Food and Chemical Toxicology 62 (2013) 436–447

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Toxicological evaluation of peroxy sulfonated oleic acid (PSOA) in subacute and developmental toxicity studies

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ARTICLE INFO

Article history:
Received 28 June 2013
Accepted 24 August 2013
Available online 3 September 2013

Keywords:
Peroxy sulfonated oleic acid
PSOA
Subacute toxicity
Developmental toxicity
Hydroperoxide
NOEL

ABSTRACT

Peroxy sulfonated oleic acid (PSOA) is a new coupler used in sanitizing solutions primarily for the food and beverage industry. The toxicity of PSOA was evaluated in a 28-day repeat dose study according to OECD 407 guidelines with a 14-day recovery period and a developmental toxicity study according to OECD 414 guidelines. In both studies, PSOA was administered once daily via gavage at 0, 5, 15 and 50 mg/kg/day to Sprague–Dawley rats. Due to its corrosive properties, the highest test concentration was restricted to 0.5%. No findings related to PSOA administration were observed for the 28-day repeat-dose study and the NOEL is 50 mg/kg/day. Additionally, no impairment of the mucous membranes of the gastrointestinal tract was observed up to 0.5%, which is considered the NOEC in terms of local toxicity. For the developmental study, an embryo-fetal NOEL of 50 mg/kg/day was identified and the maternal NOEL is considered to be 15 mg/kg/day, based on slight reductions in maternal body weight and food consumption, as well as a modest increase in the incidence of clinical observations at the high dose. These findings demonstrate that PSOA appears to have minimal potential to induce toxicity associated with repeat-dose or developmental exposures.

1. Introduction

Peroxy sulfonated oleic acid (PSOA) was developed for use as a coupler used in sanitizing solutions primarily in the food and beverage industry to clean and sanitize processing equipment. PSOA functions both as a surfactant and coupler in formulated sanitizers. PSOA also promotes the effectiveness of the product by increasing the surface penetration of these components. In addition to its role as a surfactant and coupler, PSOA is also used as a bleaching agent in various industrial applications.

PSOA is an organic peroxide that is characterized by the presence of one or more oxygen–oxygen bonds and is a derivative of hydrogen peroxide (Sanchez and Meyers, 2000). Organic peroxides can be classified according to their peroxide structure, with PSOA being part of the class referred to as hydroperoxides. Hydroperoxides are commonly sub-classified as organic (alkyl) hydroperoxides and organomineral hydroperoxides, with PSOA being a member of the former (Sanchez and Meyers, 2000). As an organic (alkyl) hydroperoxide, PSOA has four key structural moieties that influence its functionality and toxicity, a hydroperoxide structure, an acid component, a sulfonate moiety and a monohydroxy structure.

PSOA is derived by reacting sulfonated oleic acid and hydrogen peroxide under acidic conditions. The parent compound for PSOA production is manufactured via sulfonation of either animal (tallow) or plant-based oleic acid feed stocks. Biologically-based feed stocks may address sustainability in terms of supply sourcing and can be price competitive relative to non-renewable feed stocks (Nordhoff et al., 2007). Due to the variability inherent in the natural feedstock used to produce sulfonated oleic acid, PSOA falls into the regulatory definition of an Unknown, Variable Composition or of Biological origin (UVCB) substance (ECHA, 2011). Commercial PSOA preparations contain approximately 70% of the peroxysulfonated fatty acid in water (approximately 20%) with the remaining composition being hydrogen peroxide and acid.

PSOA represents a new generation of chemistry for sanitizers used in food and beverage applications as well as other industrial uses. As part of the chemical stewardship for this new chemistry, this article summarizes the results of two studies, a 28-day repeat dose study following OECD 407 guideline with a 14-day recovery period and a developmental toxicity study following OECD 414 guidelines. This represents the first published report of the toxicological properties of PSOA.

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http://dx.doi.org/10.1016/j.fct.2013.08.080

2. Materials and methods

2.1. Test material

PSOA was provided by Ecolab Inc. as a slight yellow liquid with a purity of 100%. The test material properties are summarized in Table 1.

2.2. Testing facilities and regulatory compliance

The studies described in the following sections were performed by Charles River Laboratories (Edinburgh, Scotland and Frederick, Maryland). All studies were performed according to OECD Guidelines for the Testing of Chemicals and Good Laboratory Practices.

2.3. Animal handling and husbandry

Animal husbandry and use were in accordance with applicable local and international regulations and guidelines. Animals were housed singly (Developmental toxicity study) or in groups of 2–3 (Repeat dose oral toxicity study) in suspended cages with stainless steel grid tops and solid bottoms. Cages were suspended on moveable racks and fitted with water bottles and integrated steel food hoppers. Sterilized white wood shavings were used as bedding material. A Certificate of Analysis accompanied the shavings ensuring that no additional substances had any influence on the outcome of the study. Food (SDS Rat and Mouse Breeder Diet No. 3) and water were provided *ad libitum* throughout the study and were analyzed for contaminants. For psychological and environmental enrichment, animals were supplied with a hiding device and chewing object, except during study procedures and activities. A certificate of analysis was provided for the hiding device and chewing object. There was automatic control of temperature and continuous monitoring of humidity with target ranges of 19–23 °C and 40–70%, respectively. Light cycles were automatically controlled, which provided 12 h of light and 12 h of dark, except if interrupted by study procedures and activities.

2.4. Dosing formulation and analysis

Test item dosing formulations were prepared daily, stored in a refrigerator set to maintain 4 °C and dispensed daily. The dosing formulations were removed from refrigeration and stirred for at least 30 min prior to and during dosing. Duplicate top, middle and bottom samples for each sampling time point were collected and refrigerated at 4 °C for storage prior to analysis for concentration and homogeneity. Sampling time points were weeks 1 and 4 for the 28-day oral toxicity study and weeks 1 and 2 for the development study. Concentration results were considered acceptable if mean sample concentration results were within ±15% of the theoretical concentration and individual sample concentration results were within or equal to ±20%. Homogeneity results were considered acceptable if the relative standard deviation of the mean value at each sample location was $\leqslant$5% for each group.

2.5. 28-Day oral toxicity study in rats with a 14-day recovery period

PSOA was evaluated in a 28-day oral toxicity study with a 14-day recovery period in Crl:CD(SD) rats and performed according to OECD Guideline 407 (*Repeated Doses 28-day Oral Toxicity Study in Rodents* (*adopted 03 October 2008*)). PSOA was administered by oral gavage once daily for 28 consecutive days to groups of male and female rats (5/sex/group) at doses of 5, 15 and 50 mg/kg/day, plus an additional 5 rats/sex/group for the control and high dose groups for the recovery period cohort. A vehicle control group received deionized water. Dose volumes were 10 mL/kg. Table 2 summarizes the experimental design for the 28-day oral toxicity study.

At initiation of dosing, the experimental animals were approximately 7–8 weeks old. Once a week, all animals received detailed clinical examination, which included appearance, movement and behavior patterns, skin and hair conditions, eyes and mucous membranes, respiration and excreta. All animals were examined for reaction to treatment daily during the course of dosing in the study. The onset, intensity and duration of any signs were recorded, with particular attention noted up to 4 h after dosing until Day 9 and, as no clinical signs were observed, up to 2 h post dose from Day 10 onward. Body weights were recorded twice during pretrial, daily throughout the treatment period and twice weekly during the recovery period. Food consumption was recorded twice during pretrial, daily throughout the treatment period, then twice weekly during the recovery period. Water consumption was monitored by visual inspection of the water bottles regularly throughout the study. All animals during pretrial, control and high dose main study animals during Week 4 and all recovery animals during Week 2 of the recovery period underwent an ophthalmoscopic examination. A functional observation battery to assess hearing ability, pupillary reflex, static righting reflex, grip strength and motor activity was performed on all animals during Week 4 of the treatment period and during Week 2 of the recovery period. Hematology (see Table 3 for hematology parameters assessed), coagulation parameters (activated partial thromboplastin time, fibrinogen and prothrombin time), clinical chemistry (see Table 4 for clinical chemistry parameters assessed) and urinalysis (See Table 5 for urinalysis parameters) evaluations were made for all animals during Week 4 of the treatment period and for the control and high dose groups during Week 2 of the recovery period.

Table 6 summarizes the terminal procedures. Organ weights were collected for the brain, epididymis, adrenal gland, pituitary gland, prostate gland, thyroid gland, heart, kidney, liver, lung, ovary, spleen, testes, thymus and uterus. Histopathologic examinations were performed on 55 tissues (see Table 7 for a list of tissues) for all early deaths and control and high dose groups during Week 4. Histopathological examinations were conducted for gross lesions of low and middle dose groups after Week 4 and gross lesions and target tissues of control and high dose groups after Week 6 (Week 2 of the recovery period). Statistical analyses were performed using the F-Max test for homogeneity of variance, parametric ANOVA for homogeneous data and the Kruskal–Wallis nonparametric ANOVA for non-homogeneous data. All statistical tests were two-sided and performed at the 5% significance level ($p < 0.05\%$).

2.6. Developmental toxicity study in rats

PSOA was evaluated in a developmental toxicity study with Crl:CD(SD) rats and performed according to OECD Guideline 414 (*Prenatal Developmental Toxicity Study* (*adopted 22 January 2001*)). PSOA was administered by oral gavage once daily to groups of pregnant female rats (24/group) from Days 6–19 of gestation at doses of 5, 15 and 50 mg/kg/day. A vehicle control group received deionized water. Dose volumes were 10 mL/kg. Table 8 summarizes the experimental design for the developmental toxicity study.

Once during pretrial and daily during treatment, all pregnant dams received a detailed clinical examination as described in the 28-day study. Dams were examined for reaction to treatment daily during the course of dosing in the study. The onset, intensity and duration of any signs were recorded, with particular attention noted during and for the first hour after dosing. Maternal body weights and food consumption were recorded daily throughout the treatment period. Table 9 summarizes the terminal procedures.

Study termination occurred on Day 20 of gestation for all females, except one dam in the 15 mg/kg/day dose group which was found dead on Day 18 of gestation. Premature death is suspected to have been caused by a gavage injury. All dams were subjected to a complete necropsy examination that included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. The reproductive tract was dissected from the abdominal cavity and the gravid uterus weighed. The uterus was opened and its contents were examined. The fetuses were removed from the uterus and weighed. The ovaries and uterus were examined for the number and distribution of corpora lutea, implantation sites, placentae (size, color or shape), live and dead fetuses, and early and late fetus deaths. Fetuses were examined for external abnormalities. Late deaths and dead fetuses were examined for external abnormalities to the extent possible. In addition to body weight, fetuses were individually identified with the study number, litter number and uterine distribution. Half of the viable fetuses from each uterus were fixed in methylated ethyl alcohol and the remaining half in Bouin's fluid. Following initial fixation, the fetuses fixed in alcohol were sexed and examined by open dissection for abnormalities of the thoracic and abdominal viscera. Fetuses fixed in Bouin's fluid were sexed and examined for soft tissue abnormalities by a freehand section technique as described by Wilson (1965). Fetal carcasses fixed in methylated ethyl alcohol were treated with potassium hydroxide, the skeletons stained with Alizarin Red S and the fetuses cleared with aqueous glycerol solutions. Following preparation, the skeletons were examined for abnormalities and for the extent of ossification. Abnormalities noted during the fetal examination were classified as malformations, variations or skeletal ossification parameters. Representative samples were collected from any abnormal tissues observed in the dams and preserved in 10% neutral buffered formalin. Statistical analyses were performed using the F-Max test for homogeneity of variance, parametric ANOVA for homogeneous data and the Kruskal–Wallis nonparametric ANOVA for non-homogeneous data. All statistical tests were two-sided and performed at the 5% significance level ($p < 0.05\%$).

3. Results

3.1. 28-Day oral toxicity study in rats with a 14-day recovery period

3.1.1. Mortality, clinical signs, body weight and food consumption

All rats survived to the end of the study (see Table 10). No clinical signs of toxicity related to PSOA were observed. In addition, results showed no test substance-related changes in body weight or consumption of food and water. One male rat in the recovery group was replaced by a spare male rat on Day 1 of the study due to the original animal chewing through and swallowing a piece of the gavage tubing used for dosing. This animal was humanely euthanized