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13-week oral toxicity study of vinyl laurate in rats

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ABSTRACT

Vinyl laurate (VL) is used as a monomer in the production of polyvinyl acetate vinyl laurate copolymer, a component of chewing gum base. The safety of VL was examined in a 13-week oral toxicity study in Wistar rats. VL was administered in corn coil by daily gavage (5 ml/kg bw/d) to four main groups (10 rats/sex) at doses of 0 (vehicle only), 50, 250 and 1000 mg/kg bw/d, respectively. The control and high-dose group comprised an additional 5 rats/sex which were kept untreated for a further 4 weeks until sacrifice (recovery groups). In addition to standard parameters, male and female fertility parameters were determined as well. There were no mortalities and treatment-related clinical signs. Neurobehavioral observations and motor activity assessment, ophthalmoscopic examinations, body weights, feed and water intakes, blood cell counts, coagulation time, standard clinical chemical parameters and urinalyses, absolute and relative organ weights at the end of the treatment as well as macroscopic examination at necropsy and microscopic examination of standard organs and tissues did not show any treatment-related changes. Female and male fertility parameters (estrus cyclicity, testicular and epididymal sperm counts, sperm motility and morphology) were not affected by the treatment. Accordingly, the no-observed-adverse-effect level (NOAEL) for VL was determined to be 1000 mg/kg bw/d, i.e. the highest dose level tested.

1. Introduction

Vinyl laurate (VL) is a monomer used in the manufacture of polyvinyl acetate vinyl laurate copolymer (PVAcVL). The use of this copolymer as a component of chewing gum base provides food technological benefits such as a better amalgamation with other typical components of gum base and a reduced stickiness (Hoegl et al., 2008; Hoegl et al., 2013; Wimmer and Gruber, 2013). However, PVAcVL may contain several 100 ppm of free, i.e. not polymerized VL (Wacker Chemie AG, 2013). Therefore, a standard 13-week oral toxicity study was performed in rats for examining the safety of oral VL at high doses (Lina, 2013). The results of this study are here published in fulfilment of a basic requirement according to which the pivotal safety data of substances that are generally recognized as safe (GRAS) for use in foods must be publicly available (here PVAcVL).

While there exists a large body of safety data for vinyl acetate (VAc) including data from chronic oral rodent toxicity studies (Toxnet, 2014), the safety of VL has been examined so far only in a battery of in vitro and in vivo genotoxicity tests (van Acker et al., 2014), a combined repeated dose and reproduction/developmental toxicity screening study (OECD test guideline 422) and a prenatal developmental toxicity screening study (OECD test guideline 414) (Messinger and Bär, 2014).

VAc is a colorless liquid with a solubility in water of about 23 g/L (at 20 °C) (Chemical Book, 2010). Accordingly, vinyl acetate could be administered to the test animals with the drinking water at concentrations of 10,000 or even 24,000 ppm (Valentine et al., 2002). In contrast, VL is a colorless liquid with a very low water solubility (0.24 mg/L). Accordingly, VL has to be dissolved in a suitable lipophilic carrier, such as a corn oil, for application in rodent oral toxicity tests.

2. Materials and methods

2.1. Compliance with ethical and procedural standards

This study was performed at TNO Triskelion BV (Zeist, Netherlands) with the approval of TNO's IACUC and in compliance with

Abbreviations: AA, acetaldehyde; BSA, bovine serum albumin; FOB, functional observational battery; GRAS, generally recognized as safe; IACUC, Institutional Animal Care and Use Committee; MAA, motor activity assessment; PVAcVL, polyvinyl acetate vinyl laurate; VAc, vinyl acetate; VL, vinyl laurate.

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TNO's ethical standards, in accordance with Good Laboratory Practice (GLP) and in line with the applicable guidelines for the repeated dose 90-day oral toxicity study in rodents (OECD Guideline 408; EU Commission Directive 2001/59/EC, Annex 5D, Method B.26).

2.2. Animals

A total of 108 pathogen-free, male and female about 4-week old Wistar Outbred rats (RccHan:WIST) were obtained from Harlan (Horst, Netherlands). On arrival, the animals were examined for signs of ill health. They then entered a 15-day acclimatization period prior to start of the study. During the quarantine period, the microbiological status was checked by serology in sample rats from the same shipment. On the first day of dosing, the rats were about 6 weeks old with a weight range of ±20% from the mean weight for each sex and ranging from 172 to 216 g (mean 195 g) for males and from 129 to 167 g (mean 149 g) for females.

One day before start of the treatment, the animals were allocated to the four main groups and the two recovery groups proportionally to body weight by computer randomization. With this procedure those surplus animals were automatically excluded from the study which had a body weight that deviated most from the mean. After allocation, each rat was uniquely identified by ear tattoos. Remaining rats were not used in the study but kept as sentinel animals.

The animals were kept in makrolon cages with wood shavings (Lignocel) as bedding material and strips of paper (Enviro-dri) and a wooden block as environmental enrichment. They were housed in groups of five, separated by sex. Towards the end of the treatment period, the animals were temporarily kept singly in a macrolon cage on the day of functional observational battery (FOB) testing and motor activity assessment (MAA), or in a stainless steel cage during the collection of urine.

An artificial light/dark cycle with a sequence of 12 h was applied. The room was ventilated with about 10 air changes per hour and was maintained at a temperature of 20–24 °C. The relative humidity varied between 45% and 65%.

2.3. Test substance and dosing solution

Vinyl laurate (Batch No. S207006), a colorless liquid with a purity of 99%, was obtained from Wacker Chemie AG (Burghausen, Germany). For dosing, dilutions of VL with fully refined, winterized corn oil obtained from Oliehoorn BV (Zwang, Netherlands) were prepared in weekly intervals. Analyses to determine the stability of VL in the dosing solution kept for 7 days in the refrigerator, the homogeneity of VL in the dosing solution (duplicate analyses of samples drawn at 3 levels) and the VL content of the different test dilutions (four batches) were conducted using a validated GC-method.

The dose volume was 5 ml/kg bw/d for all animals. To maintain a constant dose level in terms of the animal's body weight, the dose volumes were adjusted to the latest recorded body weights for each individual rat.

2.4. Diet

Feed and drinking water were provided *ad libitum* from the arrival of the rats until the end of the study. The rats received a powdered, cereal-based rodent diet (Rat and Mouse No.3 Breeding diet; RM3) of a commercial supplier (SDS Special Diets Services, Witham, England). Routine physical, chemical and microbiological examination of the drinking water and analysis of the feed for contaminants was performed by the water and the feed supplier, respectively.

2.5. Experimental design

The study comprised four main groups of 10 males and 10 females each, namely a vehicle (corn oil) control group and three test groups receiving 50, 250 and 1000 mg/kg bw/d of VL in corn oil for 13 weeks (7 days/week). In addition, there was a control and a high-dose recovery group each consisting of 5 males and 5 females. The rats of the recovery groups were treated for 13 weeks and were kept subsequently untreated for another 4 weeks.

Each animal was observed for clinical signs daily. All cages were checked twice daily for dead or moribund animals. The individual body weight was recorded at start of the treatment (day 0), in weekly intervals thereafter, and at necropsy (after overnight fasting). Feed consumption was measured per cage in weekly periods. Water intake was measured daily per cage for 5-day periods in weeks 1, 6 and 12 of the main study.

Detailed clinical examinations outside the home cage were performed on all rats of the main groups prior to the first exposure and then once weekly throughout the study. The parameters of the FOB and the MAA were recorded for all rats of the main groups in week 13.

Ophthalmoscopic examinations were performed prior to the start of the treatment (day-7 for males and day-6 for females) in all rats of the main and recovery groups, and on day 85 in rats of the control and high dose group (low-dose and mid-dose rats were not examined because there were no treatment related ocular changes in the high dose group).

Blood was collected from all fasted animals scheduled for termination (on day 91 for males and day 92 for females). EDTA and heparin were used as anticoagulants for the blood samples collected for the hematological and clinical chemical examinations, respectively. The parameters examined are shown in Tables 2 and 3.

On day 84–85, urine was collected from all rats of the main groups. For determining the concentrating ability of the kidneys, the animals were deprived of water for 24 h and of feed during the last 16 h of this period. During these 16 h, the rats were kept individually in stainless-steel metabolism cages for urine collection in glass tubes. The examined urinary parameters are shown in Table 3.

After deprivation from food overnight, all animals of the main groups were randomly killed on day 91 (males) and day 92 (females), and all animals of the recovery groups on day 119, by exsanguination from the abdominal aorta under CO_2/O_2 anaesthesia and subjected to a complete macroscopic examination. The following organs of all rats were weighed (paired organs together) and the relative organ weights were calculated on the basis of the final body weight for the adrenals, brain, epididymides, heart, kidneys, liver, prostate, spleen, testes, thymus, seminal vesicles (with coagulating glands), uterus (with cervix) and ovaries.

Samples of the following tissues and organs of all animals were preserved in suitable fixatives: adrenals; aorta; sternum with bone marrow; brain (brain stem, cerebrum, cerebellum); cecum; colon; epididymides; esophagus; eyes; heart; colon; duodenum; gross lesions, gut associated lymphoid tissue/Peyer's patches, ileum; jejunum; rectum; kidneys, liver; lungs; axillary lymph nodes; mesenteric lymph nodes; mammary glands; skeletal muscle; peripheral (sciatic) nerve; ovaries; oviducts; pancreas; parathyroid glands; pituitary gland; prostate (ventral and dorsal lobes), salivary glands (parotid, submaxillary and sublingual), seminal vesicles plus coagulating glands; skin/subcutis; spinal cord; spleen; sternum with bone marrow, stomach; testes, thymus; thyroid gland; trachea/bronchi; urinary bladder; uterus with cervix; vagina. After embedding in paraffin wax, sectioning and staining with hematoxylin and eosin, these tissues were examined by light microscopy in all rats of the main control group and the high dose group.