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A comparative 90-day toxicity study of allyl acetate, allyl alcohol and acrolein

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ABSTRACT

Allyl acetate (AAC), allyl alcohol (AAL), and acrolein (ACR) are used in the manufacture of detergents, plastics, pharmaceuticals, and chemicals and as agricultural agents. A metabolic relationship exists between these chemicals in which allyl acetate is metabolized to allyl alcohol and subsequently to the highly reactive, α,β-unsaturated aldehyde, acrolein. Due to the weaker reactivity of the protoxicants, allyl acetate and allyl alcohol, relative to acrolien we hypothesized the protoxicants would attain greater systemic exposure and therefore deliver higher doses of acrolein to the internal organs. By extension, the higher systemic exposure to acrolein we hypothesized should lead to more internal organ toxicity in the allyl acetate and allyl alcohol treated animals relative to those treated with acrolein. To address our hypothesis we compared the range of toxicities produced by all three chemicals in male and female Fischer 344/N rats and B6C3F1 mice exposed 5 days a week for 3 months by gavage in 0.5% methylcellulose. Rats (10/group) were dosed with 0–100 mg/kg allyl acetate, 0–25 mg/kg allyl alcohol, or 0–10 mg/kg acrolein. Mice (10/group) were dosed with 0–125 mg/kg allyl acetate, 0–50 mg/kg allyl alcohol, or 0–20 mg/kg acrolein. The highest dose of allyl acetate and acrolein decreased survival in both mice and rats. The primary target organ for the toxicity of all three chemicals in both species and sexes was the forestomach; squamous epithelial hyperplasia was observed following exposure to each chemical. In both species the highest allyl acetate dose group exhibited forestomach epithelium necrosis and hemorrhage and the highest dose of acrolein led to glandular stomach hemorrhage. Liver histopathology was the most apparent with allyl acetate, was also observed with allyl alcohol, but was not observed with acrolein. All chemicals had effects on the hematopoietic system with allyl acetate having the most pronounced effect. When dosed at quantities limited by toxicity, allyl acetate and allyl alcohol produce higher levels of urinary mercapturic acids than the minimally toxic dose of acrolein. This observation is likely due to biotransformation of allyl acetate and ally alcohol to acrolein that occurs after absorption and suggests that these chemicals are protoxicants that increase systemic exposure of acrolein. Increased systemic exposure to acrolein is likely responsible for the differences in hepatic toxicological profile observed with these chemicals.

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1. Introduction

Allyl acetate is an important intermediate in the synthesis of many industrial chemicals and has several industrial applications and can be found in consumer products, hair conditioners and detergents. Allyl alcohol is an industrial chemical used in synthesis of glycerol and acrolein (Liao et al., 1969) and in pesticide formulations (Scorecard, 2005). Human dietary exposure to allyl alcohol, as well as several allyl esters, has occurred through their use as food additives (WHO, 1997). Acrolein is a naturally occurring chemical found in food and is also used as an industrial chemical and herbicide (Ghilarducci and Tjeerdema, 1995). Although the toxicology of acrolein has been studied extensively (Kehrer and Biswal, 2000), less is known about allyl acetate and allyl alcohol.

Metabolism studies of allyl acetate and allyl alcohol strongly suggest these chemicals are protoxicants that are metabolized to acrolein, a highly reactive, α,β-unsaturated aldehyde. Upon oral administration allyl acetate is rapidly reduced by carboxyl esterases in the stomach, liver and blood to yield allyl alcohol and acetic acid. Allyl alcohol is then further oxidized to acrolein by alcohol dehydrogenase. Acrolein can subsequently be detoxified by aldehyde dehydrogenase to acrylic acid or be conjugated to glutathione. The glutathione adducts can potentially be converted by cytochromes P450 to the hard electrophile and known mutagen/carcinogen, glycidaldehyde (Barros et al., 1994; Feron et al., 1991). Alternatively, degradation products of glutathione adducts,

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S-(3-hydroxypropyl) mercapturic acid and *S*-(2-carboxyethyl) mercapturic acid, are found in the urine of rats administered acrolein, allyl alcohol, allyl chloride, allyl amine, or allyl bromide (Kaye, 1973; Sanduja et al., 1989). In support of this proposed mechanism of toxicity, pharmacological inhibition of carboxylesterase or alcohol dehydrogenase was shown to attenuate allyl ester mediated hepatotoxicity (Silver and Murphy, 1978). Similar studies with allyl alcohol demonstrated that inhibition of alcohol dehydrogenase leads to a reduction in hepatotoxicity (Reid, 1972). Furthermore, mice hypomorphic for alcohol dehydrogenase are resistant to allyl alcohol induced liver injury (Belinsky et al., 1985). Inhibition of aldehyde dehydrogenase enhances the toxicity of allyl alcohol suggesting that not only does the production of acrolein affect toxicity, but its metabolic breakdown also plays a role in liver injury (Rikans, 1987). Collectively, the data from previous studies strongly support the role of acrolein as the primary reactive species and toxicant following allyl acetate or allyl alcohol exposure.

Conflicting results have been obtained in studies assessing the carcinogenic potential of acrolein. Increased papillomas of the urinary bladder were observed in an initiation/promotion study in male F344 rats that involved 6 weeks of acrolein intraperitoneal injection followed by 28-week uracil (Cohen et al., 1992). In another study increased incidences of adrenal cortical adenomas were observed in F344 rats administered acrolein in drinking water (Lijinsky and Reuber, 1987). In a more recent study, male and female Sprague-Dawley rats were given doses of 0.05, 0.50, or 2.4 mg/kg acrolein in water by gavage daily for 24 months (Parent et al., 1992). Although survival of the 2.4 mg/kg animals was reduced compared to vehicle controls, no chemical related increases in the incidences of neoplastic or nonneoplastic lesions were observed in this study. The carcinogenic potential of acrolein has also been examined in CD-1 mice (Parent et al., 1991). Groups of 70 males and female mice received doses of 0, 0.5, or 2.0 mg/kg acrolein in distilled water daily by gavage for 2 years, and additional groups of 75 males or females received 4.5 mg/kg. Survival and mean body weights of the 4.5 mg/kg males were reduced, but survival and mean body weights of all groups of females were similar to those of the vehicle controls. Complete histopathology conducted on the 4.5 mg/kg animals and vehicle controls revealed no carcinogenic response associated with acrolein administration. Neither allyl acetate nor allyl alcohol has been evaluated for carcinogenic potential in a traditional bioassay.

Allyl acetate is mutagenic in *Salmonella typhimurium* strains TA 1535 and TA100 in the absence of activation, but not in the presence of activation (Dean et al., 1985; Irwin, 2006). Allyl alcohol was mutagenic in V79 cells, but there are no reports of bacterial mutagenicity (Smith et al., 1990), however it was not mutagenic to four strains of *S. typhimurium* with or without activation (Irwin, 2006). Acrolein is a mutagen in bacteria and V79 cells and forms DNA adducts in human fibroblasts (Foiles et al., 1989; Irwin, 2006; Smith et al., 1990; Wilson et al., 1991). In the absence of S9 activation, acrolein has been demonstrated to be a clastogen in cultured CHO cells (Irwin, 2006). Recently published cell culture studies have demonstrated preferential formation of acrolein-DNA adducts at lung cancer mutational hotspots in the p53 tumor suppressor gene in normal human bronchial epithelial cells and lung fibroblasts (Feng et al., 2006).

Because of the high production volume and widespread use of these compounds, the potential for occupational and consumer exposure, and the lack of adequate toxicity and carcinogenicity data, allyl alcohol and allyl acetate were selected for prechronic studies. Because allyl acetate and allyl alcohol are metabolized to acrolein, which is significantly more acutely toxic than either parent compound, similar toxicities between the three compounds would suggest that the effects are a byproduct of metabolism to acrolein. If this is the case, carcinogenicity studies of allyl acetate and allyl alcohol may not be necessary, because acrolein was not carcinogenic following oral exposure (Parent et al., 1991, 1992); however, substantially different toxicities would point to the need for additional testing. To address this question, a comparative toxicology study of allyl acetate, allyl alcohol, and acrolein was conducted in the same animal strains and at the same laboratory and the data are presented here. For a detailed breakdown of the study the reader is directed to the NTP toxicity report, NTP TOX 48 (Irwin, 2006).

2. Materials and methods

2.1. Chemicals

Technical grade allyl acetate (CAS no. 591-87-7; lot 0425EF), allyl alcohol (CAS no. 107-18-6; lot 00501TF) and acrolein (CAS no. 591-87-1; lot 11163AG) were obtained in single lots from Aldrich Chemical Company (Milwaukee, WI). Based on the analyses performed by the manufacturer, allyl acetate, allyl alcohol and acrolein had a purity of approximately 93.3%, 98.8% and 98.8%, respectively. All preadministration dose analyses indicated concentrations were within 10% of the target concentration, except in one case in which a dose of allyl acetate was administered at 11% below the target dose.

2.2. Animals

All animals were obtained from Taconic Laboratory Animals and Services (Germantown, NY). F344/N rats and B6C3F1 mice were shipped at approximately 5 weeks of age, were quarantined for 10–14 days, and were approximately 6–7 weeks old at the start of the study. Animals were randomly assigned to dose groups by sex and body weight. There were no statistically significant differences between group mean body weights prior to initiation of these studies. Rats and female mice were housed five per cage. Male mice were individually housed. NIH-2000 pelleted diet (Zeigler Bothers, Inc., Gardeners, PA) and tap water were available *ad libitum*. The animal room temperatures were approximately 70 ± 3 °F and humidity was 50 ± 15%. Fluorescent lights were on for 12 h/day, and there were a minimum of 10 room air changes per hour.

2.3. Experimental design

In the core toxicology study, groups of 10 animals of each species and sex were given allyl acetate, allyl alcohol or acrolein by gavage, once a day, 5 days/week, for 14 weeks. Doses for each chemical were selected following review of previous studies and are shown in Table 1. For a more detailed discussion of the dose selection process the reader is referred to the NTP toxicity report, NTP TOX 48 (Irwin, 2006). All dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice in 0.5% methylcellulose. Animals were checked twice each day for signs for moribundity or morbidity, and were examined once weekly for clinical signs of toxicity. The individual animal's body weights were recorded once weekly, and the most recent weight was used to determine the dosing volume. At study termination, survivors were weighed, anesthetized with carbon dioxide, and were bled for clinical pathology studies prior to euthanasia and necropsy. Animals received at least two consecutive dose administrations, with the last dose administered approximately 30 min prior to bleeding. Blood was collected into microcollection tubes (Sarstedt, Inc., Newton, NC) containing potassium-EDTA for hematology studies and into serum separator tubes to obtain samples for clinical chemistry. Clinical chemistry parameters included urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile salts. Hematological analyses included hematocrit; hemoglobin concentration erythrocyte, reticulocyte and platelet cell counts; mean cell volume; mean cell hemoglobin concentration; and total leukocyte counts and differentials.

In the clinical pathology study, groups of 10 male and 10 female rats were administered allyl acetate, allyl alcohol or acrolein in identical fashion to the core study rats. Blood was collected from the retroorbital sinus of these rats on days 4 and 23 and subjected to hematological and clinical chemistry analysis as described above.

All animals, including early death animals, received a necropsy. During each necropsy, all tissues were examined *in situ* for gross lesions. At study termination, selected organ weights (spleen, liver, thymus, heart, lung, right testis and kidney) were determined. All collected tissues were preserved in 10% neutral buffered formalin. Tissue examined microscopically can be found in Irwin (2006).

2.4. S-(3-Hydroxypropyl) mercapturic acid

Urine was collected from all core study rats and mice after the first dose and again after 45 doses. Approximately 15–30 min after dosing, animals were placed in metabolism cages for a 24-h period where feed and water were available *ad libitum*. Urine collection tubes were kept on ice throughout the 24-h collection period and were frozen at −20 °C after conclusion of the collection period. Sam-