

Appendix 2

Review of NTP Technical Report on the
Toxicology and Carcinogenesis Studies of
TRIM® VX
in Wistar Han [CrI:WI (Han)] Rats and B6C3F1/N Mice
(Inhalation Studies)
NTP TR 591

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February 2, 2016

These comments are focused on providing additional information and thoughts on Trim VX and the toxicology reported as it relates to the NTP inhalation study of Trim VX in rats and mice.

- The oil in the product is severely refined and has been tested in a modified Ames assay with negative results (Mutagenicity Index < 1.0). This result supports the conclusion that polycyclic aromatic hydrocarbon levels are below that of concern for carcinogenicity.
- When Master Chemical first developed this metalworking fluid (1983), they conducted acute toxicity tests on the formulated product to assess potential skin and eye irritation, oral, dermal and inhalation LD/LC50s. LD/LC50s results were in the non-toxic range (>2000 mg/kg/ >5mg/L per current GHS classifications). The concentrate was classified as a skin and eye irritant; a 10% solution in water (recommended use concentration) resulted in slight dermal irritation (PDI index = 0.42), but not eye irritation in rabbits. These results are provided on the Trim VX MSDS.
- Consistent with these results, Master Chemical has received occasional reports of skin irritation from customers (primarily when higher than the 10% working concentration has been used), however, no reports of eye or respiratory irritation have been reported from customers or employees.
- These results are also consistent with the statements made in this report (pg 22-23) that occupational metalworking fluid exposures are typically less than 1.0 mg/m³ and often less than 0.5 mg/m³, the NIOSH Recommended Exposure level for total particulate mass (NIOSH, 1998), well below the exposures used in the NTP Trim VX inhalation study.

- NTP provides a good overview of the epidemiology data available on the various classes of metalworking fluids (pg 29-30) and that the study with the most statistical power and specific exposure information (Tolbert, et al, 1992), found less evidence that soluble oil metalworking fluid exposures were associated with cancer at any specific site, as compared to straight oils or synthetic fluids.
- On page 33 and again in Appendix H, the pH of Trim VX is reported as approximately 7.5. This value seems to be questionable since Trim VX has a low water content. Additionally, adequate analytical detail is not provided in the report to determine the method used and its accuracy. However, as stated on the MSDS, when mixed with water, the pH increases to 8.3-9.3. Although the NTP study tested the concentrate, it is believed that when the aerosol mixed with the moisture in the respiratory tract, it would become alkaline. This alkalinity (along with the oil viscosity/surface tension) would have contributed to the respiratory tract irritation and inflammatory responses as well as potential tumor formation reported at the site of aerosol contact in the test animals.
- Based on the lack of systemic toxicity along with negative genotoxicity results *in vitro* and *in vivo*, tumors appear to be related to irritation/inflammation and non-specific effects at the site of contact. Additionally, NTP writes (pg 100) that this lack of systemic toxicity/carcinogenicity indeed implies that Trim VX toxicity may be limited to site-of-contact. This would seem to support a nongenotoxic mechanism for the lung tumors reported in mice. Since tumors were only statistically significant at the highest dose (100 mg/m³), this would also suggest a threshold exposure, below which tumors do not occur.
- Trim VX is very susceptible to fungal growth and as is stated in the report “contaminated metalworking fluids and aerosols increase the potential for respiratory effects when inhaled” (pg 22). The report states that testing was conducted for bacterial and fungal growth, but no data are provided.
- In Appendix H, the diagram depicts the Exposure Chemical Cabinet where the chemical drum was located. It shows a mixer in the drum, but provides no details on this set-up. Trim VX is a viscous material, prone to settling, therefore the details of this configuration are critical to assure that what was aerosolized and how samples were collected for analysis, is representative of the product.
- Chemical analysis data regarding “Measured Components” in Table 1 (pg 34) indicate that chemical degradation of Trim VX may have started to occur as of the date of the analysis described in Table 1 (Table 1 does not provide the date of the analysis of either Lot 101607N or Lot 011509N). NTP was advised

in writing prior to the start of the study that Trim VX has a useful shelf life of just 12 months. Even with this information, a single Lot of VX was used for the 2-year study. By the end of the 2-year exposure period of the study (August 5, 2011: Table 2, pg 42) the Trim VX used (Lot 011509N: Manufactured 01/15/2009) was almost 31 months old - more than 2.5 times the known useful shelf life of this product. A request was made to NTP to obtain more detailed chemical characterization and stability analytical information mentioned on page 33 and H-2 as on file at the National Institute of Environmental Health Sciences (NIEHS). This information would establish the chronology of the analysis and changes in the tested chemistry over time. NIEHS Central Data Management responded "the data presented in Appendix H is all that is releasable at this time". Given that response, the only conclusion that can be made is that the Trim VX fluid tested had chemically degraded over the time of the study.

- As stated above and in the NTP comments (pg100), the effects reported appear to be due to a site of contact reaction and not systemic or Trim VX product-specific toxicity. Although it is common practice in NTP inhalation toxicology studies to expose the control animals only to air, in this study, since this is an alkaline and oil-based product, it would have been useful to have a second control group exposed to a comparable oil-based, alkaline aerosol of a non-carcinogenic material (such as a pH adjusted mineral oil/baby oil). Using a pH adjusted oil-based control would have adjusted for the impact of inhaling a viscous oil material as well the pH effects. Comparing the results of both control groups to the Trim VX exposed groups would have then controlled for the alkaline pH and oil aerosol effects, and would have allowed for a differentiation of product-specific chemical effects from the non-specific effects.
- Based on the available information presented in the report and the fact that the Trim VX used in the 2-year study was more than 2.5 times beyond the product shelf life, the results do not represent Trim VX as manufactured and used. Due to the lack of detailed analytical information, denied release by NIEHS, but necessary to validate the conclusions of the draft report, NTP should consider classifying this study as an "inadequate study".