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Two-year carcinogenicity study of acrylamide in Wistar Han rats with in utero exposure

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ABSTRACT

Acrylamide is an important chemical with widespread industrial and other uses in addition to generalized population exposure from certain cooked foods. Previous rat studies to assess the carcinogenic potential of acrylamide have been carried out exclusively in the Fischer 344 rat with identification of a number of tumors amongst which mesotheliomas of the tunica vaginalis is an important tumor endpoint in the classification of acrylamide as a 'probably human carcinogen. In a rat carcinogenicity study to determine the human relevance of mesotheliomas Wistar Han rats were exposed to 0, 0.5, 1.5, or 3.0 mg acrylamide/kg body weight/day in drinking water starting at gestation day 6. At the end of two years, mammary gland fibroadenomas in females and thyroid follicular cell tumors in both sexes were the only tumors increased in acrylamide treated rats. These tumor endpoints have rat-specific modes of action suggesting less likelihood of human cancer risk than previously estimated. This study demonstrates that tunica vaginalis mesotheliomas are strain specific and not likely of genotoxic origin.

1. Introduction

Acrylamide (ACR) is a monomer used in the manufacture of polymers for mining, oil and natural gas processing, paper production, waste processing, as well as hospital, laboratory and other uses. Adverse health effects from industrial emissions and exposure has been extensively studied Lipworth et al., 2012) and no adverse effects have been reported with daily exposure up to 2.1 mg/kg/day (Erdreich and Friedman, 2004). Nevertheless, due to various risk assessments, several of the workplace permissible exposure limits (PEL) are under evaluation and may be lowered considerably.

In addition to industrial exposure to acrylamide, generalized population exposure to acrylamide in foodstuffs has been documented (Vesper et al., 2008, 2010). This exposure to ACR has become a worldwide concern because of its generation in a variety of carbohydrate rich foods when cooked at temperatures exceeding 120 °C (Mottram et al., 2002; Friedman, 2003). At these temperatures, Maillard reaction of sugars with asparagine residues produce acrylamide (Friedman, 2005). The 64th Joint FAO/WHO Expert Committee on Food Additives concluded that an intake of 1 μg/kg body weight/day of ACR could be taken to represent the average for the general population (JECFA, 2005). The USFDA estimated a mean intake of 0.4 μg/day for ages 2 and up with a 90% confidence limit of >2 μg/kg (http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM197239.pdf). This equates to an adult male exposure of 1.4 μg/day.

Acrylamide has previously been shown to cause fibroadenomas of the mammary gland and thyroid gland follicular tumors in rats (Johnson et al., 1986; Friedman et al., 1995; Beland et al., 2013). Since these tumor sites have well documented rat-specific modes of action (Neumann, 1991; Ben-Jonathan et al., 2008; Alison et al., 1994), a primary consideration in classification of acrylamide as a "probable human carcinogen" centers on the induction of tumors of the tunica vaginalis mesotheliomas (TVMs) in F344 rats (Johnson et al., 1986; Friedman et al., 1995; Beland et al., 2013). These unique mesotheliomas in acrylamide-treated F344 rats might be considered a potential human health risk if they were caused by a genotoxic mechanism (Wall, 2005). However, F344 rats have an elevated incidence of TVMs secondary to their known high spontaneous incidence of Leydig cell tumors (Maronpot et al., 2009). TVMs found in the acrylamide studies have the same biological behavior, ultrastructure, and morphology as TVMs found in control rats, prompting the conclusion that acrylamide accelerates the appearance of these background tumors (Damjanov and Friedman, 1998; Maronpot et al., 2009). It has also been shown that

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the time-to-tumor and incidence data for TVMs are consistent with a non-genotoxic mechanism (Maronpot et al., 2009). The mechanism of action of acrylamide induction of TVMs has been elaborated (Shipp et al., 2006).

The EPA calculation of the cancer risk for ACR relies on 2 important considerations. The first is that acrylamide acts as a mutagen. We will not discuss this assumption here. Second, that tunica vaginalis mesotheliomas (TVMs) are added to thyroid tumors in males (EPA, 2010). Depending on which study is used (Johnson, Friedman, or Beland) there are either twice as many TVMs as thyroid tumors or the same number. While the biochemistry supports an endocrine mechanism for TVMs, there are no data directly demonstrating this mechanism (EPA, 2010). In order to clarify the mode of action of acrylamide induced neoplasms, we sought out a strain of rats which did not get Leydig cell tumors and had a low level of background TVMs to examine a potential endocrine mode of action. If acrylamide acted by a genotoxic mode of action, it would necessarily produce TVMs in a second strain. Wistar Han rats were selected due to their longevity and low incidence of Leydig cell tumors. We report here the oncogenic response of Wistar Han rats to orally administered acrylamide. Furthermore, concern has been expressed that children might be at higher risk to acrylamide due to greater dietary acrylamide intake (EPA Science Advisory Board, 2008). This parameter was evaluated in the current research by initiating dosing on gestation day 6 in pregnant rats and continued to termination of the F1 offspring 2 years later.

2. Materials and methods

2.1. Study conduct

This study was conducted under GLP guidelines enacted in Germany in the 'Chemikaliengesetz', current edition and OECD Principles of Good Laboratory Practice' Document Nos. 1, 8 and 13 ENV/MC/CHEM (98)17, ENV/JM/MONO(99)24, and ENV/JM/MONO (2002)9 and was externally audited. The following guidelines were considered: OECD Guideline for the Testing of Chemicals No. 453: Combined chronic toxicity/carcinogenicity studies, adopted 7 September 2009; and EC method 8.33. Combined chronic toxicity/carcinogenicity test, 88/303/EEC; Official Journal of European Communities, L 133 1988.

2.2. Test article

Acrylamide (C3H3NO, CAS no 79-06-1, 1,2-propenamide; >99.9% pure) was purchased from Sigma Aldrich as a white, odorless, crystalline solid which is stable at room temperature for at least 2 years. Acrylamide was administered daily in drinking water at the following doses: 0, 0.5, 1.5 and 3.0 mg/kg/day. Water was available ad libitum. In a separate study, solutions of acrylamide were prepared in tap water and evaluated for stability at room temperature at 6, 13, 20, 27, 41, 55 or 90 test days after preparation and recovery ranged from 96.9% to 102.6%. In the present study acrylamide solutions were prepared weekly and after concentration adjustment for body weight, water bottles were changed weekly. Aliquots for analysis were taken at the beginning and end of exposure to determine stability. Acrylamide concentration in the drinking water was determined at test week 4, 10, 16, 22, 28, 34, 40, 46, 52, 65, 78, and 91. Fig. 1 shows that acrylamide was stable in the animal cages for the weeks of exposure.

2.3. Animals

Sperm positive female Wistar Han™/RccHan™:WIST rats were obtained from Harlan Laboratories GmbH, Serumweg 48, 27324 Eystrup, Germany in multiple deliveries. At gestation day 6 pregnant dams were provided acrylamide in their drinking water with exposures continuing in F1 offspring through postnatal day (pnd) 722. Rats were housed 1 per cage in MACROLON cages with granulated wood bedding (Brandenburg, 49424 Goldenstedt/Arkeburg). Study design is summarized in Table 1. Sixty male and sixty female rats were selected for the chronic study as well as 5 sentinel animals. Five rats per sex per group were sacrificed at 12 months and 18 months. Animal rooms were alternately lit (about 150 lx at approximately 1.50 m room height) and darkened in a 12-h lighting cycle. Cage side observations were conducted twice per day during the week and once per day on weekends. Ophthalmological and auditory examinations were conduct at the interim kills (12 and 18 months) and at termination.

On day 4 after birth, the weights of the pups were determined. The size of each litter was adjusted by eliminating extra pups to yield, as nearly as possible, five males and five females per litter. The remaining animals were allowed to remain with the dams until day 21 of lactation (weaning). On lactation day 21, the F1 animals were randomized using a computer randomization program to assign the animals to the subsets within each group.

2.4. Assessment of physical and functional development

Neurological determinations were conducted on 5 rats per sex per group. Ear opening, eye opening, cleavage of the balanopreputial gland, vaginal opening and upper incisor eruption were assessed. No significant changes were seen in any of these parameters. Functional tests that included open field behavior and passive avoidance (learning) were conducted at pnd 27 and passive avoidance (learning) at pnd 34. Functional tests included grip strength and locomotor activity. No changes were seen in these parameters.

2.5. Gross necropsy

After 52, 78 or 104 test weeks the respective animals were sacrificed under ether anesthesia by cutting the abdominal aorta, exsanguinated, weighed, and necropsied under the direction of a pathologist. For rats that died or were sacrificed prematurely, necropsy examinations were performed immediately after the animals were found dead or after sacrifice. All superficial tissues were examined visually and by palpation and the cranial roof removed to allow observation of the brain, pituitary gland and cranial nerves. After a ventral midline incision and skin reflection, all subcutaneous tissues were examined. The condition of the thoracic and abdominal viscera was noted with due attention to the thymus, lymph nodes and heart.

The weights of the following organs of all animals assigned for interim dissection and the first 10 surviving main study animals/sex/group of the main dissection were determined before fixation: adrenal gland (2), brain, epididymis (2), heart, kidney (2), liver, ovary (2), spleen, testis (2), thyroid including parathyroid (1), and uterus. Adrenals, gonads and kidneys were weighed individually and identified as left or right. Organ weights of rats that died or were sacrificed prematurely were recorded but not included into the mean value comparison.

2.6. Histopathological examination

The following organs of all animals (including deceased or sacrificed animals) were preserved in 7% buffered formalin: adrenal (2), aorta abdominalis, bone (os femoris with joint), bone marrow (os femoris, sternum), brain (cerebrum, cerebellum, medulla/pons), caecum, clitoral gland (2), coagulating gland with seminal vesicle, epididymis (2), extraorbital lacrimal gland (2), eye with

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