NTP
National Toxicology Program

Draft NTP Technical Report TR591
on
TRIM® VX

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Metalworking Fluids (MWFs)

- MWF use
  - Reduce friction, minimize heat buildup, and extend tool life in metalworking operations
- MWF exposure
  - Dermal
    - Splashes and aerosols from handling parts, tools, and equipment covered with metalworking fluids
  - Inhalation
    - Aerosols (mist) produced during machining operations

MWF Classes

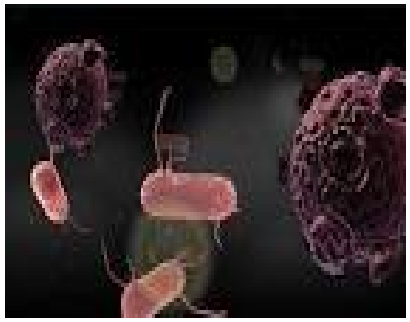
- Oil-based MWF
  - Straight oils (petroleum or mineral oils) - no water
- Water-soluble MWF
  - Soluble oils (30-85% oil, emulsifiers and blending additives)
  - Semisynthetic (5-30% oils, hybrid of straight and synthetic)
  - Synthetic (no mineral oils, 70-95% water)

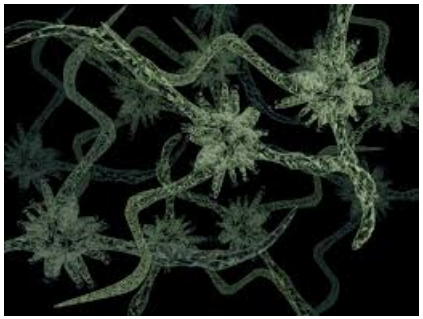
Typical Components in Water-Soluble MWFs

- Water
- Oil
- Anti-foaming agents
- Antioxidants
- Anti-weld agents
- Biocides
- Buffers (alkaline)
- Chelating agents
- Coupling agents
- Detergents
- Dyes
- Emulsifiers
- Extreme pressure additives
- Lubricity additives
- Plasticizers
- Odorants
- Surfactant
- Corrosion inhibitors

“In-Use” MWF Contaminants

- Metal particles, shavings
- Tramp oils
- Hydraulic fluids
- Bacteria and endotoxins
- Fungi and mycotoxins





MWF Nomination & Selection

- MWFs were nominated by the National Institute for Occupational Safety and Health
  - Decision to study unused MWF to assess potential toxicity directly associated with formulations

- Selection from ~30 MWFs:
  - Narrowed down to 18, based on composition and elimination of redundant formulations
  - Further reduced to 9, based on chemical screening and genetic toxicity testing
  - Final selection of 4 water soluble MWFs based on class, estimated usage, chemical composition and correspondence with NIOSH

MWFs Selected for NTP Studies

MWF	Genetic Toxicity	3-month	2-year
CIMSTAR® 3800 - semisynthetic	√	√	√
TRIM® SC210 - semisynthetic	√	√	-
Syntilo 1023 - synthetic	√	√	-
TRIM® VX - soluble oil	√	√	√

Characterization of TRIM® VX

- Test article characterization completed within a month of receipt
  - pH, specific gravity, and refractive index
  - *n*-hexane extractables
  - Bacteria and fungi
  - Water
  - Fourier Transform-Infrared (FT-IR) analysis
  - Elemental analysis
  - General organic identification
    - Alkanolamines
    - Oil constituents

Stability of TRIM® VX

- Drums were analyzed prior to, during, and after the completion of the studies to demonstrate stability of test material
  - Drums stirred to ensure homogeneity
  - Triplicate samples per drum
  - Data compared to frozen reference sample taken upon receipt of material

- Data demonstrated that the bulk test article was stable during the course of the study

3-Month Study Design

- Male and female Wistar Han rats and B6C3F1/N mice
- Whole body inhalation exposure: 6 hr/d, 5 d/wk, 3 months
  - Liquid aerosols (1.7-2.2 μm MMAD)
  - Concentrations: 0, 25, 50, 100, 200, 400 mg/m^3
- 10 animals/species/sex/concentration
- Endpoints:
  - In life (survival, clinical observations, body weights)
  - Gross/histopathology, organ weights, clinical chemistry
  - Genotoxicity

MMAD = mass median aerodynamic diameter

3-Month Study Results

	Rats	Mice
Survival	no effect	no effect
Clinical Observations	no effect	no effect
Body Weight	↓ 11%: 400 mg/m^3 (M)	↓ 8%: 400 mg/m^3 (M)
Organ Weight *Lung* *Liver* *Spleen*	↑ 20%: ≥ 100 mg/m^3 (F) ↑ 18%: ≥ 200 mg/m^3 (M & F)	↑ 28%: ≥ 100 mg/m^3 (M & F) ↑ 49%: ≥ 200 mg/m^3 (M & F) ↑ 21%: ≥ 50 mg/m^3 (M & F)
Hematology	no effect	↓12%: ≥ 200 mg/m^3 (M) erythrocytes, reticulocytes, hematocrit, and hemoglobin
Clinical Chemistry	no effect	N/A

M = male; F = female
N/A = not applicable

3-Month Study Results

Nose Histopathology:

	Rats*	Mice*
Respiratory epithelium - Hyaline droplet accumulation	≥ 25 mg/m^3 (minimal-mild)	≥ 25 mg/m^3 (minimal-mild)
Olfactory epithelium - Hyaline droplet accumulation	≥ 25 mg/m^3 (minimal-mild)	≥ 25 mg/m^3 (minimal-mild)
Suppurative inflammation	≥ 25 mg/m^3 (minimal)	≥ 25 mg/m^3 (minimal)
Respiratory epithelium - Hyperplasia	≥ 200 mg/m^3 (minimal)	
Respiratory epithelium - Squamous metaplasia	≥ 200 mg/m^3 (minimal)	
Goblet cell hyperplasia	≥ 200 mg/m^3 (minimal)	

*Similar lesions observed in males and females

3-Month Study Results

Larynx Histopathology:

	Rats*	Mice*
Squamous metaplasia	≥ 25 mg/m^3 (mild-moderate)	≥ 25 mg/m^3 (mild-marked)
Squamous hyperplasia	≥ 25 mg/m^3 (minimal-mild)	≥ 100 mg/m^3 (minimal-mild)
Chronic inflammation	≥ 25 mg/m^3 (minimal)	≥ 25 mg/m^3 (minimal)[a]

*Similar lesions observed in males and females

[a] female mice only

3-Month Study Results

Lung Histopathology:

	Rats*	Mice*
Fibrosis	≥ 50 mg/m^3 (minimal-mild)	≥ 100 mg/m^3 (minimal-moderate)
Histiocytic infiltration	≥ 100 mg/m^3 (minimal-mild)	≥ 100 mg/m^3 (minimal)
Chronic active inflammation	≥ 50 mg/m^3 (minimal-mild)	≥ 50 mg/m^3 (minimal-moderate)
Bronchiolar hyperplasia		≥ 50 mg/m^3 (minimal-mild)

*Similar lesions observed in males and females

Genetic Toxicity

- Erythrocyte Micronucleus Test
  - Negative in male and female mice and rats (i.e., no structural or numerical chromosomal alterations)

- Bacterial Mutagenicity Test
  - TRIM® VX was not mutagenic in *Salmonella typhimurium* strains TA100 or TA98, or *Escherichia coli* strain WP2 *uvr*A/pKM101, with or without exogenous metabolic activation (induced rat liver S9)

3-Month Study Summary

- Target of TRIM® VX exposure - respiratory tract
  - Similar toxicity in both sexes and both species
  - Lung fibrosis
    - Distinct finding in NTP comparison studies with MWFs

→ TRIM® VX selected for 2-year chronic toxicity and carcinogenicity studies

2-Year Exposure Concentration Selection Rationale

- Highest exposure concentration in rats and mice = 100 mg/m^3
  - Incidence and severity of lung fibrosis in rats and mice
  - Same concentrations used in 2-year CIMSTAR® 3800 study
    - Allows for direct comparison between MWF classes (soluble oil and semisynthetic) with the same exposure levels

2-Year Study Design

- Male and female Wistar Han rats and B6C3F1 mice
- Whole body inhalation exposure: 6 hr/d, 5 d/wk, 2 years
  - Liquid aerosols (1.7-2.2 μm MMAD)
  - Concentrations: 0, 10, 30, 100 mg/m^3
- 50 animals/species/sex/concentration
- Endpoints
  - In life (survival, clinical observations, body weights)
  - Gross/histopathology

MMAD = mass median aerodynamic diameter

Rats: 2-Year Study Results

- Survival
  - No effect on male or female rats
- Clinical observations
  - No effect on male or female rats
- Body weights
  - No effect on male or female rats
- Histopathology
  - Respiratory tract was the major target in males and females

Lung – Equivocal Evidence

Concentration (mg/m^3):	0	10	30	100
Males	50	50	50	50
Alveolar/Bronchiolar Adenoma	0	0	0	1
Alveolar/Bronchiolar Carcinoma	0†	0	0	2
Alveolar/Bronchiolar Adenoma or Carcinoma[a]	**0†**	**0**	**0**	**3**
Females	50	50	50	50
Alveolar/Bronchiolar Adenoma[b]	**0†**	**0**	**1**	**3**

† Significant (p≤0.05) Poly-3 trend test

[a] Historical Control in male Wistar Han rats
Inhalation = 4/150 (range 0-6%); All routes = 4/299 (range 0-6%)

[b] Historical Controls in female Wistar Han rats
Inhalation = 0/150; All routes = 0/300

Rats: Nonneoplastic Lesions

Lung

Concentration (mg/m^3):	0	10	30	100
Males	50	50	50	50
Fibrosis	4(1.0)	43**(1.0)	45**(1.4)	49**(1.3)
Infiltration Cellular, Histiocyte	14(1.0)	50**(1.4)	50**(2.0)	50**(2.6)
Inflammation, Chronic Active	7(1.0)	46**(1.0)	46**(2.0)	48**(3.0)
Alveolar/bronchiolar Epithelium, Hyperplasia	4(1.0)	22**(1.0)	39**(1.2)	46**(1.8)
Alveolar Epithelium, Hyperplasia	11(1.2)	43**(1.3)	45**(1.5)	49**(2.1)
Alveolar Epithelium, Metaplasia, Squamous	0	0	0	5*(1.4)
Alveolus, Proteinosis	0	1(1.0)	31**(1.2)	45**(2.2)
Bronchus-associated Lymphoid Tissue, Hyperplasia, Lymphohistiocytic	0	1(1.0)	4(1.3)	6*(1.0)

* Significantly different ($p \leq 0.05$; **$p \leq 0.01$) from chamber control by Poly-3 test
Data in parentheses are average severities (1=minimal; 2=mild; 3=moderate; 4=marked)

- **Similar lesions observed in female rats**
- **A spectrum of nonneoplastic lesions were also seen in the nose, larynx, and lymph nodes**

Mice: 2-Year Study Results

- Survival
  - No effect on male or female rats
- Clinical observations
  - No effect on male or female rats
- Body weights
  - No effect on male or female rats
- Histopathology
  - Respiratory tract was the major target in males and females

Mice: Neoplastic Lesions

Lung – Clear Evidence

Concentration (mg/m^3):	0	10	30	100
Males	50	50	49	50
Alveolar/Bronchiolar Adenoma (includes multiples)	6	8	5	9
Alveolar/Bronchiolar Adenoma, Multiple	1	1	0	3
Alveolar/Bronchiolar Carcinoma (includes multiples)	10	8	9	17
Alveolar/Bronchiolar Carcinoma, Multiple	2††	0	2	8*
Alveolar/Bronchiolar Adenoma or Carcinoma[a]	**14†**	**14**	**11**	**23***

† Significant (p≤0.05; †† ≤ 0.01) Poly-3 trend test

* Significantly different p≤0.05 from chamber control by Poly-3 test

[a] Historical controls for male B6C3F1/N mice
Inhalation = 69/250 (range = 26-32%); All routes = 147/550 (range = 16-38%)

Lung – Clear Evidence

Concentration (mg/m^3):	0	10	30	100
Females	50	50	50	50
Alveolar/Bronchiolar Adenoma (includes multiples)	4	5	3	8
Alveolar/Bronchiolar Adenoma, Multiple	0	0	0	2
Alveolar/Bronchiolar Carcinoma (includes multiples)[a]	5††	3	6	14*
Alveolar/Bronchiolar Carcinoma, Multiple	2	0	1	5
Alveolar/Bronchiolar Adenoma or Carcinoma[b]	**9††**	**8**	**8**	**20***

†† Significant ($p \leq 0.01$) Poly-3 trend test

* Significantly different $p \leq 0.05$ from chamber control by Poly-3 test

[a] Historical controls for female B6C3F1/N mice
Inhalation = 17/249 (range = 2-10%); All routes = 24/549 (range = 0-10%)

[b] Historical controls for female B6C3F1/N mice
Inhalation = 28/249 (range = 6-18%); All routes = 50/549 (range = 2-18%)

Mice: Nonneoplastic Lesions

Lung

Concentration (mg/m^3):	0	10	30	100
Males	50	50	49	50
Fibrosis	0	2(1.0)	5*(1.2)	45**(1.3)
Infiltration Cellular, Histiocyte	5(2.0)	9(1.6)	15*(1.5)	49**(2.0)
Inflammation, Chronic	5(1.4)	12(1.0)	16**(1.3)	50**(2.4)
Alveolar/bronchiolar Epithelium, Hyperplasia	3(3.0)	7(1.4)	15**(1.3)	50**(2.3)
Alveolar Epithelium, Hyperplasia	3(2.0)	3(1.0)	7(1.1)	47**(1.7)

Significantly different ($p \leq 0.05$; $^{**}p \leq 0.01$) from chamber control by Poly-3 test
Data in parentheses are average severities (1=minimal; 2=mild; 3=moderate; 4=marked)

- **Similar lesions observed in female rats**
- **A spectrum of nonneoplastic lesions were also seen in the nose, larynx, and lymph nodes**

TRIM® VX: Conclusions

- Male Rats: **Equivocal Evidence**
  - based on the combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung
- Female Rats: **Equivocal Evidence**
  - based on the occurrences of alveolar/bronchiolar adenoma of the lung
- Male Mice: **Clear Evidence**
  - based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung
- Female Mice: **Clear Evidence**
  - based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung

TRIM® VX: Conclusions

- Nonneoplastic lesions:
  - Lung, nose, and larynx in male and female rats and mice
  - Bronchial lymph node in male and female rats and male mice, and the mediastinal lymph node in male and female rats

Questions