIR speculates that it is a combination of alveolar proteinosis and microcytic anaemia that results in testis toxicity of GaAs. The RAC is of the opinion that there is not sufficient evidence to support such a mechanism, and should it indeed be proven, it would qualify as a combination of rather specific effects and not a secondary, non-specific consequence of the other toxic effects.

The RAC notes that although there is moderate (in mice) or severe (in rat) lung damage at the highest exposure levels, the reduction in haemoglobin concentration is not very serious as the relatively small degree of anaemia would not result in hypoxia in the testes. Furthermore, there are no signs in these studies of a clinically relevant hypoxaemia (e.g. pallor of mucous membranes, poor general health status, weakness, laboured breathing) or signs of hypoxia in the testes (such as increased number of blood vessels or decrease in vessel diameter in the testes, as reported in rats under hypobaric conditions (Farias et al., 2005) or in other vulnerable organs). However, it is acknowledged that the oxygen tension of the blood has not been measured in any of the GaAs studies.

The RAC is of the opinion that there is not sufficient evidence to consider the testis toxicity solely as a secondary consequence of the combination of alveolar proteinosis and microcytic anaemia, and even if proven, it would qualify as a rather specific effect and not a secondary, non-specific consequence of other toxic effects.

Other potential modes of action for the testicular toxicity have also been discussed, considering the distribution of gallium or arsenic ions to the testes and direct toxic action in the testes as one potential mode of action. The RAC has concluded that the available data base does not allow concluding on which single MoA or combination of MoAs that is responsible for the testicular toxicity.

Because of the clear evidence of testicular toxicity in two or more species, the original proposal to classify gallium arsenide as Repr. 1B - H360F (CLP) is maintained.

List of new references, not included in the previous RAC opinions, or in the additional information in the AIR or submitted by IND during the PC on carcinogenicity:

Bomhard, E. M. and Gelbke, H.-P. (2013). Hypoxaemia affects male reproduction: a case study of how to differentiate between primary and secondary hypoxic testicular toxicity due to chemical exposure. Arch. Toxicol. Feb 21. (Epub ahead of print).

Bernstein, L. R. (2005). Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine. Edited by Gielen and Tiekink. John Wiley & Sons, Ltd.

Farias, J. G., Bustos-Obregon, E., Reyes, J. G., (2005). Increase in testicular temperature and vascularization induced by hypobaric hypoxia in rats. J. Andrology 26(6):693-697.Pant, N., Kumar, R., Murthy, R. C. and Srivastava, S. P. (2001). Male reproductive effects of arsenic in mice. Biometals 14(2):113-117.

Pant, N., Murthy, R. C. and Srivastava, S. P. (2004). Male reproductive toxicity of sodium arsenite in mice. Hum. Exp. Toxicol. 23(8):399-403.

Yamazaki, K., Tanaka, A., Hirata, M., Omura, M., Makita, Y., Inoue, N., Sugio, K. and Sugimachi, K. (2000). Long term pulmonary toxicity of indium arsenide and indium phosphide instilled intratracheally in hamsters. J. Occup. Health 42:169-178.

VI ANNEXES

Annex 1 RAC Opinion of 25 May 2010 on a dossier proposing harmonised classification and labelling at Community level for gallium arsenide.

Annex 2 Requests from the Executive Director of ECHA to RAC of 30 November 2011 (D(2011)5595) and of 17 April 2011 (I(2012)0213) – 'the mandate'.

Annex 3 Comments and Response to Comments (RCOM)

Annex 4 Additional Information Report (AIR)

o0o