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Evaluation of 4-methylcyclohexanemethanol (MCHM) in a combined irritancy and Local Lymph Node Assay (LLNA) in mice

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ABSTRACT

4-Methylcyclohexanemethanol (MCHM) is a flotation reagent used in fine coal beneficiation. On January 9, 2014, crude MCHM, a mixture containing predominantly MCHM, was inadvertently released into the Elk River, a municipal water source that serves about 300,000 people in the Charleston, WV area, resulting in temporary contamination of 15 percent of the state's tap water and causing significant dermal exposure. The current studies were undertaken to determine whether crude MCHM or MCHM has the potential to produce dermal irritancy and/or sensitization. BALB/c female mice were treated daily for 3 consecutive days by direct epicutaneous application of 25 μL of various concentrations of crude MCHM or MCHM to the dorsum of each ear. A mouse ear-swelling test was used to determine irritancy potential and was undertaken in combination with the standardized Local Lymph Node Assay (LLNA) to determine skin sensitizing potential. MCHM was found to produce skin irritation at concentrations above 20% and did not produce sensitization. Crude MCHM also produced irritation, although weaker, and in addition was found to be a weak to moderate skin sensitizer. The results are discussed in terms of potential human health hazard.

1. Introduction

4-Methylcyclohexanemethanol (MCHM; CASRN: 34885-03-5) is a flotation reagent used in fine coal beneficiation and was the primary chemical contaminant spilled from a storage tank into the West Virginia Elk River on January 9, 2014. The Elk River is a municipal water source that serves about 300,000 people in the Charleston area. The chemical spill temporarily contaminated 15 percent of the state's tap water. The leaking tank contained crude MHCM, a commercial mixture that is mostly MCHM (e.g. 68–88%), but also contains other related chemicals [detailed lists of chemical constituents is provided on the National Toxicology Program (NTP) website (NTP, 2015)]. The greatest measured concentration of MCHM in the spilled liquid entering or leaving the water treatment facility was 3.35 mg/L (Whelton et al., 2015). Concentrations of crude MCHM in tap water following the spill and prior to flushing were much lower, ranging from <10 to 420 ppb (Whelton et al., 2015); the upper level being slightly below the short-term drinking water limit of 1 ppm for a 10 kg child established by the Centers for Disease Control and Prevention (CDC) in response to the spill (Schade et al., 2015). In the days immediately following January 9, 2014, the West Virginia Poison Center received calls from over 1900 local residents reporting various health effects (West Virginia Poison Center Fact Sheet, 2014). Based upon syndromic surveillance records conducted through telephone interviews (Schade et al., 2015) and household surveys (CDC, 2014a), dermal effects were the most common reported symptom manifested by transient skin irritation ($p < 0.001$) and mild rash ($p < 0.002$). CDC further grouped symptoms based upon three exposure scenarios (more than one exposure may have been reported for a given subject):

Abbreviations: ACD, allergic contact dermatitis; AOO, acetone:olive oil (4:1, v/v); CDC, Centers for Disease Control and Prevention; DNFB, 0.15% 1-fluoro-2,4-dinitrobenzene; DPM, disintegrations per minute; ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods; ^{125}IUdR, ^{125}iodo-deoxuridine; LLNA, local lymph node assay; MCHM, 4-methylchclohexanemethanol; NTP, National Toxicology Program; SI, stimulation index.

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bathing, showering or other skin contact (52.6%); eating, drinking or other oral exposure (43.9%); and inhalation from vapor or mist (14.6%). These findings of dermal irritation are consistent with several experimental animal studies conducted on crude MCHM by Eastman Chemical Co. (Eastman Chemical Co, 1997). In these studies, acute dermal exposure in rabbits or Sprague-Dawley rats, as well as 24-day dermal exposure in rats showed that crude MCHM produced skin irritancy characterized by erythema and desquamation at the application site. Skin hypersensitivity testing, (i.e. allergic contact dermatitis, ACD), was also conducted on crude MCHM and was negative in a guinea pig model.

The Local Lymph Node Assay (LLNA) is a murine model used extensively to predict the potential for a chemical to induce hypersensitivity. The acceptance of the LLNA as a stand-alone alternative to the Guinea Pig Maximization Test/Buehler Assay by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) demonstrates the commitment of using more humane testing models for screening chemicals that may elicit contact hypersensitivity in humans (ICCVAM, 2011). Measurement of irritancy is incorporated into the LLNA protocol used by the NTP. Sensitization potential is evaluated by measuring the differential induction of lymphocyte proliferation in the draining lymph nodes relative to appropriate controls following dermal application of the chemical. Similar to other animal models used to assess dermal sensitization, the LLNA is approximately 72% predictive of skin sensitization in humans when compared with results from human skin prick or patch test data (ICCVAM, 1999), however, a recent evaluation suggests that one-third of strong human sensitizers may be under classified as weaker sensitizers by the LLNA method (ICCVAM, 2011).

The objective of the current study was to evaluate and compare the irritancy and sensitization potential of crude MCHM and MCHM in a combined irritancy/sensitization LLNA model. For dermal irritancy assessment, mouse ear swelling was measured on days 3 and 6 during the in-life phase of the study and punch biopsy ear weights were determined at termination. Sensitization was assessed by measuring lymphocyte proliferation in the draining lymph node.

2. Materials and methods

These studies were conducted in compliance with the U.S. Food and Drug Administration Good Laboratory Practices for Nonclinical Laboratory Studies (Title 21 of the Code of Federal Regulations, Part 58).

2.1. Chemicals and dose formulations

MCHM (lot KDY3F) was supplied by TCI America (Portland, OR) and crude MCHM (lot TP14044373) was supplied by Eastman Chemical Company (Kingsport, TN). The chemical identity and purity was determined at MRI Global (Kansas City, MO). The identity of MCHM was confirmed by Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry. The purity of MCHM estimated by gas chromatography (GC) with flame ionization detection (FID) was >99% with respect to *cis*- (~68%) and *trans*- (~32%) isomers of MCHM. The identity and purity of crude MCHM were determined by GC with mass spectrometry detection. Two major peaks representing *cis*- (~33%) and *trans*-isomer (~57%) of MCHM accounted for ~ 90% of the chemical composition of crude MCHM.

Dose formulation development and stability validation studies of crude MCHM were conducted at RTI International (Research Triangle Park, NC) using GC-FID. Crude MCHM (1%, v/v) in acetone:olive oil (4:1, v/v) (AOO) was stable for up to 14 days when stored ambient or refrigerated in glass amber vials. Assessment of the crude MCHM dosing solution under simulated dosing conditions (exposed to air and light) for 3 days revealed no significant loss of the test chemical in either case.

MCHM and crude MCHM were tested for solubility and ability to be delivered through a syringe in AOO vehicle. Both forms were determined to be soluble at 50% in AOO and the viscosity of the 50% solutions was acceptable based on lack of resistance when drawing or dispensing formulations using a syringe. Formulations were prepared in AOO from 0.45% to 20% and concentrations were analyzed. Dilutional linearity ($r = 0.9990$) was demonstrated for crude MCHM with an average percent recovery of 100%. Dilution verification of 40%, 80%, and neat formulations were also acceptable (relative error <10% for recovery). All dose formulations for MCHM and crude MCHM were analyzed for concentration and met acceptance criteria. Two studies were conducted. For Study 1, formulations of MCHM and crude MCHM were prepared at nominal concentrations of 0 (vehicle), 2, 20, 50 or 100% (v/v) and 0 (vehicle), 1, 2, 5, 20, 40, or 100% (v/v), respectively, in AOO by the study laboratory (Burleson Research Technologies). Due to excessive toxicity of neat (100%) MCHM and crude MCHM on study Day 1, the concentrations for the high dose group were reduced to 50% and 80%, respectively, for the remainder of the study. An aliquot of each of the dose formulations that were prepared on Day 3 was shipped to RTI International for analysis. Formulation concentrations, determined by GC-FID, were within 77–105% of target for MCHM and 76.8–103% of target for crude MCHM. For Study 2 only crude MCHM was tested. Formulations of crude MCHM were prepared by RTI International at nominal concentrations of 0 (vehicle), 1, 5, 25, 50, and 75% (v/v) in AOO and shipped to Burleson Research Technologies. Formulation concentrations of crude MCHM were 95.2–100% of target concentration.

2.2. Test system

Female BALBc mice were purchased from Taconic Biosciences Inc., (Hudson, NY) and were 8–12 weeks of age at the start of treatment. The National Toxicology Program has historically used BALB/c mice for hypersensitivity testing and has an extensive database on the performance of this model (ICCVAM, 2009,2011). Recent studies have compared responses in BALB/c and CBA/J mice in the LLNA using bromodeoxyuridine with flow cytometry and shown that both strains provide comparable results (Lee et al., 2017).

Based upon the vendor health reports and the study sentinel health assessments, the mice were deemed specific pathogen free. The animals were quarantined/acclimated for ~8 days and randomized by body weight 1–3 days prior to the start of test article application. Mice were group housed in individually ventilated cages with up to 5 mice from the same treatment group per cage. The cages contained irradiated Sanichip® woodchip bedding. The environmental conditions of the animal room, where all animals on study were housed, were recorded daily and had a temperature range of 69–75 °F, relative humidity range of 35–65%, and a 12-h light/dark cycle. Food, (irradiated NTP-2000 diet) and tap water were provided *ad libitum*. Body weights were recorded on study Days 1 and 6, and the animals were monitored twice daily (once before 10am and once after 2pm) for signs of toxicity. The studies were reviewed and approved by the Institutional Animal Care and Use Committee for adherence to the *Guide* and the applicable policies of the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were conducted in compliance with Nonclinical Laboratory Studies Good Laboratory Practice Regulations issued by the U.S. Food and Drug Administration (Title 21 of the Code of Federal Regulations, Part 58).