



Comments on the 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

February 16, 2016

ILMA's Presenters:

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Master Chemical's Presenters:

Dr. Patricia Beattie

Dr. Steven Florio

Introduction

- Walden Dalbey, MA, PhD, DABT (DalbeyTox, LLC)
- Reviewed NTP's report on TRIM[®] VX as consultant to ILMA
- >40 years in toxicology including >25 years running inhalation studies
- Comments presented here were selected from several provided to NTP.

Selection of TRIM[®] VX

- Selection of TRIM[®] VX for 2-year studies was based in part on pulmonary fibrosis during 3-month studies (p. 31).
 - The use of fibrosis as a criteria to select a unique low-volume MWF for a 2-year study seemed unusual.
 - Was a nongenotoxic mechanism for production of tumors being considered at the time?
 - Clarification of the rationale for selection of TRIM[®] VX would be helpful.

Handling of TRIM[®] VX Sample

- Recommended shelf life of TRIM[®] VX is 12 months, but sample was ~30.5 months old at end of 2-year exposures.
 - A number of compounds were not reported in NTP analyses and concentrations of some compounds reported by NTP differed from formula.
 - Separation or degradation might have occurred.
 - How well did lab aerosol represent workplace aerosol?
- In 2005 ILMA recommended normal dilution of “soluble oil product concentrates” with water (1:20) before use in inhalation studies (normal workplace procedure).
 - The intent was to allow any normal changes in product chemistry upon dilution.
 - Undiluted TRIM[®] VX was used in inhalation exposures, contrary to ILMA’s recommendation. This again raises a question of representativeness of the lab aerosol.

Analyses of TRIM[®] VX

- Airborne fatty acid methyl esters (~8% of MWF) were used to determine particle size and to calibrate RAMs to monitor total aerosol concentration.
 - Rationale and validation of these methods were not provided and are needed.
- Report states that undiluted TRIM[®] VX contained mainly “water, alkanolamines, and oil”
 - Unstated implication was that the hexane-extractable material (HEM) was mineral oil.
 - NTP reported that HEM was 85% of MWF, but MSDS gives mineral oil content as 30-40%.
 - Clarification of this discrepancy is needed.

Analyses of TRIM[®] VX

- Reported pH of ~7.5 for TRIM[®] VX is not accurate; the pH for diluted Lot 011509N was 8.70 (Certificate of Analysis).
 - The unspecified method used at Battelle might have been inappropriate (close to a nonaqueous solution).
 - Alkalinity might be important to observed toxicity.
 - Integrity of MWF is questionable if pH is aberrant.
- MWFs are prone to growth of fungi and bacteria, often a major concern for respiratory effects in people.
 - TRIM[®] VX was used beyond its recommended shelf life.
 - Results of determinations on lab sample have not been available when requested by ILMA.

Results: Local Effects

- Nonneoplastic effects (aside from spleens in male mice) were restricted to the respiratory tract at 100 mg/m³ or below.
 - Also, tumors were observed only in the respiratory tract.
 - Can a more explicit statement be made on the lack of systemic effects than the statement on page 100 that the evidence “implies that TRIM VX-related toxicity may be limited to the site of contact”?

Results: Mode of Action

- Consider the following:
 - Increased incidence of tumors in mice only at 100 mg/m³
 - Equivocal evidence of tumors in rats only at 100 mg/m³
 - No trends in either species for increased tumors at lower doses
 - Negative genotoxicity screening assays on TRIM[®] VX and components
 - Prominent nonneoplastic lesions in the respiratory tract
- Collectively these results are suggestive of a nongenotoxic mechanism involving irritant or other nonspecific properties of the aerosol and possibly having a threshold.
- Can NTP address this possibility more than already stated in the report? (Trying to understand possible MOA)

Review of NTP Technical Report on the
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in Wistar Han [Crl:WI (Han)] Rats and B6C3F1/N
Mice
(Inhalation Studies)
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Patricia Beattie, PhD, DABT, SciVera LLC
on behalf of
Master Chemical Corporation

Introduction

- Patricia Beattie, PhD, DABT, Vice President, Scientific Development, SciVera LLC; this includes leading the toxicology team. Comments are on behalf of Master Chemical Corporation
- Over 35 years in toxicology, much of which as a toxicologist in the automotive manufacturing sector, involved in hazard and risk assessment of metalworking fluids
- My focus is to provide NTP with additional information on Trim VX as it relates to this study

Comments on Trim VX

- The oil in Trim VX is severely refined and has tested negative in a modified Ames assay, indicative of PAH levels of low concern for carcinogenicity
- Trim VX has been tested in acute toxicity tests; the concentrate is a skin and eye irritant. When diluted as used (10% solution), it is a mild skin irritant, with a pH of 8.3-9.3 (8.7 based on Certificate of Analysis for the NTP Lots tested). This information is provided on the MSDS
- Over the history of use, occasional reports of skin irritation but no eye or respiratory irritation reported from employees or customers
- The tested concentrate (reported as pH 7.5 by NTP) would become alkaline when mixed with the moisture in the respiratory tract; this and the viscosity/surface tension of the oil is likely causing the irritation, inflammation and tumors formed at the site of contact

Comments on NTP Report

- Negative results in genotoxicity tests, lack of systemic toxicity/tumors and tumors only at the site of contact at the highest dose tested, suggest a nongenotoxic mechanism and possible threshold
- 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 NIEHS
- NIEHS denied release of the additional analytical information; these data would provide chronology of analyses and changes in chemistry, critical for study interpretation

VX Chemical Considerations

8 February 2016

Dr. Steven M. Florio, PhD
Chief Technology Officer
Master Chemical Corporation

Background

- VX is a complex, 17-component engineered formulation that is designed around creating specific chemical interactions between its various components
- It is specified to be utilized in concentrations varying from 5-20%
 - No basis for use at 100% concentration
- VX has enjoyed only limited commercial utility; its manufacture and sale has been discontinued
 - 0.12% of commercial sales
- The major factor limiting the utility of VX is its lack of stability, especially outside of its 1 year recommended shelf life
 - NTP utilized material that had aged outside of this recommended shelf life
- Significant differences were noted in the as-measured components listed in Table 1 p.34 of the Peer Review Draft and the actual components contained in a fresh sample of VX

VX Study - Analytical Concerns

- Chemical degradation of VX occurs, contrary to the study conclusion (p.33)
 - Known, demonstrable differences by FTIR between fresh and 11 month samples of VX (Appendix 1)
 - FTIR spectra from NTP requested and denied
 - Depletion of key formulation component versus time by GC/MS and HPLC (Appendix 1)
 - Sample settling notable by Specific Gravity (Appendix 1b)
- The analysis of the two VX samples as reported in Table 1 p.34 identified some, but not all, of the 17 manufacturing materials contained in a fresh sample of VX (Appendix 2)
 - The analysis as reported identified 13 compounds and a hexane extract
 - The actual VX formulation contains 17 ingredients
- 8/13 of the materials identified were qualitatively identical/nearly identical to those in the as-manufactured product (Appendix 2)
 - In those 8 instances where the materials were qualitatively correct, the quantities shown varied substantially in some cases from the manufactured composition of the VX. Any dose dependent results or conclusions are questionable.
- The analysis did not detect 4 other critical components of the manufactured material
- The general conclusion is that the two lots of material that were tested were not chemically equivalent to the VX as produced and marketed by Master Chemical Corporation
 - The study is inadequate as to VX and any generalized conclusions about it or other “soluble oil” metal working fluids

Appendices

Appendix 1. Aging Studies

Sample Description	Sample ID	Comments
Top of freshly made lab control	WB3201-126A-T	Made by BJM on 1/11/2016
Bottom of freshly made lab control	WB3201-126A-B	Made by BJM on 1/11/2016
Top of oldest QC retain sample	Lot 022515H-T	QC retain from 02/25/2015
Bottom of oldest QC retain sample	Lot 022515H-B	QC retain from 02/25/2015
Top of oldest undisturbed drum	Lot 071015H – UT	Manufactured 07/10/2015
Bottom of oldest undisturbed drum	Lot 071015H – UB	Manufactured 07/10/2015
Top of oldest undisturbed drum mixed 5 minutes	Lot 071015H – M5T	Manufactured 07/10/2015
Bottom of oldest undisturbed drum mixed 5 minutes	Lot 071015H – M5B	Manufactured 07/10/2015
Top of oldest undisturbed drum mixed 1 hour	Lot 071015H – M1HT	Manufactured 07/10/2015
Bottom of oldest undisturbed drum mixed 1 hour	Lot 071015H – M1HB	Manufactured 07/10/2015

FTIR Aging Study Key Result

“Eight of the samples gave similar FTIRs. Two of the samples (**Lot 022515H-T** and **Lot 022515H-B**) were slightly different from the other eight. The formation of a new peak, although slight, can be seen at 1710 cm^{-1} . The appearance of this peak at 1710 cm^{-1} is not unusual considering **Lot 022515H-T** and **Lot 022515H-B** are the oldest samples in the group. Peaks in that area of the FTIR spectrum refer to carbonyl stretches, most likely of carboxylic acids or esters. The fact that ester or acid is changing over time is not that unusual and is probably occurring in all ten of the samples. However in the two samples, the change has occurred to a greater extent, allowing the change to be seen in the FTIR.”*

GC/MS Aging Study Key Result

“In these experiments, a new batch of Trim VX (WB3201-126A) was compared to an older batch (Lot 022515H).

When the experiment was conducted by GC/MS, it was established that the 4-chloro-3-methyl-phenol depleted by 2 % .”*

*Results from MCC Technical Report JHM January 19, 2016 “Test Protocol to Determine Stability of TRIM® VX Concentrate over Time”

Appendix 1b

January 19, 2016

Steve,

Please note the Specific Gravity from the top and bottom of the oldest and newest plant manufactured Quality Control retains. We ran the bottom sample twice for Lot # 081915N just to double check because the value was unusually high. In my experience a variation of greater than +/- 0.01 units is a concern.

Best regards,

Brian

VX 022515H

Top = 1.001

Bottom = 0.998

VX 081915N

Top = 1.003

Bottom run #1 = 1.033

Bottom run # 2 = 1.031

Appendix 2. Compositional Analysis

Reverse Engineered Chemistry Identified	Lot 101607N	Lot 011509N	Manufacturing Component?	% Delta v Manufactured VX	
				Lot 101607N	Lot 011509N
Water	7.1	6.8	Yes	39	33
Triethanolamine	3.7	3.2	Yes	19	2
4-Chloro-3-methyl-phenol	3.59	2.49	Yes	88	31
Diethylene glycol	0.87	1.07	No****	NA	NA
Diethylene glycol monobutyl ether	1.02	1.11	No****	NA	NA
Methyl palmitate	1.18	1.2	Yes*	1	4
Methyl oleate	5.65	5.81			
Methyl stearate	0.89	0.93			
Myristic acid	0.49	0.23	No****	NA	NA
Oleic acid	3.18	1.23	Yes	-20	-69
Palmitic acid	1.01	0.31	No****	NA	NA
Propylene glycol	0.2	0.2	No****	NA	NA
α -Terpineol	0.6	0.5	Yes**	0	-17
Hexane extractable material	80.2	85	Yes***	17.6	24.6

* Manufacturing raw material varies from lot to lot in terms of specific amounts of esters

** α -Terpineol is the key component of the manufacturing raw material

***4 specific manufacturing materials not identified

****The 5 materials identified by NTP that were not specifically added by Master Chemical Corporation to the VX formulation may have been present in some unknown amount as secondary/trace products contained in the intended raw material

Introduction

- John K. Howell, B.S., M.A., Ph.D., C.M.F.S.
- 45 years experience in metal finishing and metalworking/lubricant R&D, safety, health & environmental affairs, hazard communication
- Member of OSHA Metalworking Fluid Standards Advisory Committee, 1997 – 1999
- GHS Resources, Inc. – President
 - Consultant, Independent Lubricant Manufacturers Association

General Information on Complexity of MWFs and NIOSH-NTP Candidate MWF Selection Process:

MWF Selection Process

- MWFs are an array of complex mixtures. Substances in MWFs vary with the MWF; thousands of formulations are commercially available¹.
- NIOSH-NTP “top down” selection process between 2001 – 2005 for candidate fluids for chronic inhalation studies identified 5 or 6 products each from the estimated top 5 producers to produce a list of 29 possible candidates for testing. ILMA cooperated with NTP in the process.²
- NTP determined 18 fluids to be commercially available, including examples of all three classes of water-miscible metal removal fluids: “soluble oils;” “semi-synthetics;” and “synthetics.”
 - Ultimately, 9 fluids were selected for further evaluation
 - Trim[®] VX identified as “unique among the six soluble oils.”

¹ – Independent Lubricant Manufacturers Association, Comments to NIOSH, January 31, 2001, regarding November 14, 2000, draft, Metalworking Fluids: Recommendation for Chronic Inhalation Study

² - Independent Lubricant Manufacturers Association, October 21, 2005, letter to Dr. Daniel L. Morgan

NTP should find the TRIM[®] VX Study Inadequate

There are significant issues regarding the characterization of TRIM[®] VX:

- Bacterial and fungal growth may have occurred. If fungus had grown in the fluid to which the animals were exposed, any finding cannot be attributed to TRIM[®] VX
- The variations in the chemical characterization of TRIM[®] VX, including:
 - finding of chemical compounds not formulated into the product,
 - significant variation in pH,
 - incomplete characterizationtaken together, strongly suggest that the chemical composition of the product had changed, further suggesting that any finding cannot be attributed to TRIM[®] VX
- Using the product well beyond its stated shelf life:
 - stratification of the product likely occurred resulting in a composition which varied depending upon which part of the container from which it was drawn
 - study was conducted with a material that was 2 1/2 times its published shelf life.

Taken together, all of these issues surrounding the identification of just what was the substance to which the animals were exposed strongly suggest that NTP can only conclude that this study is an **“Inadequate Study.”**

OSHA “HCS 2012” Appendix A6³ and Appendix F⁴

- OSHA provides rules for classification of chemicals or chemical mixtures as carcinogens and whether/how those results can be extended to other untested materials
 - A6.3.2 Classification of mixtures when data are available for the complete mixture
 - A mixture may be classified based on the available test data for the mixture as a whole. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations and analysis (e.g., statistical analysis, test sensitivity) of carcinogenicity test systems

³- See <https://www.osha.gov/dsg/hazcom/hazcom-appendix-a.html>

⁴- See <https://www.osha.gov/dsg/hazcom/hazcom-appendix-f.html>

OSHA “HCS 2012” Appendix A6³ and Appendix F⁴

- OSHA provides rules for classification of chemicals or chemical mixtures as carcinogens and whether/how those results can be extended to other untested materials
 - A6.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles
 - When the mixture itself has not been tested to determine its carcinogenic hazard, but there are **sufficient data** on both the **individual ingredients** and **similarly tested mixtures** to adequately characterize the hazards of the mixture, these data will be used with the following bridging principles as found in paragraph A.0.5 of this Appendix: Dilution; Batching; and Substantially similar mixtures.

NTP Cannot Extend the Study Results to Other MWFs

- Trim[®] VX, like every other MWF, is a unique formulation
- From the guidance in HCS 2012, Appendix A.6, we conclude:
 - Any testing results from Trim[®] VX cannot be extended to other MWFs, individually or as a class, unless the compositions of these other MWFs are so similar and there are sufficient data on both the individual ingredients and similar tested mixtures so as to allow application of “bridging principles” as described in 29 CFR 1910.1200, Appendix A.6.3.2 and A6.3.3³.