Minority position on divanadium pentaoxide – Julie Séba

I am of the opinion that divanadium pentaoxide should have been classified for Germ Cell Mutagenicity in Category 1B based on a weight-of-evidence assessment :

- Several *in vitro* micronucleus and comet assays showed positive results ;
- This was further confirmed in a positive mouse micronucleus via inhalation, which I consider the most reliable route of exposure in this case. Positive results in a dominant lethal study, a micronucleus and two comet assays through intraperitoneal route are also considered supportive evidences, among others. I do not see sufficient justification to disregard these positive findings from *in vivo* studies ;
- Toxicokinetic studies clearly demonstrated the distribution of divanadium pentaoxide to the testes ;
- Finally, adverse effects were reported in sperm cells after divanadium pentaoxide inhalation in repeated-exposure studies in rodents.

There are clear evidences of genotoxicity *in vitro*, including positive micronucleus and comet assays in human and animal cells. Aneuploidy was also reported in human primary lymphocytes after exposure to divanadium pentaoxide. In animals, studies using three different routes were described, all routes showing some or clear evidences of genotoxicity.

The *in vivo* inhalation mutagenicity/genotoxicity dataset shows a positive micronucleus assay in male mice (Rojas 2014). Schuler et al. (2011) also demonstrated in mice a statistically significant concentration-related increase in 8-oxoGua DNA lesions although the related Comet assay was found negative. These results are further supported by the observations of oxidative stress in hepatocytes (Cano-Gutiérres et al., 2012). Additionally, structural DNA damage were microscopically observed in testicular cells of mice after inhalation of divanadium pentaoxide in a recent investigation (Rodriguez-Lara et al., 2016). All these studies were found to have some limitations. However, I consider that these limitations are not sufficient to disregard the positive findings in mice after divanadium pentoxide inhalation. On the other hand, I question the results from an older micronucleus assay found to be negative (NTP, 2002). Indeed, in this NTP micronucleus study, there is no demonstration that the bone marrow was adequately reached, possibly resulting in a false negative result.

For the oral route, the available dataset is very limited, with only two studies in rat described. A gavage micronucleus assay in rats which was considered reliable although the purity of the test compound was not assessed by RAC (Anonymous, 2011). This is in line with the findings described in the 1988 WHO report, showing positive results in a mice micronucleus assay for all routes (inhalation, intraperitoneal and subcutaneous injection) but not after oral exposure (Sun et al, 1987 cited from WHO, 1988). Toxicokinetic studies also indicated that divanadium pentaoxide is poorly absorbed by the gastrointestinal tract. The second oral study showed significant increase in length of DNA migration in liver, kidney, heart, lung, spleen and brain of rats after exposure to 70 mg/kg divanadium pentaoxide by gavage (Paramanik and Rajalakshmi et al., 2013). Overall, there are indications that the oral route might not be the most relevant to assess the genotoxic potential of divanadium pentaoxide.

The intraperitoneal route was also investigated. Five *in vivo* mutagenicity/genotoxicity studies using the i.p. route were available in the CLH report and four of them were found to be positive. Two mouse comet assays showed positive results in all investigated tissues, including testicular cells for one of them (Altamirano-Lozano et al., 1996 and 1999). A dominant lethal test and a micronucleus test also showed positive results in mice after intraperitoneal injection (Altamirano-Lozano et al., 1996 and Garcia-Rodriguez et al., 2016). On the other hand, a bone marrow SCE assay was negative in the same species (Altaminaro-Lozano et al., 1993). Although an intraperitoneal injection is not considered as a physiological route and should therefore be interpreted with caution, I noted when assessing the toxicokinetic dataset that divanadium pentaoxide cannot be metabolized. I am therefore of the opinion that the clear evidence of a genotoxic potential of divanadium pentaoxide after peritoneal injection in mice should be considered as a supportive evidence in the whole evaluation of the compound.

Regarding the toxicokinetic behaviour of the compound, numerous studies demonstrated with high confidence that divanadium pentaoxide is distributed to the testes after exposure through various routes in animals. Among others, this affirmation is supported by toxicokinetic studies showing distribution of divanadium pentaoxide in testes of mice after inhalation exposure (Mussali-Galante et al., 2005; Fortoul et al., 2007). Vanadium was also detected in testes or ovaries of rats after intra-tracheal installation of divanadium pentaoxide (Edel and Sabbioni, 1988; Greim, 2006).

Finally, adverse effects on germ cell and testes were observed in test-animals after divanadium pentaoxide exposure in various studies. Fortoul et al., (2007) reported an increase of vanadium concentration in testes of mice after one week of exposure to the test compound via inhalation as well as necrosis of spermatogonium, spermatocytes and Sertoli cells, pseudo-nuclear inclusion and disruption of cellular junctions in these test-animals. Mechanistic studies from the same group showed a decrease of the percentage of gamma-tubulin in all analyzed testicular cells of mice (Sertoli, Leydig and germ cells) starting with the first week of treatment in a time dependent manner as well as an accumulation of vanadium in the testes of mice starting with the initial inhalation (Mussali-Galante et al., 2005). Reduced membrane connexin 43 in seminiferous tubules or actin content time-dependently reduced in testes cells of mice were also reported after inhalation to the test compound (Bizarro-Nevares et al., 2016; Rodríguez-Lara et al., 2016). The NTP (2002) study also reported hypospermia in rats and reduced sperm motility in mice after exposure via inhalation, although general toxicity might have been associated. In Guinea pigs, supportive evidence include altered sperm parameters and spermatogonia and mild necrosis of testicular tissue after intraperitoneal injection (Uche et al., 2008).

In conclusion, I consider that the overall weight-of-evidence is appropriate to warrant a Muta. 1B classification for divanadium pentaoxide.