a **science group** company

Inhalation Exposure to Triethylene Glycol (TEG) in Grignard Pure Products

Executive Summary

The Grignard Pure products are intended for use as mold and mildew deodorizers and as antimicrobial air sanitizers. The active ingredient, triethylene glycol (TEG), comprises approximately 50% by weight of the product. Pesticidal products containing TEG were first registered in 1947 for use in hospitals as an air disinfectant. The Grignard Pure mold and mildew odor elimination and airborne antimicrobial sanitization products are intended for use in commercial, institutional and residential settings whereby the product is dispersed into indoor spaces either via a device placed in the space or through integration with the building HVAC system. The airborne concentration of TEG is maintained up to 2.0 mg/m^3 for a maximum of 12 hours per day. This present assessment was commissioned to evaluate the science and safety of TEG as the active ingredient in the Grignard Pure antimicrobial products.

Although the U.S. EPA Office of Pesticide Programs (OPP) has concluded since 2005 that existing information is adequate to understand the risk associated with exposure to TEG, the EPA has more recently expressed interest in understanding better the likelihood of harmful effects from longer-term exposure to airborne TEG. TEG has very low acute inhalation toxicity and has been categorized by the US EPA as Toxicity Category IV ("practically non-toxic") as a result. The available repeat dose inhalation studies with TEG have very clear limitations with respect to understanding the potential effects of long-term exposure. As a result, a safety assessment on TEG by the inhalation route of exposure has not been completed to date.

Historically, gaps in toxicology data needed to conduct a safety assessment have been filled by initiating animal studies specifically designed to address these gaps. In 2013, the EPA OPP prepared a guidance document addressing the neurotoxicity, subchronic inhalation, subchronic dermal and immunotoxicity data requirements for pesticide registration. The goals of the guidance document were to "ensure that there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision." Doing so, according to the EPA "avoid[s] unnecessary use of time and resources, data generation costs, and animal testing." Among the alternatives to animal testing

TSG Consulting ¬
1150 18th Street, NW, Suite 475
Washington, DC 20036
T +1 (202) 828-8990
E info@tsgconsulting.com
www.tsgconsulting.com

routinely considered by the Agency is read-across. Generally speaking, read-across involves using already available data for one or more data-rich chemical surrogates to fill data gaps for a more data-poor substance.

In 2013, the EPA announced the decision to group triethylene glycol, propylene glycol (PG), and dipropylene glycol (DiPG) in the same registration review case pursuant to 40 CFR Part 155.42(a) which gives the Agency the authority to group related active ingredients when the active ingredients are "so closely related in chemical structures and toxicological profile as to allow common use of some or all required data for hazard assessment." Consideration of a read-across approach to date for TEG has been entirely based on this decision by EPA to include PG (and DiPG) in the same registration review with TEG. Although there are no repeat dose inhalation studies available for DiPG, repeat dose inhalation studies have been published with PG. Like the inhalation toxicity studies conducted with TEG, there are some limitations in the study design and results reporting for the repeat dose inhalation studies conducted to date with PG. Unlike TEG, however, all of the repeat dose inhalation studies conducted with PG were of sufficient duration and quality to allow for a better evaluation of potential adverse effects from repeated exposure. Importantly, there were no significant effects following repeated exposure to PG at air concentrations well above that experienced from use of TEG in Grignard Pure applications.

Although the limitations with the TEG studies cannot be dismissed, the results from longer-term evaluations of PG reduce the uncertainty with the TEG studies and provide support for the conclusion that harmful effects from longer-term inhalation exposure to TEG are unlikely. The concentrations of airborne TEG from use of the Grignard Pure products (≤ 2.0 mg/m^3) are at least 100 times less than the human equivalent concentration (~200 mg/m^3) of the established limit dose for TEG in repeat-exposure animal inhalation toxicity studies.

Background - Grignard Pure Products

The Grignard Pure, LLC products are intended for use as mold and mildew deodorizers and as antimicrobial air sanitizers. The composition of the air deodorizing and sanitizing products is identical to that of lighting effects products widely used during live events, stage performances, film and television production, and in other venues such as houses of worship and trade shows in the United States for more than twenty years. The active ingredient, triethylene glycol (TEG), comprises 52.25% by weight of the product; the balance of the product is 1% propylene glycol (PG) and water.

Similarly, the odor eliminating and antimicrobial products are also applied by the same types of dispersion equipment as the lighting effects products, and thus, produce the same type of aerosols. Specifically, the products used for deodorizing and sanitizing indoor air are introduced into indoor spaces as an aerosol either through the HVAC system or by portable aerosolizing equipment.

Grignard Pure Odor Elimination and Air Sanitization Product Use Scenarios

The mold and mildew odor elimination and airborne antimicrobial sanitization products produced by Grignard Pure are intended for use in commercial, institutional and residential settings. Currently, there are three use scenarios for the Grignard Pure products used for deodorizing or sanitizing indoor air: for occupied spaces a continuous treatment regimen, for unoccupied spaces via a controlled, time-release treatment and finally, also for unoccupied spaces, a single shot treatment.

In addition to potential for inhalation exposure, the opportunity for dermal exposure exists for the product handler/applicator when it is required that the handler/applicator pour a liquid concentrate of the product into the device ahead of use. The potential for routine dermal exposure is negligible, however as not every device used to disperse the product requires that the device to be filled prior to use; it takes no longer than five seconds to fill a device when the task is required; only 100 to 200 mL of the concentrate are used in select devices; and the devices that need to be filled are only filled approximately once monthly.

Grignard Pure Products U.S. Regulatory History

Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) authorizes EPA to allow Emergency Exemptions for unregistered uses of pesticides during emergency conditions. This allows state and federal agencies to permit the unregistered use of a pesticide in a specific geographic area for a limited time if emergency conditions exist (e.g., COVID pandemic). As part of this process, the EPA assesses potential risks to human health and the environment to confirm that the pesticide use meets required safety standards. On January 15, 2021, Grignard Pure, LLC was granted a Section 18 Public Health Emergency Exemption program allowing the use of the Grignard Pure product in Georgia and Tennessee in specific indoor locations as determined by the EPA as part of its evaluation. The Section 18 exemption was extended to Pennsylvania, Maryland, Texas, and Nevada in July 2021.

As it is a requirement of a Section 18 exemption to demonstrate progress towards a Section 3 FIFRA registration of the pesticide for use throughout the United States, Grignard Pure LLC initiated the process to submit a Section 3 application for their products for use in occupied and unoccupied spaces. Registration requires a comprehensive evaluation of efficacy and toxicology data for the product (i.e., Grignard Pure), as well as the active ingredient (i.e., TEG).

Triethylene Glycol (TEG) – Pesticidal and Non-Pesticidal Uses

Products containing TEG for use in hospitals as an air disinfectant were first registered in 1947 when it was recognized that TEG had antimicrobial properties. As an active ingredient in pesticides, TEG is formulated primarily as a pressurized liquid used as an air sanitizers/hospital disinfectant and for pest (mites and red lice) control on caged birds. As an inert ingredient, TEG facilitates delivery of formulated herbicides, fungicides, insecticides, growth regulators and attractants on a wide variety of agricultural commodities (EPA, 2005). TEG has numerous listed use sites as an air sanitizer, including household or domestic residences, vehicles, hospitals, commercial and industrial equipment, laundry equipment, bathroom premises, waste containers, and hard, non-porous surface treatments. Products containing TEG are registered for use as a disinfectant on hard, nonporous, food-contact surfaces may result in indirect dietary exposure (EPA, 2017). Effective May 2020, the EPA has exempted residues of TEG from the requirement of a tolerance when used on or applied to food-contact surfaces in public eating places, dairy-processing equipment, and food-processing equipment and utensils (40 CFR 180.940a). TEG is also exempted from the requirement of a tolerance when used as an intentionally-added inert ingredient (deactivator) in pesticide formulations (40 CFR 180.920).

TEG is also an approved indirect food additive as a component of adhesives (21 CFR 175.105), a component of paper and paperboard in contact with aqueous and fatty foods (21 CFR 176.170), a surface lubricant used to make metallic food contact articles (21 CFR 178.3910), a defoaming agent used in coatings (176.200), a component of cellophane (21 CFR 177.1200), in packaging materials for food to be irradiated (21 CFR 179.45), as a component of resinous and polymeric coatings (21 CFR 175.300), as an adjuvants and production aid as plasticizers in polymeric substances (21 CFR 178.3740), and as an optional adjuvant in resinous and polymeric coatings for polyolefin films. TEG is likewise listed as an US FDA approved excipient in Sustol, an antiemetic medication. Other varied applications of TEG include its use as a viscosity-decreasing agent in cosmetics, in plastics to increase pliability, in dehydration of natural gas, as a humectant in printing inks, as an extraction solvent and as the main ingredient in brake fluid (CIR, 2006).

Triethylene Glycol – Toxicology

The focus of the toxicology overview provided below is on the existing inhalation exposure studies on TEG. It is of note, however, that several toxicology studies have been conducted with TEG as the test substance by the oral route of administration. Some of these studies were designed to understand local and systemic effects of repeated doses of TEG by the oral route (both in the diet and drinking water) over several exposure durations (short-term to chronic), while other studies focused specifically on the effects of oral exposure to TEG on reproductive and developmental parameters. Summaries of these studies are available in multiple published reviews (e.g., CIR, 2006; EPA, 2013a).

a) Acute Toxicity and Irritation Profile

The acute toxicity of TEG is well characterized. An acute inhalation LC_{50} of >5.2 mg/L in the rat was reported following a single 4-hour exposure which allows for the chemical to be categorized as EPA Toxicity Category IV ("practically non-toxic"; Nachreiner, 1991; cited in EPA, 2005). TEG also has very low acute oral toxicity (rat LD_{50} = 15-22 g/kg; Category IV), is a slight skin irritant (Category III), a mild eye irritant (Category IV) and is not a skin sensitizer (EPA, 2005).

b) Repeat Dose Toxicity – Inhalation

The available repeat dose toxicity studies on TEG by the inhalation route of exposure include two short-term (subacute) evaluations in the rat, one subchronic study in the rat and two subchronic/chronic studies in the monkey.

In the first rat study, groups of ten male and ten female Sprague-Dawley rats were administered 0, 494, 2,011, or 4,824 mg/m^3 TEG[1] (99.9% pure; ~0.5, 2.0, or 5.0 mg/L day) aerosols via whole body inhalation six hours per day, five days per week over 11 days (Ballantyne et al., 2006; previously Sun, 1992; cited in EPA, 2013a, EPA, 2014). The mass median aerodynamic diameter (MMAD) of TEG ranged from 1.9-2.9 µm. All of the high-dose animals died between died between exposure days 2 and 5. Prior to death, these animals were ataxic and were observed with labored breathing, swollen periocular tissues, ocular discharge, perinasal and periocular encrustation, and blepharospasms; body weights were significantly reduced. None of the low or mid-dose animals died during the exposure period. However, periocular swelling and perinasal encrustation were observed on exposure days 2 through 5. Body weight was statistically significantly decreased in high-dose males and females on day 2 , in the mid and high-dose males on day 5 and in mid-dose males on days 8 through 12. Food consumption was statistically significantly increased in all treatment animals. Serum alkaline phosphatase and inorganic phosphorous were significantly increased in all treated females. A number of statistically significant changes in clinical chemistry were reported in the mid-dose females, including increased erythrocyte count, decreased mean erythrocyte corpuscular volume, decreased serum glucose, decreased serum chloride, increased alanine aminotransferase activity, increased urine volume, decreased urine osmolality, and decreased urine pH. In the mid-dose males, alanine aminotransferase activity was statistically significantly increased, urine volume was statistically significantly increased, and urine pH and N-acetyl-β-D-glucosaminidase were both statistically significantly decreased. Absolute liver weight was statistically significantly increased in mid-dose males. Relative liver weight was statistically and biologically (>10%) significantly increased in mid-dose males and females. Absolute kidney weight was statistically significantly increased in low, mid and high-dose males and in mid-dose females. A NOAEL of 494 mg/m^3 and a LOAEL of 2,011 mg/m^3 were defined by the study authors based on clinical chemistry changes (i.e., increased serum alkaline phosphatase and alanine aminotransferase activities) indicative of liver toxicity and accompanied by an increase in liver weights greater than 10%.

Whole body inhalation studies allows for exposure through other exposure routes (e.g., oral exposure through preening). Consequently, a companion nose-only study was conducted by the same study authors to isolate any effects attributable to inhalation exposure alone (Ballantyne et al., 2006; previously Norris, 1994; cited in EPA, 2013a, EPA, 2014). In the companion study, groups of ten male and ten female Sprague Dawley rats were exposed nose-only to TEG aerosol at measured concentrations of 0, 102, 517, and 1,036 mg/m^3[2] (approximately 0.1, 0.5, 1.0 mg/L/day). The MMAD ranged from 1.2−1.4 µm. The same endpoints examined in the whole body exposure study were examined in the nose-only study. One mid-dose male and one mid-dose female died during the exposure period. Because the deaths were not accompanied by any signs of toxicity or any other abnormal findings, the study authors did not consider the deaths as exposure-related. No exposure-related effects were observed at any exposure concentration. The study authors concluded that the toxicity noted in the whole-body exposure study was likely due to oral

[1] Equivalent to human equivalent concentrations (HECs) of 0, 101, 411, and 987 mg/m^3 based on extrarespiratory effects adjusting for continuous exposure and a blood-gas partition coefficient of 1 (EPA, 2014).

[2] Equivalent to HECs of 0, 21, 106, and 212 mg/m^3 are estimated based on extrarespiratory effects adjusting for continuous exposure and a blood-gas partition coefficient of 1 (EPA, 2014)

exposure through preening. However, lower concentrations were used for the nose-only study which reduces the confidence in the authors conclusion. A NOAEL of 1,036 mg/m^3 was defined based on the absence of treatment-related effects at all concentrations.

In a much earlier evaluation, a group of 24 male and 12 female rats was housed in a chamber containing supersaturated TEG vapor in air (approximately 4 mg/m^3) for 6 to 13 months (Robertson et al., 1947; cited in EPA 2013a, EPA, 2014). A group of four male and two female rats were housed in a separate chamber containing untreated air for the same study duration. As the male and female rats were housed together during the study, the populations increased in the TEG and control chambers to 60 and 14 animals, respectively. Interval sacrifices were performed at 3, 4, 5, and 6 months. The growth rates of the adult and offspring rats exposed to TEG were similar to the growth rates in the control group. The overall health of the rats was not affected by TEG exposure and the evaluated hematology parameters were likewise similar between control and treated animals. No exposure-related lesions were observed at necropsy. A NOAEL of 4 mg/m^3 can be defined.

Two companion studies by these same authors were conducted by with rhesus macaque monkeys (Robertson et al., 1947; cited in EPA 2013a, EPA, 2014). In the first study, 17 monkeys (sex unspecified) were administered approximately 4 mg/m^3 supersaturated TEG vapor in air continuously for one to 10 months; eight control monkeys were housed in a separate chamber containing untreated air for 5 to 8 months. The study authors reported decreased body weight, browning of the skin of the face, and crusting of the ears in the treated animals. Evaluated hematology, blood chemistry, and urinalysis parameters were not affected by TEG exposure. Significant mortality or moribund sacrifices was reported in both the treated (7 of 17 monkeys) and control (5 of 8 monkeys) groups. No quantitative data were provided by the study authors. As a result, it is not possible to identify a LOAEL or NOAEL for this study. In the second study, eight rhesus macaque monkeys (sex unspecified) were administered approximately 2–3 mg/m^3 TEG vapor from continuously for 2 weeks to 10 months; eight monkeys were kept in a separate chamber containing untreated air for the same study duration (Robertson et al., 1947). No adverse reactions or histopathological changes in the examined tissues (not specified). A NOAEL of 3 mg/m^3 can be defined for this study.

Triethylene Glycol – US EPA Conclusions

There are very clear limitations with the repeat dose inhalation toxicity studies available for TEG to date. Although the studies by Ballantyne (2006) in the rat appeared to generally adhere to current day study guideline requirements, they were short-term studies which provide very limited information to help understand the likelihood of adverse effects from longer-term exposure to TEG. Although longer in duration, the studies by Robertson et al. (1947) were also limited in their ability to assess the effects of inhalation exposure to TEG given deficiencies in study design and results reporting, which is typical of studies conducted well before current study guidelines and protocols were developed and normalized. As a result, no quantitative risk assessments have been published by the Agency specific to inhalation exposure to TEG. Notably, EPA NCEA (2014) concluded that neither subchronic nor chronic provisional reference concentrations (p-RfC) could be derived for TEG based on the available studies. EPA NCEA (2014) did derive subchronic and chronic p-RfD based on a much more robust set of subchronic, reproductive, developmental and chronic oral studies conducted with TEG.

The US EPA OPP has based their conclusions on TEG toxicity as it relates to pesticide registration on the weight-of-evidence from the complete TEG toxicity dataset with consideration of studies completed by

the oral, dermal and inhalation routes of exposure. The following was included in the 2005 Reregistration Eligibility Decision (RED) for TEG completed by the Agency:

> "Based on a review of the available toxicology data, the Agency has concluded that triethylene glycol is of very low toxicity by the oral, dermal, and inhalation routes of exposure. The toxicology database is adequate to characterize the hazard of triethylene glycol, and no data gaps have been identified. There are no indications of special sensitivity of infants or children resulting from exposure to triethylene glycol… The Agency has not identified toxicological endpoints of concern for the active and the inert uses of triethylene glycol. Therefore, a quantitative human health risk assessment was not conducted for this RED document. The Agency has no risk concerns for triethylene glycol with respect to human exposure."

This conclusion was based on the review of the TEG toxicity data conducted by the Antimicrobials Division Toxicological Committee (ADTC) in 2003 and revised in 2005 (EPA, 2005).

In the 2013 Propylene Glycol, Dipropylene Glycol and Triethylene Glycol Final Work Plan, the Agency confirmed their low concern for TEG exposure from pesticide use by concluding:

> "There is no evidence of adverse effects at doses of triethylene glycol, propylene glycol or dipropylene glycol up to the established limit dose in repeat-exposure dermal (1,000 mg/kg/day) and inhalation (1 mg/L or 1,000 mg/m^3) toxicity studies. Thus, no toxicological endpoints of concern have been established for all three chemicals based on review of the available mammalian toxicity data. Due to the low order of toxicity and low application rates from the current uses of these chemicals, no risks associated with potential exposures have been quantified for use of triethylene glycol, propylene glycol or dipropylene glycol as active ingredients in pesticide products."

This conclusion was reiterated in the 2017 Interim Registration Review Decision published by EPA in which the Agency noted:

> "Based on data reviewed in support of the REDs for triethylene, propylene and dipropylene glycols, there is no evidence of adverse effects at doses of triethylene glycol, propylene glycol or dipropylene glycol up to the established limit dose in repeat-exposure dermal (1,000 mg/kg/day) and inhalation (1 mg/L or 1,000 mg/m^3) toxicity studies. Thus, no toxicological endpoints of concern have been established for all three chemicals based on review of the available mammalian toxicity data. Due to the low order of toxicity and low application rates from the current uses of these chemicals, no risks associated with potential exposures have been quantified for use of triethylene glycol, propylene glycol or dipropylene glycol as active ingredients in pesticide products."

Propylene Glycol - Read-Across Considerations

Historically, gaps in toxicology data needed to conduct a safety assessment have been filled by initiating animal studies specifically designed to address these gaps. In 2013, the EPA OPP prepared a guidance document addressing specifically the neurotoxicity, subchronic inhalation, subchronic dermal and immunotoxicity data requirements for pesticide registration (EPA, 2013b). The goals of the guidance document were to "ensure that there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision." Doing so,

according to the EPA "avoid[s] unnecessary use of time and resources, data generation costs, and animal testing."

Among the alternatives to animal testing under consideration is read-across. Generally speaking, read-across involves using already available data for one or more data-rich surrogate chemicals to fill data gaps for a more data-poor substance. In principle, read-across data on data rich compounds can be used to predict certain physicochemical properties, mammalian toxicity, environmental fate and ecotoxicity of the chemical of interest. It is critical, however, that the use of read-across is substantiated for each endpoint for which it is employed. In other words, while data for a particular endpoint for data-rich chemical may be suitable to use in an assessment of a data-poor chemical, this alone does not provide justification to use data for the data-rich chemical as a surrogate for all endpoints. The read-across approach is often aided by computational tools, such as ChemID Plus and the OECD QSAR Toolbox, but also requires expert judgement of the suitability of a surrogate (data rich chemical) for each endpoint of interest.

Prior to 2013, two separate Registration Review cases were scheduled by the Agency to address triethylene glycol (Case 3146) and propylene glycol and dipropylene glycol (Case 3126). However, the Agency determined that, "because of their similar use patterns, comparable chemical, physical, and environmental fate characteristics, low mammalian toxicity, and low toxicity to non-target aquatic and terrestrial organisms this document will address propylene glycol (PC code 068603), dipropylene glycol (PC code 068604), and triethylene glycol (PC code 083501)" (EPA, 2013a). The decision by the Agency to group these active ingredients together and merge them into the Propylene Glycol, Dipropylene Glycol and Triethylene Glycol Registration Review case was pursuant to 40 CFR Part 155.42(a) and 40 CFR Part 155.42(b)(4). As stated in 40 CFR 155.42(a), "the Agency may group related active ingredients into a registration review case when the active ingredients are so closely related in chemical structures and toxicological profile as to allow common use of some or all required data for hazard assessment."

Propylene glycol, dipropylene glycol and triethylene glycol are all alcohols and dipropylene glycol and triethylene glycol are also esters. They are all considered semi-volatile to volatile compounds with boiling points ranging from 188° C (PG) to 288°C (TEG) and vapor pressures ranging from 1.32×10^{-3} mmHg (TEG) to 1.3×10^{-1} mmHg (PG). All three compounds inactivate target pests by denaturing proteins found in cell membranes and viral protein coats which renders target pests unable to cause infections by disrupting structural integrity (EPA 2004, 2006; cited in EPA, 2013a).

No repeat dose studies by the inhalation route of exposure exist for dipropylene glycol (DiPG). Consequently, consideration of a read-across approach for the repeat-dose inhalation toxicity end point has been focused on propylene glycol (PG); repeat dose inhalation studies are available on PG and mixtures with PG as the test substance. It is noteworthy that consideration of a read-across approach to date for TEG has been entirely based on the decision by EPA to include PG (and DiPG) in the same registration review with TEG. A detailed analysis has not been conducted to provide further support for the read-across approach.

Propylene Glycol – Inhalation Toxicology

a) Acute Toxicity and Irritation Profile

Like TEG, PG is very low in acute toxicity, is not a skin or eye irritant and is not a dermal sensitizer (EPA, 2013a). PG is EPA Toxicity Category IV for all acute toxicity end points. The acute inhalation LC_{50} for PG in the rat is >2.0 mg/L (Konradova, 1978; cited in EPA, 2013a).

a) Repeat Dose Toxicity – Inhalation

In the first subchronic inhalation study, groups of 19 males and 19 females were administered target aerosol concentrations of 0, 0.1, 1.0 or 2.2 mg/L PG five days per week, 6 hours per day for 13 weeks by nose-only inhalation (Suber et al., 1989). The daily aerosol exposure concentrations were determined to be 0.16 ± 0.04, 1.01 ± 0.11 and 2.18 ± 0.31 mg PG/L. The MMADs of the diluted aerosol were less than 2.22 µm and 1.96 µm for the medium and high dose groups, respectively. Animals were observed daily for evidence of toxicity; feed consumption and body weights were recorded weekly. All animals were necropsied at study termination. Organ weights were recorded for lungs, thymus, spleen, liver, heart, kidneys, adrenals, ovary, urinary bladder, uterus, testes, prostate and brain. Thirty nine organs and tissues were removed and fixed for pathological examination. The respiratory tract, including nasal passages, lungs, trachea and larynx were examined by light microscopy. Respiratory rates and tidal volumes were measured in four male and four female rats from each treatment group. Clinical chemistry and hematology measurements on all animals were made before the start of exposures and before necropsy.

There were no significant differences in respiratory rates, tidal volume or minute volume between the treated animals and controls. Treatment-related nasal hemorrhaging was noted starting in the second week of exposure; this continued for the remainder of the study with up to 75% of the animals in the high dose group being affected. Similar trends were observed with ocular discharge with up to 40% of the animals in the medium and high-dose groups being impacted. Body weights in the medium and high dose animals were reduced. There were no consistent treatment-related trends in observed hematology with the exception of the erythrocyte profile at the end of the study in high dose females. Statistically significant decreases in serum sorbitol dehydrogenase and α-glutamyl transferase in the medium and high-dose animals. Sporadic statistically significant changes in serum glucose, albumin and creatinine were observed in the medium dose males and for serum protein, albumin, and cholesterol in high-dose males. Only serum protein was elevated in medium dose females. Absolute organ weights were significantly decreased for lungs of high-dose females, spleens of low and high dose males, livers of medium and high-dose males, kidneys of high and medium dose males and females. When expressed as relative to body weights or brain weights, high dose male spleen weights were significantly decreased and low dose male lung weights were increased. There were no treatment-related changes in gross pathology in any of the animals at necropsy. Microscopic evaluation of the nasal cavity revealed thickening of the respiratory epithelium noted as increased numbers of goblet cells or as an increase in the mucin content of the goblet cells in the medium and high dose males and females. No histological changes were observed in the trachea, lungs or larynx.

Nasal hemorrhaging was thought by the authors to be indicative of the dehydrating nature of PG. The observations in clinical chemistry and hematology parameters did not present a clear relationship between PG exposure concentrations. The statistically significant changes in absolute organ weights were not considered by the authors to be biologically significant when the weights for all of the treatment

groups were compared and when the absence of gross and histopathological findings were considered. The increase in number of goblet cells or increase in mucin content in the nasal turbinates of male and female rats appeared to the authors to be exposure related and were thought to be related to the physical effects of PG. The authors concluded that the results from this study demonstrated that PG is not a systemic toxin when administered by inhalation or other routes of administration. A NOAEL of 2.2 mg/L (2,200 mg/m^3) can be defined based on an absence of significant treatment-related systemic (respiratory) effects.

Two more recent subchronic inhalation studies were initiated to understand the toxicity PG and PG as a mixture with vegetable glycerin (VG). These studies have not yet been considered by the EPA OPP.

A 90-day repeat dose nose-only inhalation study was conducted in rats in general adherence with the OECD 413 guideline to determine potential toxicological effects of exposure to PG and VG mixtures. The evaluation included three test atmospheres of the PG/VG mixture: 0.174/0.210 mg/L (low); 0.520/0.630 mg/L (medium) and 1.520/1.890 mg/L (high)[3]. Groups of ten male and ten female SD rats were administered the test substances 6 hours per day, 5 days per week for 13 weeks (Phillips et al., 2017). A time adaptation phase was included during week 1 in which the animals were exposed to increasing exposure durations over the seven days (1.5 hours days 1 and 2, 3 hours per day for days 3 and 4, 4.5 hours for days 5-7). Weekend exposures (7 days/week) were performed prior to scheduled necropsy as necessary to ensure that all animals experienced a minimum of two consecutive exposure days before necropsy. In addition, six females per treatment group were randomly assigned to eight additional experimental groups to characterize exposure related effects at the molecular level. Weights of OECD 413-specified organs were measured at necropsy as absolute values and the relative weights were then calculated according to body weights after exsanguination. Bronchoalveolar lavage was performed. Respiratory tract organs (nose larynx, trachea, left lung) were prepared for histopathological examination. Non-respiratory tract organs were collected; histopathological examination was performed only in the high test and reference item groups. Further analysis of the low and medium were performed only if findings in high dose group were identified.

According to the study authors, histopathological evaluation of the respiratory tract of the treated animals demonstrated a low incidence of treatment-related effects that were thought to be adaptive changes, such as those caused by dehydration. Most of these observations were restricted to the larynx. Infiltration of unpigmented macrophages in the lung occurred at the low and medium doses of PG/VG. No clear treatment-related effects from exposure to PG/VG were observed on the measured endpoints in the respiratory system, including histopathology, lung inflammation and molecular responses measured by transcriptomics and proteomics. These mild effects did not reach statistically significance. Based on the absence of significant local and systemic (respiratory) effects, the high dose of 1.520/1.890 mg/L (or 1,520 / 1,890 mg/m^3) for the PG/VG mixture can be defined as the NOAEL.

In a subchronic study by Langston et al. (2021), groups of ten male and ten female SD rats were exposed nose-only inhalation to aerosol concentrations of 5 mg/L TPM (total particulate matter) PG (90% v/v), 5 days per week for 13 weeks. The daily exposure dose was adjusted based on exposure duration – 1 hr (low), 3 hr (mid) or 6 hr (high) per day. In the mid and high-dose groups, daily exposure was increased from 1 to 2 to 3 hours over the first three days to help acclimate the animals to daily exposure regimens. The mass median aerodynamic diameter (MMAD) ranged from 1.7-2.6 µm. An additional 10 females were

[3] The concentrations in the test atmospheres corresponded to approximate daily delivered doses of 50 PG/60 VG mg/kg day (low); 150 PG/181 VG mg/kg day (medium); and 438 PG/544 VG mg/kg day (high).

added to each treatment group for assessment of 'systems toxicology endpoints.' Five animals per sex were randomly assigned to the 6-week recovery groups. All study animals were observed twice daily for mortality or moribundity. Clinical examinations were conducted prior to exposure and within an hour post-exposure. On non-exposure days and during recovery period, the animals were observed once daily. Individual body weights were measured prior to the start of the study, weekly during study period and on the day of necropsy. Food consumption was recorded weekly. Blood samples were collected from 5 animals/sex/group during weeks 4 and 12 for plasma PG analysis. Blood samples were collected from 5 animals/sec/group immediately after exposure during weeks 5 and 11 for carboxyhemoglobin analysis. Bronchoalveolar lavage was performed on left lung of all animals designated for standard endpoints.

No deaths considered by authors to be related to PG/W or PG/VG/W exposures were reported. Non-exposure related early deaths included one male from PG/W 3-hour exposure group and one female from the PG/W 1-hour exposure group. No macroscopic or microscopic findings related to exposure were observed in these animals and the cause of death was undetermined. Dried red material was noted around the nose and occasionally around the eyes at one hour post exposure clinical observations. These observations did not correlate with microscopic findings in nasal cavity. The authors concluded that the red material was not blood, but rather harderian gland secretions. No other significant clinical observations were reported. Statistically significant decreases in minute volume were noted for the 3-hour PG/W group when compared with the sham control in pooled sexes at week 12. The PG/W low (1-hour) and PG/W mid (3-hour) males showed a statistically significant decrease in relative lung weights compared with controls. Dose-related mucous cell hyperplasia characterized by increased numbers of closely packed goblet cells with areas of pseudostratification or intraepithelial gland formation that minimally thickened the nasal mucosa were observed. These effects were not well defined In the males. The dose-related response was more pronounced in females. The nasal mucosa cell hyperplasia in PG/W resolved in both sexes in the 6 week recovery phase (incidence and severity). The authors reported that there were notable histopathological changes were observed in the PG exposed groups compared with the controls. The lack of pulmonary inflammation was consistent with the lack of changes in the enzymes and cytology parameters of the BALF. No alterations were found in hematology, cytology, coagulation, serum chemistry and urinalysis parameters between the exposed and controls. The authors calculated the PG delivered dose of 1,152 mg/kg day based on 5 mg/L (or 5,000 mg/m^3), 6 hours/day dose group and referred to this dose as the NOAEC.

Propylene Glycol - Conclusions

Like the inhalation toxicity studies conducted with TEG, there are some limitations in the study design and results reporting for the repeat dose inhalation studies conducted to date with PG. Unlike TEG, however, all of the repeat dose inhalation studies conducted with PG (or PG/VG mixtures) were of sufficient duration and quality to allow for a better evaluation of potential long-term adverse effects from exposure to the test substance. Specifically, there was a reported absence of significant treatment-related systemic effects following subchronic inhalation exposure to PG and PG/VG mixtures in each of the three studies at aerosol concentrations well above that experienced from use of TEG in Grignard Pure applications. These results provide additional support to the conclusion reported by the EPA in the Interim Registration Review Decision (2017) for PG, DiPG and TEG:

> "There is no evidence of adverse effects of doses of triethylene glycol, propylene glycol or dipropylene glycol up to the established limit dose in repeat-exposure dermal (1,000 mg/kg day) and inhalation (1 mg/L or 1,000 mg/m^3) toxicity studies. Thus, no toxicological endpoints of concern have been established for all three chemicals based on review of the available mammalian toxicity data. Due to

the low order of toxicity and low application rates from the current uses of these chemicals, no risks associated with potential exposures have been quantified for use of triethylene glycol, propylene glycol or dipropylene glycol as active ingredients in pesticide products."

Conclusions

The US EPA Office of Pesticide Programs has concluded since 2005 that existing information is adequate to understand the risk associated with exposure to TEG and that there are no concerns associated with use of TEG as an ingredient in pesticide products. More recently, the EPA has expressed interest in understanding better the likelihood of harmful effects from longer-term exposure to airborne TEG as the existing animal studies that evaluated potential effects from inhalation of TEG have some limitations and, therefore, resulted in some hesitation by the Agency surrounding use of these studies to inform product registration decisions. Importantly, studies evaluating the potential for harmful effects following longer-term exposure to a related compound, propylene glycol (PG) have been completed which may reduce the hesitation voiced by the Agency about the TEG inhalation studies. Specifically, no harmful effects were observed in longer-term studies with PG. Although the limitations with the TEG studies cannot be dismissed, the results from longer-term evaluations of PG reduce the uncertainty with the TEG studies and provide additional support for the conclusion that harmful effects from longer-term inhalation exposure to TEG are unlikely. The concentrations of airborne TEG from use of the Grignard Pure products ($\leq$ 1.5 mg/m^3) are more than 100 times less than the human equivalent concentration (~200 mg/m^3) of the established limit dose for TEG in repeat-exposure animal inhalation toxicity studies (1,000 mg/m^3).

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