Science-based commentary of the Proposal for Harmonised Classification and Labelling of Divanadium pentaoxide (CLH Report, Version 02, August 2019) by Professor Len Levy

The CLH proposal for carcinogenicity of divanadium pentaoxide is based principally on the results from the NTP 2002 Report on the toxicology and carcinogenicity of vanadium pentoxide (divanadium pentaoxide) in rats and mice and thus, I will restrict my comments to this study (NTP 2002) and its interpretation.

The CLH Report states in the following section:

10.9.*3 Conclusion on classification and labelling for carcinogenicity*

*"With a weight of evidence approach, considering the clear evidence for carcinogenicity in mice in both sexes, also supported by results in rats (NTP, 2002) with the various other CLP criteria to be considered, a classification to **Carc. 1B, H350: May cause cancer** is warranted."*

"Classification should not be limited to a single exposure pathway. Although the data available only relate to inhalation exposure, a decision to restrict carcinogenicity classification to the inhalation pathway of exposure would not be justified, in the absence of carcinogenicity data by the oral or dermal pathway for divanadium pentaoxide."

I would disagree with the interpretation of the available data as presented in the CLH Report and its use in its conclusion in relation to the CLP criteria and CLH Guidelines. In my opinion, a weight of evidence approach, still primarily based on the NTP 2002 study, as in the Report, would be **Carc. 2 – suspect human carcinogen** and further, I would propose that this is restricted to inhalation and not to the dermal and oral routes, as proposed in the Report, for the following five reasons:

1. A number of published papers (Assem and Levy, 2009; Starr et al. 2012) have clearly demonstrated that there is no increase in lung tumours in either male of female rats in the NTP 2002 study when the data from the appropriate historical background rates are applied and the data are analysed statically. In any case, there never was a statistical increase in lung tumours, even in the original NTP 2002 publication.
2. There were no tumour increases at sites, other than the lung in mice, in the rats or mice in the NTP 2002 study but there is ample evidence to show that the inhaled divanadium pentaoxide, reached all tissues and organs (measured as vanadium) over the two-year exposure duration. In terms of marked inflammatory responses at the respiratory epithelium, this demonstrates that the exposure was clearly greatly in excess of the maximum tolerated dose (MTD) in rat and mice for the whole of the exposure duration at all exposure levels yet no systemic tumours or even tissue pathology were reported. For this reason, I propose that any carcinogenicity classification is restricted to inhalation, even in the absence of studies via the oral and dermal route.
3. It is noted that the increases in lung cancer in mice at the three exposure levels in the NTP 2002 study were almost maximal and at the same level in the low, medium and high exposure groups (Starr and Macgregor 2014). This lack of a dose response curve is both puzzling and problematical. Normally, a positive dose-response curve gives confidence to the researcher and regulator of a causal link between the exposure to a substance and an effect. This is not possible here. However, there was a marked inflammatory response to the lungs (principally alveolar epithelial bronchiolar epithelial hyperplasia) which was dose related but moderate to severe in all three groups. This response was also seen in all three rat groups where it was even more marked.

4. A major drawback in the interpretation of the similar degree of lung tumour increase in the low, medium and high exposure groups of mice in the NTP 2002 study is that the low, medium and high groups all experienced marked and persistent inflammatory damage to the bronchial and alveolar epithelium, presumably for the full experimental exposure duration. This is not surprising as it is well known that divanadium pentaoxide is highly irritating and points to a poor dose selection for the NTP 2002 study. This is surprising as the 16-day and 3-month range finding studies, reported in NTP 2002, give evidence that marked inflammatory changes could be expected in the two-year study at the selected doses. It is important to note that both the OECD and the USEPA give guidance about the selection of doses used in chronic studies and note that excessive toxicity may "compromise the usefulness of the study and/or quality of the data generated" (OECD 2012). In general, international guidance for the design and conduct of hazard evaluation explicitly state that doses, where irritation or inflammation occur at the site of contact should be avoided (OECD 2012). In the case of chronic studies (as is NTP 2002) it states: "Principle 5 - Physicochemical factors (e.g., solubility, vapour pressure), the bioavailability of the compound, the palatability of the compound in food or drinking water, and other factors such as the potential for the substance to cause adverse effects at the site of administration (e.g., irritation, erosion, and ulceration) will influence the selection of the highest dose for chronic rodent bioassays. It is recommended that doses for chronic rodent bioassays be selected **to minimize or avoid adverse** nutritional, physical, organoleptic, and **irritant effects."** More recently, the OECD test guidelines for chronic toxicity/carcinogenicity testing state that potentially corrosive or irritating substances need to be diluted to avoid severe local effects (OECD 2018a,b). Clearly, these important study design factors were not adhered to in the case of the NTP 2002 study in rats and mice, as evidenced by the generally severe and persistent inflammatory changes, seen in both tested rodent species and which presumably occurred over the whole exposure duration. I believe this both "comprises the usefulness of the study" and confounds a clear interpretation of the lung tumour findings in the mice.
5. A further concern is the lack of concordance between the and mice and rats in the NTP 2002 study. It has been proposed that when a compound is non-genotoxic and associated with an increase in lung tumours in mice only, the relevance of the finding for human lung cancer risk is greatly diminished (Smith and Anderson 2017; Smith et al. 2018). In this context, non-genotoxic is intended to mean non-DNA-reactive.

Discussion:

The CLP Regulation states in Table 3.6.1: "*The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from* ***limited evidence of carcinogenicity in animal studies."***

My reasoning for proposing **Cat.2** is that my evaluation of the results of the NTP 2002 studies lead to an opinion of ***limited evidence of carcinogenicity in animal studies*** for the reasons given in 1-5 above. I acknowledge that the IARC evaluation of divanadium pentaoxide (IARC 2006) as *Group 2B possibly carcinogenic to humans* and that this was based on *sufficient evidence* in experimental animals.

However, the IARC Working Group did not have the benefit of the recent publications, noted above(Assem and Levy, 2009; Starr et al. 2012), which have shown that there is no increase in lung tumours in rats in the NTP 2002 study and, as is the working practice of IARC Monograph meetings, the animal cancer findings were evaluated by the animal carcinogenicity sub-group and the inflammatory effects

in the lung (from the same NTP 2002 study) by a separate sub-group (Other Effects) and thus may not have received sufficient consideration for confounding, caused by chronic inflammation, that they deserved.

In *section 3.6.2.2* (see above) of the CLP Regulation, the following is provided to assist in the evaluation:

Annex I*: 3.6.2.2.6.* *"Some important factors which may be taken into consideration, when assessing the overall level of concern are:*

(a) tumour type and background incidence;

(b) multi-site responses;

(c) progression of lesions to malignancy;

(d) reduced tumour latency;

(e) whether responses are in single or both sexes;

(f) whether responses are in a single species or several species;

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

(h) routes of exposure;

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

(j) the possibility of a confounding effect of excessive toxicity at test doses;

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity."

Where I disagree with the interpretation of the CLH Report for proposing **Cat. 1B** is based on three of these above important factors in particular and as follows:

(b) multi-site responses; - there is no evidence that there was an increase in tumours at any site other than the lung in male and female mice. This is clearly site-of-contact response related to the inflammation caused by the well-known irritant properties of divanadium pentaoxide. The divanadium pentaoxide is completely and rapidly concerted to the soluble vanadate ion and transported to all tissues and organs where no increase in tumours or tissue or organ damage was seen in mice or rats (NTP 2002).

(f) whether responses are in a single species or several species – it is a single species; only in mice. There is no increase in lung tumours in the rat (male or female) Starr et al. 2012.

(j) the possibility of a confounding effect of excessive toxicity at test doses – the study findings (tumours in mice) are confounded (OECD 2012) by the excessive toxicity (chronic and persistent marked bronchial and alveolar epithelial inflammation) at all three dose levels tested and coupled with a lack of dose response for the lung tumour findings in mice.

As has been noted, this chronic inflammation has led to marked oxidative damage which has resulted in increases in oxidative damage, including the formation of reactive oxygen species (ROS). This could well explain the increase in lung tumours in mice by secondary (non-DNA reactive) mechanisms, such as aneuploidy (IARC 2006, Assem and Levy 2009). However, this is an effect due to chronic inflammation due to the highly acidic local environment caused by the interaction of the inhaled

particulate divanadium pentaoxide at the mucous respiratory tract and alveolar epithelial lining and unlikely to be an intrinsic toxicological property of divanadium pentaoxide *per se*. Clearly, the persistent respiratory tract and alveolar epithelium inflammation at all three tested levels in the NTP 2002 study for the whole duration of the exposure period, greatly in excess of the MTD, has compromised the study design and confounded a clearer interpretation of the mouse lung tumour results and leads me to the conclusion of not *sufficient* but of ***limited evidence of carcinogenicity***. This is consistent with a **Category 2** classification – based on the CLP Regulation and CLH guidance document.

Conclusion:

Based on these above considerations, I would urge the authors of the CLH Report to re-evaluate the data, review relevant publications and the comments above and to reconsider their proposal for the carcinogenicity of divanadium pentaoxide.

References:

Assem FL, Levy LS. 2009. A review of current toxicological concerns on vanadium pentoxide and other vanadium compounds: gaps in knowledge and directions for future research. Journal of Toxicology and Environmental Health, Part B. 12:289-306.

ECHA (2014) Guidance on the preparation of dossiers for harmonised classification and labelling. Reference: ECHA-14-G-02-EN. Version 2.0, August 2014.

IARC. 2006. IARC Monogr. Eval. Carcinogen. Risks Hum. Vol. 86: Cobalt in Hard Metals and Cobalt Sulfate, Gallium Arsenide, Indium Phosphide and Vanadium Pentoxide, pp. 227–292. Lyon, IARC Press. 86:227–292.

National Toxicology Program (NTP). 2002. NTP Technical Report on the Toxicology and Carcinogenicity Studies of Vanadium Pentoxide (CAS NO. 1314-62-1) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program, Research Triangle Park NC. NTP TR 507, NIH Publication No. 03-4441, pp. 352, dated December 2002.

Organisation for Economic Cooperation and Development (OECD). 2012. Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452, and 453. 2nd Edition. Series on Testing and Assessment No. 116 Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2011)47&doclanguage=en.

Organisation for Economic Cooperation and Development (OECD). 2018a. Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. Available at: https://doi.org/10.1787/9789264071223-en..

Organisation for Economic Cooperation and Development (OECD). 2018b. Test No. 451: Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. Available at: https://www.oecd-ilibrary.org/environment/test-no-451carcinogenicity-studies_9789264071186-en

Smith CJ, Anderson SP. 2017. High discordance in development and organ site distribution of tumors in rats and mice in NTP two-year inhalation studies. Toxicology Research and Application 1:1-22.

Smith CJ, Perfetti TA, King JA. 2018. Bronchioloalveolar lung tumors induced in "mice only" by non-genotoxic chemicals are not useful for quantitative assessment of pulmonary adenocarcinoma risk in humans. Toxicology Research and Application 2:1-24.

Starr TB, Macgregor JA, Ehman KD, Nikiforov AI. 2012. Vanadium pentoxide: Use of relevant historical control data shows no evidence for a carcinogenic response in F344/N rats. Regulatory Toxicology and Pharmacology 64(1):155-160.

Starr TB, MacGregor JA. 2014. Vanadium pentoxide: Risk assessment implications of a treatment- but not dose-related tumorigenic response in B6C3F1 mice. Regulatory Toxicology and Pharmacology 69(3):333-337.

Professor Len Levy OBE, BSc, MSc, PhD, FFOM, FBTS

Emeritus Professor, Cranfield University/Toxicological Consultant to Vanitec,

len.levy@cranfield.ac.uk

The views expressed in this Commentary are solely those of the author and do not necessarily express the views of Vanitec or any other organisation or person.

Short Biography:

Professor Levy is Emeritus Professor of Environmental Health at the University of Cranfield. Prior to this he was Head of Toxicology and Risk Assessment at the UK Medical Research Council's Institute for Environment and Health based at the University of Leicester. He holds a doctorate in experimental pathology from the Institute of Cancer Research in London and has held academic positions at the University of Aston and the University of Birmingham's Institute of Occupational Health, where he was Reader in Occupational Health, continuing his research into the causes and mechanisms of occupational cancer.

Professor Levy has over than 300 publications on occupational carcinogenesis, occupational and environmental toxicology, biomonitoring, risk assessment and risk management and the regulatory aspects of environmental and occupational air standards. In 2000, he was awarded an OBE for Services to Occupational Health and Safety. Professor Levy's contributions have earned him a place on the list of recipients of the US ACGIH Herbert E. Stokinger Award.

Professor Levy was an independent member of the UK's Health and Safety Commission's former Working Group on the Assessment of Toxic Chemicals (WATCH) and the former Advisory Committee on Toxic Substances (ACTS), and now a member of the HSE's new Workplace Health Expert Committee (WHEC) and the HSE's Science, Engineering and Evidence Assurance Committee (SEEAC). He was Chair of the EU Scientific Committee on Occupational Exposure Limits (SCOEL), DG EMP. and was Chair of the executive committee of the UK Interdepartmental Group on Health Risks from Chemicals (IGHRC).

Professor Levy has been an invited member at 12 IARC Monograph Working Group meetings on the Evaluation of Carcinogenic Risks to Humans and chaired two of these meetings. He has conducted occupational and environmental risk assessments on many types of substances, ranging from pesticides to metals and solvents, and has led teams responsible for producing authoritative Criteria Documents used for setting Occupational Exposure Limits in the UK and the EU.