Minority opinion regarding classification for reproductive toxicity of gallium arsenide by Boguslaw Baranski, Lina Dunauskiene and Helmut Greim, July 2013

RAC in its 24th plenary proposes to classify gallium arsenide as Reprotox Cat. 1B.

Three RAC members including the rapporteurs disagreed with this C&L and propose Reprotox Category 2, because there is overwhelming evidence that the effects on spermatogenesis might be a secondary non-specific consequence of other toxic effects, especially lung toxicity.

This minority vote is based on the following information:

1. Existing evidence is not sufficiently convincing to place the substance in category Repr. 1B
2. Effects of hypoxemia on testes in humans
3. Effects of hypoxemia on testes in laboratory animals
4. Effects of inflammation on testicular function
5. Conflicting results that soluble anorganic Ga- and/or As-compounds have direct effects on spermatogenesis

1. Existing evidence is not sufficiently convincing to place the substance in category Repr. 1B

The main evidence of reproductive toxicity of Gallium arsenide comes from four studies.

First two studies where this substance has been given intratracheally to rats and hamsters at dose 7.7mg/kg for twice a week for 8 weeks (Omura at al. 1996) , which was not a recommended model for studying reproductive toxicity.

The other two short-term repeated toxicity studies were carried out on rats and mice exposed by inhalation to aerosol of GaAs at concentration 0, 0.1, 1, 10, 37, 75 mg/m^3 for 6 hours/day, 5 day/week for 14 weeks (MMAD range: 0.8-1.6µm).

Testicular effects such as testicular atrophy (minimal to marked severity) and epididymal hypospermia (mild to marked severity) were only observed in males having pulmonary lesions which could impair exchange of gases in lungs and which could lead to hypoxia. The hypothesis that testicular effects are caused by hypoxia which is a consequence of alveolar lesions is biologically plausible.

This hypothesis of the secondary, non-specific nature of testicular effects induced by hypoxia due lung lesions caused by GaAs at high doses is scientifically sound and patho-physiologically probable it cannot be neither proven nor disregarded due to lack of sufficient data. Gallium arsenide reproductive toxicity has only been tested by inhalation route, so any problems of assessment of toxic properties which might be linked to this particular route of exposure cannot be solved because we did not have data on reproductive toxicity of this substance by oral or dermal route. It should be noted that RAC has already recognized strong lung toxicity of GaAs by classifying that substance to category STOT RE 1 – H372 (lungs) which somehow supports this hypothesis.

Overall it is concluded that due to strong uncertainty concerning interpretation of secondary or not secondary non-specific nature of testicular effects caused by GaAS at high doses after intratracheal instillation or short term inhalation exposure, lack of

clear testicular effect in 2-year inhalation study (0.1 – 1mg/m^3), negative data or lack of data on testicular toxicity of other compounds of gallium or arsenic, there is not sufficient evidence to classify GaAs to category Cat. Repr. 1B – H360F, according to the CLP Regulation; and Repro. Cat. 2; R60 according to the DSD Directive].

There was consensus at RAC that inhalation GaAs exposure leads to severe lung damage, which is accompanied by testicular effects. However, according to the CLP criteria classification as Reprotox 1B is not warranted if the effects on the reproduction can be considered secondary. Since there is overwhelming evidence from human and animal studies that impairment of lung function affects spermatogenesis the rapporteurs and one RAC member conclude that it is possible that the testicular effects are secondary to severe lung damage. This is physiologically plausible because spermatogenesis requires high energy so that disturbed oxygen supply by any disturbance of lung function will affect spermatogenesis. This is further supported by a number of animal studies with soluble Ga and As compounds, which do not indicate direct effects on testicular function. Since some of them are conflicting and difficult to interpret the rapporteurs propose to classify GaAs as Repr 2 to take into account these uncertainties.

2. Effects of hypoxemia on testes in humans

Several studies describe testicular atrophy in patients with a history of chronic bronchitis and emphysema, which according to the authors supports the hypothesis that hypoxemia in lung disease suppresses the hypothalamic-pituitary-testicular axis (Semple et al 1984, Gosney 1987). Similarly, Aasebø et al (1993) described disturbance in the testosterone regulation in male patients with chronic respiratory failure. Oxygen therapy for 1 month increased arterial pO_2 and testosterone levels and 5 of the 12 patients regained sexual potency.

Verratti et al (2008) investigated spermatogenic parameters in healthy mountain trekkers after 26 days exposures to altitudes between 2,000 and 5,600 m. After returning to sea level, sperm counts and total number of motile sperm have been reduced, the number of abnormal or immature spermatozoa increased.

3. Effects of hypoxemia on testes in laboratory animals

Animal studies confirm the impact of hypoxia due to reduced partial oxygen pressure on testicular function. Thus, hypobaric conditions (with the consequence of reduced oxygen pressure) up to 60 days simulating altitudes between 4000 and 6000 m above sea level reduced Sertoli and Leydig cells in testicular tissue (Shevantaeva and Kosyuga (2006), spermatogenesis, epididymal sperm count, testicular, epididymal and seminal vesicle weights (Gasco et al 2003). More recently Liao et al (2010) described that hypoxia inhibits spermatogenesis. Farias et al (2005) described changes in testicular morphology and loss of spermatogenic cells in all stages of the spermatogenic cycle and provided some evidence that hypoxia interferes with the high oxygen and energy demand of the spermatogenic cells, which could be partly related to increased lipid peroxidation. This is further supported in that administration of ascorbinic acid ameliorated these effects (Farias et al 2010).

4. Effects of inflammation on testicular function

In addition to hypoxia, inflammatory disease-associated impairment of male fertility may add to explain testicular dysfunction (Hales et al, 1999, and Hales 2002). The authors describe inflammatory disease-associated decreases in male fertility. According to Hales (2002) both the classic endocrine hormones and inflammatory

mediators regulate spermatogenesis and maintenance of testicular interstitial tissue homeostasis. During infection or inflammation the functional activities of this regulation are disturbed by the elevated and prolonged expression of the inflammatory mediators. This explains the observation that males with critical illness, burn trauma, sepsis and rheumatoid arthritis are at least temporary infertile.

In adult rats lipopolysaccharides induce a well-defined inflammatory state and disorganization of the seminiferous epithelium. This disorganization involves an increase in apoptosis and disruption of the essential cell-cell contacts between Sertoli cells and spermatocytes or spermatids.

5. Conflicting results that soluble inorganic Ga- and/or As-compounds have direct effects on spermatogenesis

The intratracheal and inhalation studies on GaAs indicate that As and Ga ions distribute to the testes.

In the 2-year inhalation study there is no accumulation of As in the testes whereas Ga concentrations increase about tenfold between months 2 and 18 at 1 mg/m^3 exposure. However, as described in chapter 4 of the RAC opinion application of relatively high doses of Ga-salts by other routes than inhalation do not result in toxic effects in the testes (Dudley and Levine 1949, Colomina et al. 1993).

Other authors have investigated accumulation of As and Ga in the testes after oral, inhalation or subcutaneous application of GaAs or other soluble or insoluble As compounds. The RAC concludes that “the overall impression of these studies is that both Ga and As ions distribute to the testes, but that neither ion is accumulated to a higher extent than in other organs. However, whether inhalation exposure to GaAs will lead to sufficient concentrations of these ions in the testes to explain the testes toxicity is still an open question.”

Overall Conclusion

The following information allows the conclusion that the effects of GaAs on the testes can be considered secondary non-specific to severe lung damage:

- There is clear evidence from studies in humans and animals that impaired lung function and inflammation impairs male fertility.
- Whether the relatively low or absent accumulation of As and Ga in the testes contribute to impairment of spermatogenesis remains unclear.
- The publications by Dudley and Levine (1949) and Colomina et al (1993) show that application of soluble Ga-salts via other routes than inhalation does not induce testicular toxicity.

Based on this information it is concluded that the testicular effects upon inhalation or intratracheal instillation of GaAs can be considered secondary non-specific consequences of lung toxicity. Therefore classification of GaAs in Category 1B for reproductive toxicity is not warranted. Since it remains unclear whether increased Ga (and/or As) concentrations observed in the testes in the 2 years inhalation study may contribute to testicular toxicity, classification in Category 2 (H361f) is proposed.

Two recent publications in peer reviewed international journals (Bomhard et al 2012 and Bomhard and Gelbke 2013) support this conclusion.

Bomhard EM, Cohen SM, Gelbke H-P, Williams GM (2012) Evaluation of male reproductive toxicity of gallium arsenide. Reg Pharmacol Toxicol 64, 77-86

Bomhard EM, Gelbke H-P (2013) Hypoxaemia affects male reproduction: a case study of how to diferentiate between primary and secondary hypoxic testicular toxicity due to chemical exposure. Arch Toxicol

All other references are given in the RAC opinion.