UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: June 16, 2006

ACTION MEMORANDUM

SUBJECT: Inert Reassessments: One Exemption from the Requirement of a Tolerance for Dimethyl sulfoxide (CAS Reg. No. 67-68-5)

FROM: Pauline Wagner, Chief Pauline Wagner 6/19/06
Inert Ingredient Assessment Branch

TO: Lois A. Rossi, Director
Registration Division

I. FQPA REASSESSMENT ACTION

Action: Reassessment of one inert ingredient exemption from the requirement of a tolerance. Current exemption is to be maintained.

Chemical: Dimethyl sulfoxide

Table 1. CFR and CAS Registry Numbers and Names

40 CFR	Inert Ingredients	Limits	Uses (Pesticidal)	CAS Reg. No. & Name
180.920	Dimethyl sulfoxide	(none)	Solvent or cosolvent for formulations used before crop emerges from soil or prior to formation of edible parts of food plants	67-68-5 Methane, sulfinylbis-

Use Summary: DMSO is used as a solvent for many organic compounds including fats, carbohydrates, dyes, resins, and polymers. It is also used to cryopreserve and store cultured cells. When mixed with water, DMSO is used as antifreeze or hydraulic fluid. DMSO is also used as an inert ingredient (solvent/cosolvent) in pesticide products.

II. MANAGEMENT CONCURRENCE

I concur with the reassessment of the one exemption from the requirement of a tolerance for the inert ingredient Dimethyl sulfoxide (CAS Reg. No. 67-68-5). I consider the one exemption, established under 40 CFR 180.920 [formerly 40 CFR 180.1001(d)], to be reassessed for purposes of FFDCA's section 408(q) as of the date of my signature, below. A Federal Register Notice regarding this tolerance exemption reassessment decision will be published in the near future.

Lois A. Rossi

Lois A. Rossi, Director
Registration Division

Date: June 20, 2006

CC: Debbie Edwards, SRRD
Joe Nevola, SRRD

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TOXIC SUBSTANCES

June 16, 2006

MEMORANDUM

SUBJECT: Reassessment of the One Exemption from the Requirement of a Tolerance for Dimethyl Sulfoxide (DMSO; CAS Reg. No. 67-68-5)

FROM: R. Tracy Ward R Tracy Ward
Inert Ingredient Assessment Branch (IIAB)
Registration Division (7505P)

And

Linda Taylor
Reregistration Branch I
Health Effects Division (7509P)

TO: Pauline Wagner, Chief
Inert Ingredient Assessment Branch (IIAB)
Registration Division (7505P)

Background

Attached is the science assessment for dimethyl sulfoxide (DMSO; CAS Reg. No. 67-68-5). DMSO has one exemption from the requirement of a tolerance under 40 CFR 180.920. This assessment summarizes available information on the use, physical/chemical properties, toxicological effects, exposure profile, environmental fate, and ecotoxicity of DMSO. The purpose of this document is to reassess the existing exemption from the requirement of a tolerance for residues of DMSO when used as an inert ingredient (solvent or cosolvent) in pesticide formulations as required under the Food Quality Protection Act (FQPA).

Executive Summary

This report evaluates DMSO, a pesticide inert ingredient for which one exemption from the requirement of tolerance exist. The exemption is for the use of DMSO as a solvent or cosolvent in pesticide formulations applied before the crop emerges from the soil or prior to formation of edible parts of food plants under 40 CFR 180.920.

DMSO is sponsored under the U.S. EPA's High Production Volume (HPV) Challenge Program (http://www.epa.gov/chemrtk/hpvrstp.htm) by the Dimethyl Sulfoxide Producer's Association (2003). The Association submitted an International Uniform Chemical Information Database (IUCLID, 2003) summary for DMSO. The goal of the HPV Challenge Program is to collect and make publicly available a complete set of baseline health and environmental effects data on those chemicals that are manufactured in, or imported into, the United States in amounts equal to or exceeding one million pounds per year. Industry sponsors volunteer to evaluate the adequacy of existing data and to conduct tests where needed to fill the gaps in the data, and EPA (and the public) has an opportunity to review and comment on the sponsors' robust summary report. A robust summary has been submitted for DMSO and the relevant information has been made part of this assessment.

A summary of the relevant scientific information on the potential human health effects of a group of 137 flavouring agents (simple aliphatic and aromatic sulfides and thiols), including DMSO, was prepared by the 53rd meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA; WHO 2000). This reassessment document was developed from extracts of the above JECFA document as well as information in the open literature.

DMSO has low acute toxicity via the oral, and inhalation routes of exposure in animal studies, but is a dermal and gastric irritant. The chemical properties of DMSO allow it to pass rapidly through biological membranes, such as the skin. The eye has been shown to be a target tissue following subchronic and/or chronic exposure via oral and/or dermal routes of exposure. Levels exceeding the limit dose in subchronic and chronic oral and dermal exposures to DMSO resulted in lenticular changes in the eyes. Developmental toxicity (decreased fetal body weight and delayed rib ossification) was observed only at maternally toxic concentrations of DMSO that were at or greater than the limit dose. Studies suggest that the central nervous system is not a target tissue for the chemical. DMSO was not a carcinogen. There are mixed results in mutagenicity studies, but the majority of the data do not suggest a significant genotoxic risk from exposure to DMSO.

Residential (inhalation and dermal) exposures to DMSO are possible, but as an inert ingredient in pesticide formulations, it is not expected to be available at levels that would cause toxic effects or produce skin irritation. Dietary (food and drinking water) exposures of concern to residues of DMSO are not expected due to its use patterns and its physical and chemical properties, including high volatility and rapid photodegradation in the ambient air.

Taking into consideration all available information on DMSO, it has been determined that there is a reasonable certainty that no harm to any population subgroup will result from aggregate exposure to DMSO when considering exposure through food commodities and all other non-occupational sources for which there is reliable information. Therefore, it is recommended that the one exemption from the requirement

of a tolerance established for residues of DMSO when used as an inert ingredient in pesticide formulations can be considered reassessed as safe under section 408(q) of the Federal Food, Drug, and Cosmetic Act (FFDCA).

I. Introduction

This report provides a qualitative assessment for DMSO, an inert ingredient for which one exemption from the requirement of tolerance exists when used in pesticide formulations. This exemption is for the use of DMSO as a solvent or cosolvent for formulations applied before the crop emerges from the soil or prior to formation of edible parts of food plants under 40 CFR 180.920. .

II. Use Information

A. Pesticide Uses

DMSO is used as an inert ingredient (solvent/cosolvent) in pesticide products. The tolerance exemption for this chemical is presented below in Table 1.

Table 1. Pesticide Uses

CFR Citation				CAS Reg. No. 9CI Name
40 CFR	Inert Ingredients	Limits	Uses	
180.920[a]	Dimethyl sulfoxide	None	Solvent or cosolvent for formulations used before crop emerges from soil or prior to formation of edible parts of food plants	67-68-5 Methane, sulfinylbis-

[a] Residues listed in 40 CFR 180.920 are exempt from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops only.

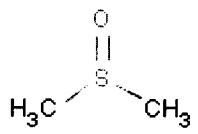
B. Other Uses

DMSO is used as a solvent for many organic compounds including fats, carbohydrates, dyes, resins, and polymers (HSDB 2006). It is also used to cryopreserve and store cultured cells. When mixed with water, DMSO is used as antifreeze or hydraulic fluid.

III. Physical and Chemical Properties

Some physical and chemical characteristics of DMSO, along with its structure and nomenclature, are found in Table 2.

Table 2. Physical and Chemical Properties of DMSO*

Parameter	Value	Reference
Structure		ChemIDPlus 2004
CAS Reg. Number	67-68-5	
Molecular Formula	C_2-H_6-O-S	ChemIDPlus 2004
Molecular Weight	78.13	HSDB 2006
Synonyms	Delton, Demasorb, Dimexide, Domoso, Methyl sulfoxide, Methyl sulfinylmethane	ChemIDPlus 2004
Odor	Slightly sulfurous odor	HSDB 2006
Physical State	Practically colorless liquid or crystals	HSDB 2006
Melting Point	18.5 °C*	ChemIDPlus 2004
Boiling Point	189 °C*	ChemIDPlus 2004
Water Solubility	1.0×10^6 mg/L*; Miscible	ChemIDPlus 2004; HSDB 2006
Other Solubility	Soluble in ethanol, acetone, ether, benzene, chloroform	HSDB 2006
Vapor Pressure	0.61 mm Hg @ 25 °C*	ChemIDPlus 2004
Log K_{ow}	-1.35*	ChemIDPlus 2004
Henry's Law Constant	1.51×10^{-9} atm-m^3/mole @ 25 °C*	ChemIDPlus 2004
Atmospheric OH Rate Constant	6.2×10^{-11} cm^3/molecule-sec @ 25 °C*	ChemIDPlus 2004

*Measured values.

IV. Hazard Assessment

The current document was developed from relevant information from the IUCLID summary (2003), the WHO (2000) summary, and select information in the open literature.

A. Hazard Profile

The available toxicity database for DMSO consists of acute, subchronic (oral, dermal, and inhalation), chronic (oral), and rat and rabbit developmental (gavage) studies in animals, as well as mutagenicity studies.

DMSO is of low acute toxicity via the oral, and inhalation routes of exposure, but it is a dermal and gastric irritant. The chemical properties of DMSO allow it to pass rapidly through biological membranes, such as the skin, and it may enhance the

penetration of other substances, serving as a carrier for compounds applied to the skin. DMSO is extremely hygroscopic, and it is a good solvent for a wide range of chemicals. The eye has been shown to be a target tissue in rats, rabbits, pigs, and dogs following subchronic and/or chronic exposure via oral and/or dermal routes of exposure. No developmental toxicity is observed following oral exposure to rats and rabbits. Predominantly negative results are found with DMSO in short-term *in vitro* and *in vivo* tests of genetic toxicity. However, DMSO was positive in *Salmonella typhimurium* strains TA1537 and TA2637 and in *E. coli* WP2uvrA at fairly high concentrations, with and without metabolic activation, and there was a dose-related increase in the frequencies of cytogenetic aberrations in an *in vivo* study.

B. Metabolism and Pharmacokinetics

Dimethyl sulfoxide is metabolized to either dimethyl sulfone or dimethyl sulfide. Dimethyl sulfide is a volatile metabolite responsible for the garlic odor of exhaled air (1%). Approximately 85% of DMSO and its metabolites are excreted in both urine (50%) and feces (50%) (IUCLID, 2003).

The absorption, metabolism and excretion of DMSO have been studied in rhesus monkeys given a daily oral dose of 3 g/kg body weight for 14 days (Layman & Jacob, 1985). DMSO was rapidly absorbed, reached a peak serum concentration after about 4 hours and was cleared from the blood within 72 hours after treatment ended. Dimethyl sulfone (DMS) was detected in the blood 2 hours after treatment and reached a steady-state concentration after 4 days and was cleared from the blood 120 hours after treatment ended. Urinary excretion of DMSO and dimethyl sulfone accounted for 60% and 16%, respectively, of the total ingested dose. Neither DMSO nor DMS was detected in the feces. The half-life of DMSO in the rhesus monkey was calculated to be about 38 hours and its elimination rate constant equaled about 2% per hour (Layman and Jacob, 1985).

Following topical application, DMSO is absorbed and widely distributed in tissue and body fluids. DMSO and DMS are excreted in the urine and feces. DMSO is eliminated through the breath and skin as dimethyl sulphide, which is responsible for the characteristic garlic odor. No residual accumulation of DMSO in tissues has occurred after treatment for protracted periods of time (Novak, 2005).

Little information is available concerning the mechanism by which DMSO enhances skin permeability. It has been suggested that DMSO: (1) removes much of the lipid matrix of the stratum corneum, making holes or artificial shunts in the penetration barrier; (2) produces reversible configurational changes in protein structure brought about by substitution of integral water molecules; and (3) functions as a swelling agent (Amdur, Doull and Klassen, 1991).

C. Toxicological Data

Acute Toxicity

DMSO administered orally is of low acute toxicity to rats and mice (Table 3). After massive single doses, experimental animals exhibited rapid breathing, restlessness, and coma, leading to hypothermia and death within a few hours (Gosselin, *et al.*, 1984). Administration of pure DMSO (100%) resulted in serious and rapid modification of the red blood cells, along with "certain coagulation defects" (Caujolle, *et al.*, 1967). Lethal oral doses caused ataxia, myasthenia, decreased motor activity, and bradypnea shortly after administration (Willson, *et al.*, 1965). Non-lethal oral doses produced only decreased motor activity, although polydipsia and polyuria were noted in rats following doses of 20 g/kg. Hyperemia and inflammation in the eyes of Sprague-Dawley rats was observed following single oral doses of ≥13 g/kg DMSO.

DMSO is of low acute toxicity by the dermal route. Studies have demonstrated that DMSO is a skin irritant when used in sufficiently high or multiple doses, but it is not a serious eye irritant. Mild pulmonary irritation was observed in an acute inhalation study in rats (Fishman, *et al*, 1969). When applied intragastrically for 10 minutes, DMSO caused extensive mucosal damage in rats, and it was concluded that DMSO is a gastric irritant (Sorbye, *et al.*, 1993).

Table 3. Summary of Acute Toxicity Data for DMSO

Parameter	Toxicity Value	Reference
Oral LD_{50}	Rat (14.5-28.3 g/kg) Mouse (16.5-21.4 g/kg)	Sommer and Tauberger, 1964; Willson, *et al.* (1965) Fishman, *et al.* (1969)
Oral LD_{50}	Rat (>20 mL/kg) Mouse (20 mL/kg)	Brown, *et al.* (1963)
Oral LD_{50}	Rat (>15.0 mg/kg (50% DMSO)) Mouse (>14.0 mg/kg (50% DMSO))	Caujolle, *et al.* (1967)
Oral LD_{50}	Mouse 21400 mg/kg	Rosenkrantz, *et al.*, (1963)
Oral LD_{50}	Mouse 7920 mg/kg	Lewis (1996)
Dermal LD_{50}	Rat (40-50 g/kg) Mouse (40 g/kg)	Mason (1971)
Inhalation, Rat	Mild pulmonary irritation (edema) 1600 mg/m^3 (4 hr) – 2900 mg/m^3 (24 hr) 2000 mg/m^3 (40 hr)	Fishman, *et al.* (1969)
Eye Irritation, Rabbit	Slight conjunctivitis at 24-hour observation period; cleared by 48 hours	Fishman, *et al.* (1969)
Eye Irritation, Rabbit	Undiluted; no effects	Brown, *et al.* (1963)
Skin Irritation, Rabbit	Slight erythema	Fishman, *et al.* (1969)
Sensitization, Guinea Pig	Allergic contact dermatitis	Wright and Winter (1966)

Subchronic Toxicity

ORAL Young adult beagle dogs (6/sex/group) received daily oral doses of 0, 2.5, 5, 10, 20, or 40 g/kg/day DMSO, 5 days/week for up to 23 weeks (Rubin and Barnett, 1967). Dose levels of 20 and 40 g/kg/day were not tolerated well and were reduced. After 9 weeks of administration, changes in the lens of the eye were observed in dogs receiving 5 g/kg/day, and by the 18th week, all dogs at the lowest dose level were affected similarly. After 23 weeks, treatment was stopped and the dogs were observed for 31 weeks. Eye changes persisted after withdrawal of DMSO but became less pronounced.

New Zealand rabbits were treated orally (drinking water) with 0.5 g/kg DMSO/day or 10 g/kg/day for 24 weeks (Wood and Wirth, 1969). The 10 g/kg rabbits exhibited progressive changes in the optical lenses when examined with the biomicroscope at 2, 6, 12, and 24 weeks. The effect was characterized by refractile changes, and there were alterations in the composition of lens proteins. No adverse effects were observed in the rabbits dosed at 0.5 g/kg/day.

DERMAL No discernable effects were observed on the skin following undiluted application of DMSO to the clipped backs of guinea pigs daily for 28 days or to the skin of hairless mice twice a week for 30 weeks (Brown, *et al.*, 1963). In a similar study (Wright and Winer, 1966), undiluted DMSO was applied to the shaved backs of guinea pigs 4 times/day for 10-42 days of dosing spread over 63 days. Erythema was observed by day 2 of treatment, which gradually decreased during the next 6-7 days; dryness (day 6) and marked scaling and local edema occurred by day 9, which were more severe by day 12. By day 21, the sites were indurated and thickened to palpation. Dryness, scaling, and thickening were present on day 63. Mild to moderate thickening was still present 6 months after treatment ceased. Microscopic changes consisted of hyperkeratosis of the stratum corneum and thickening of the stratum granulosum and stratum malpighii, spongiosis, and intracellular edema, vesiculation, and hyperkeratosis consistent with an allergic contact dermatitis.

INHALATION In an inhalation study, rats were exposed to DMSO at a concentration of 211 mg/m^3 for 7 hours/day, 5 days/week, for 30 exposures (Fishman, *et al.*, 1969). There were no overt signs of toxicity and all animals gained weight. No treatment-related effects were observed in hemoglobin concentration, microhematocrit, total leukocyte counts, reticulocyte counts, serum glutamic-pyruvic and glutamic-oxaloacetic transaminase activities, or serum urea nitrogen. There were no treatment-related gross or microscopic findings at necropsy. Non-specific inflammatory changes were noted in the lungs and livers of nearly all animals, including controls. Absorption of DMSO through the skin could not be ruled out in this study.

Chronic Toxicity

ORAL In a chronic oral toxicity study (Noel, *et al.,* 1975), 50 Sprague-Dawley rats/sex/group and groups of 5 pure-bred Pembrokeshire Corgi dogs/sex/group were administered DMSO [a 50% aqueous solution containing DMSO doses of 0, 1, 3, or 9 mL/kg/day (1100, 3300, or 9900 mg/kg/day)] via gavage 5 days/week for 18 months (rats, dogs) or 24 months (dogs). Clinical signs, body weight, and food consumption were monitored, and periodic assessments of hematological, clinical chemistry and urinary parameters, ophthalmoscopic examination, and gross and histopathological examinations were performed.

Male rats exhibited a dose-related decrease in body-weight gain and a slight reduction in hemoglobin and packed cell volume at the high dose. Although no changes were observed in the retina or vitreous humor, a small number had some degree of change in the refractive index at 9 mL/kg/day.

For the dogs, no treatment-related effects were observed on body weight. At the mid- and high-dose levels, persistent diuresis but no renal damage was observed. Increased packed-cell volume and hemoglobin levels were observed at the high dose, although the erythrocytes had normal hemoglobin concentrations and were of normal size. Lenticular changes (alterations in the refractive index of the central portion of the lens, lens opacity, opalescence in the central region of the lens, and/or changes in vitreous humor) and biochemical changes in the lens (an increase in insoluble protein and reductions in soluble protein, glutathione, and water) were observed. Ocular changes were evident before 10 weeks at the high dose, with obvious progression with continued dosing. The sequence of changes occurred at the 3 mL/kg/day dose level, although the onset of changes was delayed. At the low dose, nuclear refractive changes were observed after 6 months, but none of these dogs had opalescence. Dogs withdrawn from the study at 18 weeks showed partial (high-dose) to complete (mid-dose) recovery by two years. No other histopathological abnormalities were observed. The LOAEL is 1100 mg/kg/day, based on ophthalmologic changes, and thus, no NOAEL could be determined for this study; however, it is noted that the effects at this dose manifested only after a considerable period of time (greater than 6 months), and there were no "progression signs."

ORAL/DERMAL Rhesus monkeys (4-6 monkeys/group) were administered DMSO (90% solution) via gavage or dermal (abdominal skin) application in daily doses (administered in equally-divided portions each morning and afternoon) of 0, 1, 3, or 9 mL/kg/day (0, 0.099, 2.97, or 8.91 g/kg/day) over an 18-month (74-87 weeks) period (Vogin, *et al.*, 1970). Five of the 6 gavage high-dose monkeys died due to DMSO exposure (time of deaths not reported, but one was dosed for 15 weeks, three were dosed for 39 weeks and one for 53 weeks). These monkeys exhibited marked body-weight loss and suffered from anorexia and emesis, especially during the first 6 weeks. Histologic examination of these animals revealed emphysema and atelectasis, possibly the result of regurgitation and/or aspiration of DMSO. No other treatment-related changes were observed (clinical signs, hematology, clinical chemistry, ophthalmoscopy,

urinalysis, or organ weights). It was concluded that rhesus monkeys tolerated DMSO administered daily for 18 months at dose levels up to 3 mL/kg/day orally (2.97 g/kg/day; NOAEL). In an earlier report (Rubin and Mattis, 1966, as cited in Vogin, *et al.*, 1970; cited as 5 g/kg/day in Noel, *et al.*, 1975), no lenticular changes were observed in rhesus monkeys receiving DMSO doses of 5 g/kg/day for 100 days. It is noted that the findings in another earlier study (Barnett and Noel, 1967) of lenticular changes visible within 14 weeks (dosed 5 days/week for 6 months) in monkeys receiving 9 mL/kg/day and suspected at 3 mL/kg/day were not reproducible.

DERMAL The dermally-exposed monkeys had scaling and flaking of the skin at the site of application (Vogin, *et al.*, 1970). Treatment-related effects were not observed in any of the remaining animals (body weight, blood pressure, heart rate, respiratory rate, body temperature, water consumption, neurologic reflexes, ophthalmologic findings, electrocardiograms, hematologic studies, blood chemistry, urinalysis, and gross and microscopic examinations). It was concluded that rhesus monkeys tolerated DMSO administered daily *via* the dermal route of exposure for 18 months at dose levels up to 9 mL/kg/day (8.91 g/kg/day).

In similar but less extensive dermal studies, pigs (normal skin) and rabbits (normal and abraded skin) were treated with DMSO at dose levels of 0, 1.5, 2.7, 4.5, or 8.1 mL/kg/day for 58 weeks and 26 weeks, respectively (Noel, *et al.*, 1975). Pigs exhibited transient depression in body-weight gain at the high-dose level and had abnormalities of the lens, similar to those observed in the dog at 2.7, 4.5, or 8.1 mL/kg/day. The severity of the refractory changes was dose-related but did not increase with time. The rabbits also displayed a dose-related refractory change in the lens, similar to that in the dog.

Neurotoxicity

No relevant neurotoxicity data were identified for DMSO. *In vitro* studies have shown that moderate doses of DMSO (not specified) have resulted in nerve blockade and mild cholinesterase inhibition (Gosselin, *et al.*, 1984).

Mutagenicity

DMSO was negative for mutagenicity in the reverse mutation assays in strains TA97, TA98, TA100, TA102, TA 1535, TA1537, and TA1538 at concentrations up to 300,000 μg/plate, as summarized in Table 4 (WHO, 2000). DMSO was mutagenic in *Salmonella typhimurium* strains TA1537 and TA2637 at concentrations of 0.1-0.4 mL/plate and in *E. coli* WP2uvrA at concentrations of 0.2-0.4 mL/plate, with and without metabolic activation (Hakura, *et al.*, 1993); however, the concentrations were relatively high with some being cytotoxic. Additionally, DMSO was mutagenic in the *umu* test with *Salmonella typhimurium* strains TA1535/pSK1002 carrying the *umuC-lacZ* fusion gene (Nakamura, *et al.*, 1990). The *umu* gene expression was detected only at high-dose levels (5%-15%). The level of β-galactosidase activity, which reflects *umu* expression was increased in a concentration-related pattern (121-313 units compared to 90 units in

control). There was a dose-related increase in gene conversions at certain loci of log phase cells of the D4 strain of *Saccharomyces cerevisiae* following exposure to dose levels up to 1.4 M for 4 hours at 37°C. The gene conversions were attributed to the metabolic conversion of DMSO to a genetically active compound by cytochrome P-450 mixed function oxidation reactions.

Table 4. Genotoxicity-Mutation Assays

Bacterial strain	DMSO concentration	Results (± = w/ & w/o Metabolic Activation)	Reference
Reverse Mutation Assays in *Salmonella typhimurium*			
TA97, TA98, TA100, TA102	0.005-10 µmol/plate	Negative (±)	Karekar, *et al.*, (cited in WHO)
TA98, TA100	12.5-200 µg/plate	Negative (±)	Wang, *et al.*, (cited in WHO)
TA97, TA98, TA100	100000-300000 µg/plate	Negative (±)	Brams *et al.*, (cited in WHO)
TA97, TA98, TA100, TA1535, TA1537	100-10000 µg/plate	Negative (±)	Zeiger *et al.*, (cited in WHO)
TA97, TA98, TA100, TA102, TA104, TA1535, TA1538	0.1-0.4 mL/plate	Negative (±)	Hakura, *et al.*, (1993)
TA1537, TA2637	0.1-0.4 mL/plate	Positive at cytotoxic concentrations	Hakura, *et al.*, (1993)
Forward Mutation Assays in *Saccharomyces*			
Saccharomyces cerevisiae D4 strain	0.5, 1.0, or 1.4 M DMSO for 4 hours at 37°C	Dose-related increase in gene conversions at certain loci of log phase cells; attributed to metabolic conversion to a genetically active compound by P-450-dependent MFO reactions	Callen and Philpot; cited in Smith, *et al.*, (1983)
Schizosaccharomyces pombe P_1 strain	0.5, 2.0, 5%	Negative effect on forward mutation rate of yeast cells; ± mouse S-9; moderate toxicity demonstrated	Aravindakshan, *et al.*; cited in Smith, *et al.*, (1983)

DMSO did not induce dominant lethality in male rats injected intraperitoneally at a dose level of 1 mL/kg/day for 10 weeks (Sheu and Green, 1979), or in Swiss mice injected with doses up to 10 g/kg twice at intervals of about 20 hours (Aravindakshan, *et al.*, 1975). A summary of gene mutation studies is provided in Table 5.

Table 5. Genotoxicity-Chromosomal and Other

Cells	Dose/ Duration	Results	Reference
Chromosomal Aberrations/Chromosomal Breaks/chromatid exchanges			
Femoral bone marrow cells – SD rat; i.p. injection	5 mL/kg of 1%, 10%, 50%, or	Negative; but combining frequencies for cytogenetic	Kapp and Eventoff,

Cells	Dose/ Duration	Results	Reference
	100% for 5 days	aberrations (chromosome breaks, chromatid breaks, markers, and severely damaged cells) shows an increased incidence of aberrant cells/rat from 10% at 1% DMSO to 69% at 100% DMSO	(1980)
Chinese hamster ovary cells	*In vitro* up to 10% DMSO	Chromosome aberrations induced only in presence of rat liver S-9 at 10%; Negative at 1% DMSO ±S-9; Number of sister chromatid exchanges unaffected	Tates and Kriek, (1981)
Oocytes of Drosophila melanogaster	2% solution DMSO	Did not induce aneuploidy (numerical chromosome aberrations	Traut, (1983)
Cell cultures (trout gonads)	0.5% DMSO	No significant change in incidence of anaphase chromosomal aberrations	Kocan, *et al.* (1982)
3-day and 4-day old Chick embryos	*In vivo* application of 1% DMSO	No increase in chromosome breakage or sister chromatid exchanges	Bloom, (1982)
Other			
Male rats	*i.p.* injection of 1 mL/kg/day for 10 weeks	Did not induce dominant lethality in mated females	Sheu and Green (1979)
Male Swiss mice	*i.p.* injection with 5, 7.5, or 10 g/kg DMSO twice at interval of .20 hours	Did not induce dominant lethality	Aravindashan, *et al.* (1975)
Chinese hamster V79 cells	10 mM, *in vitro*	Did not induce DNA damage	Swenberg, *et al.* (1976)

Developmental and Reproductive Toxicity

In a developmental toxicity study, groups of 25 female Sprague-Dawley rats were administered DMSO via gavage at dose levels of 0, 200, 1000, or 5000 mg/kg/day (in purified water) on gestation days 6-15. At 5000 mg/kg/day, maternal toxicity was observed, as evidenced by decreased bodyweight gain (32%) and food consumption (11%). Fetal body weights were decreased at 5000 mg/kg/day (7%). There was an increased incidence of dilated renal pelvis (all treated groups) and an increased incidence of dilated ureter at 5000 mg/kg/day. These findings were not accompanied by any microscopic changes in the kidneys, and were not considered to be an adverse effect, but might be related to the diuretic properties of DMSO. At 5000 mg/kg/day, delayed ossification of the ribs was observed in the fetuses, which may be related to the

decreased fetal body weight. There were no treatment-related soft tissue malformations or skeletal variations or malformations. The maternal NOAEL is 1000 mg/kg/day, and the maternal LOAEL is 5000 mg/kg/day, based on decreased bodyweight gain and food consumption. The developmental toxicity NOAEL is 1000 mg/kg/day, and the developmental toxicity LOAEL is 5000 mg/kg/day, based on decreased fetal body weight and delayed rib ossification (Regnier and Richard, 1998).

In a study in rabbits (5 g/kg/day of 50% DMSO administered on days 6-14 of gestation), no teratogenic effects were reported (Caujolle et al., 1967).

On day 9 of gestation, pregnant ICR mice were exposed via a percutaneous application by dipping the lower right appendage in DMSO solutions of 0, 0.04%, 0.4%, or 4% for 20 seconds. A 20 second application of 0.04% DMSO is equivalent to a blood concentration of 19 ppm. Embryos were examined microscopically for abnormalities 1 day after exposure. No information was provided for the dams. The percentages of abnormalities in the 10-day old embryos were: control (4%); at 0.04% (60%); at 0.4% (68%); at 4% (88%). Average litter size at delivery was decreased relative to control at each dose level, but there was no clear dose response [8.5 (control); 6.4 (0.04%); 7.5 (0.4%); 6.0 (4%)]. Based on the information provided, the LOAEL for developmental toxicity would be less than or equal to 0.04% DMSO; no NOAEL was determined (Schmitt, 1988).

No reproductive toxicity studies were located on DMSO.

Carcinogenicity

From Smith *et al.*, 1983:

The potential carcinogenicity of DMSO has been evaluated using *in vivo* and *in vitro* systems. DMSO produced mild dyskeratosis but no carcinogenic transformations when applied to hamster cheek epithelium (Elzay, 1967). Negative results were found for cell transformations in cell cultures of Syrian hamster embryos (Pienta, 1980) and hamster sternal hyaline cartilage (Katoh, 1977). Oral doses of 2.5 mL and 5.0 mL of undiluted DMSO to male Wistar rats had no effect on the number of mitosis in cells of the adrenal cortex, which was considered supporting evidence that DMSO is not a tumor promotor or active carcinogen (Danz and Urban, 1979). No recent reports concerning the carcinogenicity of DMSO were found.

D. Special Considerations for Infants and Children

In acute, subchronic, and chronic studies, DMSO has been demonstrated to be of low toxicity. Developmental toxicity was observed only at maternally toxic doses at concentrations of DMSO at or greater than the limit dose. Based on this information, there is no concern, at this time, for increased sensitivity to infants and children to DMSO when used as an inert ingredient in pesticide formulations. For the same reason, a safety factor analysis has not been used to assess risk and, therefore, the

additional tenfold safety factor for the protection of infants and children is also unnecessary.

V. Environmental Fate Characterization/Drinking Water Considerations

From the HSDB (2006):

"Reduced sulfur compounds are biologically produced in soils, water, and vegetation and are major natural contributors to atmospheric sulfur. Dimethyl sulfoxide is produced and released into seawater by phytoplankton, as is dimethyl sulfide. Dimethyl sulfide, which is estimated to comprise 90% of the reduced sulfur flux from the ocean to the atmosphere, is subsequently oxidized to dimethyl sulfoxide and then sulfur dioxide and sulfate as part of the global atmospheric sulfur cycle. It has also been shown that dimethyl sulfide is readily photooxidized in aqueous solution in the presence of photosensitizers. The fact that two moles of dimethyl sulfide is consumed for each mole of oxygen is consistent with the formation of dimethyl sulfoxide."

"Dimethyl sulfoxide is part of the global atmospheric sulfur cycle and is produced when dimethyl sulfide is photooxidized. If released to air, a vapor pressure of $6.1X10^{-1}$ mm Hg at 25 deg C indicates dimethyl sulfoxide will exist solely as a vapor phase in the atmosphere. Vapor-phase dimethyl sulfoxide will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 6.2-6.6 hours. Dimethyl sulfoxide does not absorb light at wavelengths >290 nm and therefore is not expected to be susceptible to direct photolysis by sunlight. If released to soil, dimethyl sulfoxide is expected to have very high mobility based upon an estimated Koc of 4. Volatilization from water and moist soil surfaces is not expected to be an important fate process based upon a Henry's Law constant of $1.5X10^{-9}$ atm-cu m/mole. Dimethyl sulfoxide is expected to slowly volatilize from dry soil surfaces based upon its vapor pressure. A 3% theoretical biological oxygen demand (BOD) after 2 weeks in activated sludge indicates that biodegradation is not expected to be an important environmental fate process. If released into water, dimethyl sulfoxide is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. A low experimental bioconcentration factor (BCF) of <1 suggests that bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions. Monitoring data indicate that the general population may be exposed to dimethyl sulfoxide *via* inhalation of ambient air and ingestion of or dermal contact with food and water contaminated with dimethyl sulfoxide."

DMSO is highly volatile and quickly degraded photochemically in the ambient air, and is, therefore, unlikely to reach surface waters at levels of concern from its use as an inert ingredient in pesticide formulations.

VI. Exposure Assessment

Dietary (food and drinking water) exposures of concern to residues of DMSO are not expected due to its use patterns (before crop emerges from soil or before edible parts form, or on peas only) and its physical and chemical properties, including high volatility and rapid photodegradation. Residential (inhalation and dermal) exposures to DMSO are possible, but dermal exposures may be limited by its high volatility.

VII. Aggregate Exposure

In examining aggregate exposure, the FFDCA section 408 directs EPA to consider available information concerning exposures from the pesticide residue in food and all other nonoccupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

For DMSO, a qualitative assessment for all pathways of human exposure (food, drinking water, and residential) is appropriate given the lack of human health concerns associated with exposure to DMSO as an inert ingredient in pesticide formulations.

VIII. Cumulative Exposure

Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to DMSO and any other substances, and this material does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that DMSO has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative.

IX. Human Health Risk Characterization

DMSO has low acute toxicity via the oral, and inhalation routes of exposure in animal studies, but is a dermal and gastric irritant. Levels exceeding the limit dose in subchronic and chronic oral and dermal exposures to DMSO resulted in lenticular changes in the eyes. Developmental toxicity was observed only at maternally toxic concentrations of DMSO that were at or greater than the limit dose. Studies suggest

that the central nervous system is not a target tissue for the chemical. DMSO was not a carcinogen. There are mixed results in mutagenicity studies, but the majority of the data do not suggest a significant genotoxic risk from exposure to DMSO.

Residential (inhalation and dermal) exposures to DMSO are possible, but as an inert ingredient in pesticide formulations, it is not expected to be available at levels that would cause toxic effects or produce skin irritation. Dietary (food and drinking water) exposures of concern to residues of DMSO are not expected due to its use patterns and its physical and chemical properties, including high volatility and rapid photodegradation in the ambient air.

Taking into consideration all available information on DMSO, it has been determined that there is a reasonable certainty that no harm to any population subgroup will result from aggregate exposure to DMSO when considering exposure through food commodities and all other non-occupational sources for which there is reliable information. Therefore, it is recommended that the one exemption from the requirement of a tolerance established for residues of DMSO when used as a solvent/cosolvent can be considered reassessed as safe under section 408(q) of the FFDCA.

X. Ecotoxicity and Ecological Risk Characterization

DMSO appears to have very low toxicity to aquatic organisms including algae, invertebrates, and fish, based on studies found in EPA's Ecotox database (http://mountain.epa.gov/ecotox). In toxicity studies, green algae (*Dunaliella tertiolecta*) had an EC_{50} of approximately 45 g/L, water fleas (*Daphnia magna*) had an LC_{50} of 43 g/L, and brine shrimp (*Artemia salina*) had an LC_{50} of about 6.8 g/L. The LC_{50} in bluegill sunfish (*Lepomis macrochirus*) was > 400 ml/L, but 33 ml/L in rainbow trout (*Oncorhynchus mykiss*).

Using mammals as a surrogate for birds and other terrestrial phase animals (e.g., reptiles), dimethyl sulfoxide is likely to be practically nontoxic on an acute ingestion basis. However, it is important to note that due to its vapor pressure (0.61 mm Hg @ 25 °C), dimethyl sulfoxide is likely to exist in a vapor phase after application. Therefore, exposures via this route will likely be an important exposure pathway for a period following applications. Reproductive effects may occur if applications exceed 5 pounds per acre.

In a study looking at the mortality of mature earthworms (*Eisenia fetida*), no effects were reported at the highest dose tested after over 48 hours exposure to dimethyl sulfoxide in a 2-propanone carrier. The LC_{50} was characterized as >1000ug/cm^2. In a study of lethality in newts, rough-skinned newts (*Taricha granulosa*) were dosed once interperitoneally and observed for 300 days. Survival was affected at

a Lethal Threshold Concentration (LETC or an incipient LC_{50}) of 15 g/kg. In the same study, the newts were immersed in solutions of dimethyl sulfoxide ranging from 0.1% to 9% and observed for mortality over 300 days. The LETC was determined to be 2% for survival. Finally, in the same study, newts were intraveniously exposed to dimethyl sulfoxide at 15 mg/kg once and followed for 43 days; the study also included a control group. At the end of the exposure period, all animals were sacrificed and organs were weighed and compared to whole body weight for the control and treated groups.

Little difference was seen in the percent lipid fat, liver and spleen weights between control and treated groups.

In several studies looking at the effects of dimethyl sulfoxide (sulfinyl bis(methane)) over 10 days on germination of butter print, green amaranth, ragweed, quackgrass, common morninglory, narrowleaf plantain, field sorrel, curley dock, giant bristlegrass, green foxtail, Johnson grass, and Jimsonweed seeds by fumigation, no effects were reported at a dose of up to one milliliter (mL). Details of the study design were insufficient to determine the application rate equivalent or fumigant concentration. In a 28 day study of Jimsonweed seedlings, concentrations equivalent to 2% in formulation sprays applied to the plants resulted in no effects on chlorophyll production and biomass, but did should a slight decrease in stem plant size (11%) when compared to controls. In a study varying in duration from 5 to 19 days, wild carrot, soybean, and lettuce, plants were soaked/dipped in a solution of 1% to 2% dimethyl sulfoxide to determine the effects on biomass at the cellular level. Results indicated effects were likely at the 2% dose, however it was inconclusive as to whether these effects would result in long-term impacts to the plants or its ability to reproduce. Further review of the supporting literature is necessary before a definitive conclusion can be made.

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