A Lifetime Oncogenicity Study in Rats with Acrylamide

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A lifetime oncogenicity study in Fischer 344 rats was conducted to accurately characterize the carcinogenic potency of acrylamide. Acrylamide was administered in drinking water throughout the 106-week study at concentrations required to provide a dose of 0, 0.1, 0.5, or 2.0 mg/kg/day to males or 0, 1.0, or 3.0 mg/kg/day to females. Complete necropsy and gross pathology examinations were performed on all study animals. Histopathology examinations were conducted on selected tissues of all high-dose and control animals. Selected tissues from intermediate and low-dose groups were subjected to histopathological examinations as required to clarify high- and control-dose group observations. There was no visual observation of neurotoxicity in any study animal but sciatic nerve degeneration was observed in the male and female high-dose groups. Increased mortality related to acrylamide was observed in the high-dose male group from Month 17 to the end of the study and in the high-dose females during Month 24. Mesotheliomas of the testicular tunic were significantly increased in both the high-dose females during Month 24. Mesotheliomas of the testicular tunic were significantly increased in the high-dose male group. The combined incidence of mammary gland adenocarcinomas and fibroadenomas was significantly increased in both acrylamide-dosed female groups. Males and females in the high-dose groups as well as females of the low-dose group had significantly (p < 0.001) increased thyroid follicular cell adenomas and adenocarcinomas. A variety of other tumor types observed with increased incidence in a previous acrylamide oncogenicity study (i.e., combined CNS glial neoplasms, papillomas of the oral cavity, adenomas of the clitoral gland, and uterine adenocarcinomas) were not observed to be present at increased incidence in this study. This study confirms previously described acrylamide induction of benign tumors of the thyroid and mammary glands as well as mesotheliomas of the testis. By using a larger number of animals with an unbalanced study design, this study showed that acrylamide did not induce glial tumors and demonstrated that the no-observable-effect level for scrotal mesotheliomas is 0.5 mg/kg. It also demonstrated that the increased incidence of mammary tumors was again within historical control ranges. © 1995 Society of Toxicology.

Acrylamide is a monomer used to manufacture water-soluble polymers and copolymers. Approximately 43% of acrylamide production is used in the manufacture of polyacrylamides used in municipal and industrial wastewater treatment and municipal drinking water treatment as flocculants and dewatering aids (NIOSH, 1976; Conway et al., 1979). Another 20% of the polyacrylamide is used in the paper pulp industry as dry strength agents, retention, and draining aids. Miscellaneous uses of polyacrylamides include drilling muds, polyester-laminating resins, textile resins, flocculation of ores, mining tailings, and coal, friction reduction, thickening agents, soil stabilizers, oil-in-water demulsifiers, gel chromatography and electrophoresis, photography, dyeing, and ceramics (Bikales, 1973; Flock and Rasch, 1973; MacWilliams et al., 1973; NIOSH, 1976; USEPA, 1976; Conway et al., 1979).

Occupational exposure to acrylamide can occur not only during manufacture and production of acrylamide and polyacrylamides, but also during its use in the preparation of polyacrylamide gels for separation of macromolecules or as a chemical grout used to repair sewer lines, waterproof mines, tunnels, and foundations or to consolidate soil around roadbeds and dams.

Acrylamide is a well-known neurotoxin, the effects of which may be observed after chronic or acute exposure (Schaumburg et al., 1974; Spencer and Schaumburg, 1974). Neuropathology in rats characterized by axonal degeneration with a concomitant accumulation of microtubules observable by electron microscopic evaluation of peripheral nerves has been noted in response to chronic doses above 0.5 mg/kg/day.

Acrylamide has been repeatedly shown to be inactive in genetic toxicity tests which measure point mutations but is active in tests which can detect chromosomal breaks (NTP, 1985; Linjinsky and Andrews, 1980; Shiraiishi, 1978; Smith et al., 1986; Preston et al., 1986).

Acrylamide has been tested in several screening studies in mice for carcinogenic activity (Bull et al., 1984a,b; SENCAR mice treated with high intermittent doses of acrylamide (300 mg/kg) orally, dermally, or intravenously and observed for 1 year had no increased incidence of papillomas or squamous cell carcinomas. However, when treatment with acrylamide was followed by vigorous promotion with a promoting agent (TPA), there was an increase in tu-
tumors of concern; and (3) a sialodacryoadenitis virus in-
inogenicity of acrylamide and provide adequate informa-
fitation of experimental and control rats may have con-
characterization of the carcinogenic hazard associated with
male scrotal mesotheliomas, CNS tumors, and combined
el al. (1986) suggest may have
increased incidence of CNS tumors was also reported in
(node (3 mg/kg) group and low tumor incidence rates were
expected in the lowest dose (1 mg/kg) group based upon the
outcome of the Johnson et al. (1986) study.
This current study was also planned to characterize the
carcinogenic potency (dose–response) of acrylamide to fe-
male rats. This characterization was not possible based
upon the outcome of the Johnson et al. (1986) study since
a significant increase of tumors in females was only ob-
erved at the highest dose of exposure. The number of fe-
male rats in each experimental group was increased since
acrylamide-induced mortality was expected in the highest
dose (3 mg/kg) group and low tumor incidence rates were
expected in the lowest dose (1 mg/kg) group based upon the
outcome of the Johnson et al. (1986) study.

MATERIALS AND METHODS

Test material. Acrylamide (C3H7NO, CAS 79-06-1, 2-propenamide) is
a white, odorless, crystalline solid which is stable at room temperature for
at least 2 years. Ultra-Pure electrophoresis-grade crystalline acrylamide
was purchased from Polysciences, Inc. (Warrington, PA). Purity was
99.9%. Unopened bottles were stored at −20°C and opened bottles at 4°C.
The test material was analyzed (HPLC and TLC) for the presence of other
organic compounds which could affect the outcome of the study.

Animals. Weanling Fischer 344 albino rats, a total of 735 male and
410 female rats, from a specific pathogen-free colony, 25 days of age, were
purchased from Charles River Breeding Facility (Kingston, NY). The sup-
plier certified negative serum titers to Sendai virus (SEN), sialodacryoad-
nitis virus (SDAV), rat coronavirus (RCV), revirorum type 3 (RE03), Kil-
ham’s rat virus (KRV), and pneumonia virus (PV and Mycoplasma pul-
modus (MYCO)). This colony was also negative for Corynebacterium kutscheri, M. pulmonis, Salmonella spp., Streptobacillus moniliformis,
and Bordetella bronchiseptica. Paralysis tests for ecto- and endopara-
tes were also negative. Animals were acclimated for 20 days in the same
room in which they were housed for the study. They were permanently
identified by tail tattoo with a three-digit number. The body weight range of
male rats at the start of the study was 75 to 144 g while that of the
females was 75 to 122 g.

Housing and maintenance. Animals were individually housed in stain-
less steel suspended cages with food (certified AGWAY PROLAB R-M-H
3200 Roden Meal) and acrylamide-containing drinking water solutions or
charcoal-filtered tap water was provided ad libitum. The facility was a
limited access barrier facility with laminar flow HEPA filtered air displace-
ment. This room was maintained at 72°F with approximately 60% relative
humidity, a 12-h photoperiod, and 16.0 air changes per hour.

Preparation and analysis of drinking water. Tap water was filtered
through charcoal prior to mixing with the test substance. The tap water
was analyzed for pesticide residues every 6 months. No contaminants were
present in levels which would affect the outcome of the study. Drinking water solutions containing acrylamide were prepared by adding an appropriate amount of crystalline acrylamide into a small volume of charcoal-filtered tap water to form a premix at a concentration of 20 mg/ml. Measured volumes of this premix were then added to charcoal-filtered tap water to achieve the desired concentration. Fresh solutions were prepared weekly just prior to presentation to the test animals, and excess solution needed to replace the contents of spilled bottles was stored at room temperature (25°C).

Acrylamide concentrations in the freshly prepared drinking water solutions, as well as in bottles removed from the animal cages after 1 week, were determined periodically. The samples were analyzed by HPLC using a 5-μm Hamilton PRP-1 column (15 cm × 4.1 mm), aqueous effluent, and uv detection at 200 nm.

**Experimental design.** The study design has been previously described by Frankos *et al.* (1985). At randomization, a total of 585 males and 300 females were selected on the basis of acceptable physical condition and body weight and assigned to experimental groups using a weight stratification procedure. An additional group of 25 males and 25 females was selected as sentinel animals. The allocation of animals and dose groups are described in Table 1. The control groups for each sex were split into two separate groups so as to better establish the variability of low-incidence background tumors, i.e., CNS tumors (female) and scrotal tumors. Acrylamide was administered in the drinking water throughout the study at concentrations, calculated weekly, required to provide a dose of 0, 0.1, 0.5, or 2.0 mg/kg/day to male rats or 0, 1.0, or 3.0 mg/kg/day to female rats. Water consumption was measured weekly throughout the study, and no difference was observed between control and treated animals. Body weight and food consumption of each animal under study were recorded prior to the start of the study, weekly for 16 weeks, and every 4 weeks thereafter. All experimental animals received the test substance in drinking water throughout the entire study period from 44 to 45 days of age to study termination (106–108 weeks on test) except for those animals which were sacrificed in a moribund state or those that died during the course of the study. All animals were observed twice daily for mortality, morbidity, and/or obvious signs of toxicity. Physical examinations for signs of toxicity were performed weekly for the first 16 weeks, every 4 weeks for the next 24 weeks, and biweekly for the remainder of the study. Animal examinations for palpable mass detection were added to the physical exam process starting at Study Month 6.

**Sentinel animal program.** During the quarantine period, serum samples from four males and four females were screened for identification of exposure to the following: PVM, REO-3, SEN, KRV, SDAV, MYCO, Theiler's virus, murine encephalomyelitis (GD-7), lymphocytic chorion meningitis (LCM), and Toolins H-1 (TH1). In these same eight animals, *Mycoplasma, Pasteurella,* and *Bordetella* isolations were attempted from the nasopharynx and *Salmonella pseudomonas, Klebsiella,* and *Citrobacter* isolations were attempted from the cecum. These same animals were also examined grossly and microscopically for lesions prior to the start of the study.

Three animals per sex from the sentinel population were selected quarterly for serological assessment of viral titers to PVM, REO-3, SEN, KRV, TH1, and SDAV. There were only two male sentinel animals left alive at the end of the seventh quarter (21 months) so a moribund male control animal was sampled just prior to sacrifice. Three males and three females from the control groups were selected for use to detect viral exposure to these agents at the terminal sacrifice.

**Postmortem evaluations.** A complete necropsy was performed on all study animals. The terminal necropsy procedures took place during an 11-day period. The terminal sacrificed animals were randomized for necropsy according to day of sacrifice, dose group, sex, time of day sacrificed, and prosector. Rats were fasted overnight, weighed, and then sacrificed by CO2 asphyxiation. Brain, liver, kidneys, and testes were excised and weighed, paired organs weighed together. Group mean organ weights and organ-to-body weight ratios were calculated. Gross lesions and the following tissues and organs were examined, removed, and preserved in 10% buffered formalin: adrenal glands, aorta, bone (femur and sternbea with bone marrow), bone marrow smear, brain, epididymides, esophagus, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph node (mesenteric), ovaries, pancreas, peripheral nerve (sciatic), pituitary gland, prostate gland, salivary glands (submandibular), seminal vesicles, skeletal muscle (abdominal wall, femoral muscle, diaphragm), small intestine (duodenum, jejunum, ileum), skin/mammary gland, spinal cord, spleen, stomach, tes- tes, thymus, thyroid/parathyroid gland, tongue, trachea, ureters, urinary bladder, uterus, vagina, and eyes.

Selected tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Initially, microscopic evaluation was completed only on the high-dose and control animals. Clarification of these initial histopathology evaluations was completed by examination of additional tissues harvested from the intermediate and/or low-dose groups as required. In all female rats, thyroid, brain (three levels), and mammary glands were examined. The spinal cord (three levels), uterus, and gross lesions were examined in all control and high-dose female rats found dead or sacrificed moribund in the low-dose group. Thyroid and testes were examined microscopically in all male rats. The brain (three levels), spinal cord (three levels), and gross lesions were examined in all control and high-dose male rats and in all low- and mid-dose male rats that were found dead or sacrificed moribund.

**Statistical analyses.** Body weight, food consumption, and water consumption were analyzed by one-way analysis of variance (ANOVA). If ANOVA indicated possible differences between control and compound-treated groups, Dunnett's t test was used to determine which means of the compound-treated groups were significantly different from the controls at the 95% confidence interval. Statistical evaluations included comparisons of all groups relative to Control Group 1, all groups relative to Control Group 2, and all groups relative to the pooled control groups (Control Groups 1 and 2, combined data).

Pairwise t tests were used to compare the mean absolute organ weights (and mean percentage relative organ weights) between the pooled control groups and each treated group by sex and organ. Pooled standard deviations were used in testing. Two-sided trend tests were performed to determine whether the mean weights increased (or decreased) with increasing dose. The pooled standard deviation was also used in the tests.

Tumor incidence data were analyzed statistically by the following methods. Estimates of survival were obtained using the Kaplan–Meier method (Kaplan and Meier, 1958). To test for differences in survival among treated groups, the log rank test (Mantel, 1966) was applied. In addition, a test for dose-related trend in survival was also performed (Tarone, 1975). The data were also analyzed using lifetime tumor rates which were not time-adjusted utilizing the Cochran–Armitage trend test (Cochran, 1954; Armitage, 1978).
TABLE 2

| Acid | Concentration found (ppm) | Limit of detection (ppm) |
|------|---------------------------|--------------------------|
|      | 1  | 2                         |                          |
| Acrylic acid | <10 | <10 | 10 |
| Methacrylonitrile | <7 | <7 | 7 |
| Acetamide | <60 | <60 | 60 |
| Acrylonitrile | <0.2 | <0.2 | 0.2 |
| Ethyl acetate | <10 | <10 | 10 |
| 3-Hydroxy propionitrile | <50 | <50 | 50 |
| Soluble polyacrylamide | 12.7 | 10 | 10 |
| Water | 250 | 890 | 50 |

* Ultra-Pure, electrophoresis-grade acrylamide, Lot No. 54157, produced and analyzed by Polysciences, Inc. (Warrington, PA).

Since cause of death was not determined on a per-animal basis in this study, assumptions regarding tumor lethality were necessary. Testicular mesothelioma was regarded as possibly fatal or "rapidly lethal" (Tarone, 1975). For all tumor types, two other approaches were also applied, assuming the tumor to be nonfatal or incidental. These were the interval-based methods of Peto et al. (1980) and the logistic score test (Dinse and Lagakos, 1983). A continuity correction was used only for the logistic score test. In addition, tests were performed using actual doses or using "ordinal scores" (coding doses as 0, 1, 2, or 3 for males or 0, 1, or 2 for females). In the case of male mesotheliomas and the combined incidence of follicular cell adenomas and adenocarcinomas in females, the high-dose group was excluded and the tests were rerun to determine significance at the lower doses. Results are considered significant if \( p < 0.05 \), unless stated to be significant at a higher confidence level.

RESULTS

Test material. The crystalline acrylamide tested was found to contain low concentrations of some hydrocarbons, but none of these were expected to affect the outcome of the study (Table 2).

Analysis of drinking water. Acrylamide was found to be stable in drinking water for a period of 1 week at room temperature. Stability measurements of 97 to 117% were obtained for concentrations from 0.5 to 17 ppm stored over a 1-week period (Table 3).

Mortality, body weight, and clinical signs. Mortality for male and female rats is shown in Figs. 1 and 2, respectively. Mortality rates were low through Study Week 60. During Study Weeks 68-72, mortality increased in the high-dose male group and this increase continued through the end of the study. At termination of the study (106 weeks), mortality (found dead plus moribund sacrificed animals) was 75% in the high-dose male group compared to 53 and 44% in Control Groups 1 and 2, respectively. Differences in mortality between the male control groups were greater than differences in mortality between either of those groups and the low- or mid-dose treated groups. Female rats showed no differences in mortality over the first 23 months of the study. During the last month of the study, more high-dose females were sacrificed in a moribund condition than either control group. At termination, total females found dead or sacrificed moribund were 28, 40, 35, and 49% in Control Groups 1 and 2 and low-dose and high-dose female groups, respectively.

Mean body weights of male treated animals receiving the high dose of 2.0 mg/kg/day were consistently decreased from those of the control groups from Week 8 until the end of the study (Fig. 3). This decrease was statistically significant from Week 40 (high-dose group 398 g/rat vs control 408 g/rat to the study end (high-dose group 375 g/rat vs control 412 g/rat). No significant mean body weight differences were identified in the low- or mid-dose groups.

Decreases in mean body weight of female rats occurred earlier and were larger than those seen in the males (Fig. 4). Mean body weights of the high-dose female rats were significantly decreased from controls as early as Week 3 of the study and remained significantly lower throughout the study. Mean body weights of the low-dose female rats were significantly decreased from Weeks 8 to 32 with no significant differences later in the study. There were no compound-related or biologically significant differences in food or water consumption among the different experimental groups.

Palpable masses were observed among male rats beginning in the first 12 months of the study. These palpable masses were located primarily in the inguinal area and they were most likely associated with inflammation of the preputial gland. The incidence of these inguinal masses was similar among all dose groups during the second year of the study. Although there were no differences between dose groups in percentage of rats with masses at individual locations, the total percentage of rats with palpable masses was increased in the high-dose group compared to either individual or combined control groups.

TABLE 3

| Acrylamide Stability |
|-----------------------|
| Day                   |
| 0.51 | 0.67 | 0.60 | 0.55 | 0.55 |
| 3.0  | 3.0  | 3.1  | 3.1  | 3.1  |
| 5.5  | 5.6  | 5.6  | 5.5  | 5.6  |
| 14.6 | 14.9 | 15.1 | 14.7 | 14.5 |
| 17.0 | 16.9 | 17.3 | 16.7 | 17.1 |

Note: Concentration of acrylamide (ppm); room temperature storage, 25°C.
Of particular interest, since acrylamide is a neurotoxin, there were no significant occurrences of signs of neurotoxicity such as hind limb weakness, ulceration of the hind feet, and/or gait abnormalities in either males or females. Female rats in this study received the highest chronic dose of acrylamide (3 mg/kg) ever administered to rats without observation of clinical signs of neurotoxicity.

**Sentinel animal program.** The initial 4 male and 4 female rats sampled as well as the 25 sentinel rats of each sex sampled during the study were found to be free of exposure or infection by the pathogens tested.

**Final body and organ weights.** Decreased mean final body weights in both the high-dose male and female groups reflect the decreased mean body weights observed during life. While there were slight differences between group mean organ weights that were, in some cases, statistically significant, these differences generally reflected group differences in final body weight and possibly the influence of pathological processes in individual animals (i.e., testicular interstitial cell tumors) rather than treatment-related differences.

**Necropsy and pathologic examination.** A summary of the microscopic observations is shown in Tables 4 and 5. Mesotheliomas of the testicular tunic were statistically \((p < 0.001)\) increased in the high-dose male group in comparison to the combined control groups. The slight increase in the mid-dose group (0.5 mg/kg/day) was not statistically significant. There was no increase in the low-dose group. In all male rats with mesotheliomas in the present study, the neoplasm was found on the tunica vaginalis of the testes. Mesothelioma sites included the scrotal sac, testes, and the peritoneal cavity. In all cases where the mesothelioma was found in the peritoneal cavity, it was also found in the testicular tunic. No attempt was made to differentiate benign and malignant mesotheliomas on a morphological basis. All were considered as malignant.
There was no significant difference in the incidence of mammary gland adenocarcinoma between female control and treated rats. However, there was an increased incidence of mammary gland fibroadenomas in both acrylamide-treated female groups. The combined incidence of adenocarcinomas and fibroadenomas was also statistically significantly increased ($p < 0.0001$) in both dose groups (Peto et al., 1980) when compared to the combined control groups.

Microscopically, there were no significant differences in the incidence of thyroid gland C-cell adenomas or carcinomas, the most common type of thyroid neoplasm. No statistically significant increased incidences of thyroid follicular cell adenocarcinomas were seen in either sex. Both male and female rats in the high-dose groups, as well as females of the low-dose group, had statistically ($p < 0.001$) increased combined incidences of thyroid follicular cell adenomas and adenocarcinomas (Peto et al., 1980). The follicular cell adenomas were accompanied by a slight increase in incidence of follicular cell carcinomas in the high-dose female group, which was not statistically significant (Peto et al., 1980). There were no significant increases in the incidence of follicular cell hyperplasia.

In both males and females, sciatic nerve degeneration was seen with increased frequency in the high-dose groups compared to the respective control groups. The lesion was characterized by vacuolated nerve fibers, was of minimal-to-mild severity, and is typical of lesions seen after acrylamide treatment in other studies (Burek et al., 1980; Johnson et al., 1986).

**DISCUSSION**

The findings of this study both qualitatively and quantitatively confirm certain outcomes observed in the prior carcinogenicity study of acrylamide in F344 rats (Johnson et al., 1986). However, there are highly significant differences in the outcome of this study in comparison to that of John-
TABLE 4
Selected Microscopic Lesions in Male F344 Rats after Chronic Acrylamide Treatment

| Dose level | 0  | 0.1 | 0.5 | 2.0 |
|------------|----|-----|-----|-----|
| No. animals per group | 102 | 102 | 204 | 102 | 75 |
| Tissue/Lesion | Brain (glial origin) | No. examined | 102 | 102 | 98 | 50 | 75 |
| | Astrocytoma | 1 | 0 | 0 | 0 | 2 |
| | Oligodendrogloma | 0 | 1 | 1 | 1 | 0 |
| Spinal cord (glial origin) | No. examined | 82 | 90 | 68 | 37 | 51 |
| | Astrocytoma | 0 | 0 | 1 | 0 | 1 |
| Total rats with CNS tumor of glial origin | 112 | 13 |
| Testes | No. examined | 102 | 102 | 204 | 102 | 75 |
| | Tunic-mesothelioma | 4 | 4 | 9 | 8 | 13* |
| Sciatic nerve | No. examined | 83 | 88 | 65 | 38 | 49 |
| | Degeneration | 30 | 29 | 21 | 13 | 26 |
| Thyroid gland | No. examined | 100 | 102 | 203 | 101 | 75 |
| | Follicular cell adenoma | 2 | 1 | 9 | 5 | 12* |
| | Multiple | 0 | 0 | 0 | 0 | 3 |
| | Follicular cell carcinoma | 1 | 2 | 3 | 0 | 3 |
| | Follicular cell, atrophy | 1 | 3 | 1 | 1 | 3 |
| | Follicular cell, hyperplasia | 2 | 2 | 7 | 3 | 2 |
| Total rats with follicular cell neoplasms | 3 | 3 | 12 | 5 | 17 |

* Statistically significant, p < 0.001 (Peto et al., 1980).

son et al. (1986). For example, the Johnson et al. study had shown an increased mortality among female rats at the end of the chronic study. Those findings contrast with those of the present study and others (Burek et al., 1980; Keefe, 1989) which have indicated that the male is more sensitive than the female to the toxic effects of acrylamide.

One major objective of the present study was to investigate whether glial tumors in the Johnson et al. study were significant. Glial cell tumors of the CNS, though not falling into the class of common rodent tumors such as the mammary or liver tumors, are still fairly common spontaneous tumors in 2-year bioassays. The prior acrylamide bioassay by Johnson et al. (1986) had reported an 8% incidence in the control male rats with incidences in treated male rats increased but not statistically significant at 13%. Female rats in that study had a statistically significant increase of glial cell tumors at the high dose. Unlike the findings of the Johnson et al. study (Johnson et al., 1986), incidences of central nervous system tumors of glial origin for both the high-dose female and high-dose male groups in the present study were within the historical background incidence of Fischer 344 rat as reported in NCI studies (Solleveld et al., 1984; Haseman et al., 1984) and not significantly different from the concurrent controls. Although the incidence of astrocytomas alone was statistically increased for the male high-dose group (Peto et al., 1980), it is most appropriate to combine all neurological neoplasms for statistical analysis since they all arise from similar progenitor cells (McConnel et al., 1986). When this analysis was performed, there was no statistically significant increase in incidence of CNS glial neoplasms in the high-dose groups of either sex.

The incidence of glial cell tumors is quite variable, as illustrated by recent studies of several color additives that were conducted to meet FDA registration requirements and which were designed to include duplicate control groups (Haseman et al., 1984). In one of these studies, the incidence of brain gliomas in male rats was 0/69 in one control group.

TABLE 5
Selected Microscopic Lesions in Female F344 Rats after Chronic Acrylamide Treatment

| Dose level | 0  | 0  | 1.0 | 3.0 |
|------------|----|----|-----|-----|
| No. animals per group | 50 | 50 | 100 | 100 |
| Tissue/lesion | Brain (glial origin) | No. examined | 50 | 50 | 100 | 100 |
| | Astrocytoma | 0 | 0 | 2 | 2 |
| | Oligodendrogloma | 0 | 0 | 0 | 0 |
| Spinal cord (glial origin) | No. examined | 45 | 44 | 21 | 90 |
| | Astrocytoma | 0 | 0 | 0 | 1 |
| Total rats with CNS tumor of glial origin | 0 | 0 | 2 | 3 |
| Sciatic nerve | No. examined | 37 | 43 | 20 | 86 |
| | Degeneration | 7 | 12 | 2 | 38 |
| Mammary gland | No. examined | 46 | 50 | 94 | 95 |
| | Fibroadenoma | 5 | 4 | 20* | 26* |
| | Adenocarcinoma | 2 | 0 | 2 | 4 |
| | Hyperplasia | 26 | 27 | 33 | 47 |
| Total rats with mammary gland neoplasms | 7 | 4 | 21* | 30* |
| Thyroid gland | No. examined | 50 | 50 | 100 | 100 |
| | Follicular cell adenoma | 0 | 0 | 7 | 15 |
| | Follicular cell adenoma, bilateral | 0 | 0 | 0 | 1 |
| | Follicular cell carcinoma | 1 | 1 | 3 | 7 |
| | Follicular cell, hyperplasia | 0 | 1 | 5 | 1 |
| Total rats with follicular cell neoplasms | 1 | 1 | 10 | 23* |

* Statistically significant, p < 0.001 (Peto et al., 1980).
adenocarcinomas. It is, therefore, unclear whether the ap-
study was still well within the historical range at a dose
ever, the incidence of these mammary tumors in the present
an incidence of these tumors which would be outside the
increased dose did not produce an increased incidence of
was biologically significant (John-
et al., 1986). If these tumors were occurring in a dose-
son (1985), who reported an incidence of total mammary
1984). A similar increase in incidence of
mesotheliomas in the mid-
dose group, 0.5 mg/kg/day, was not statistically signifi-
cantly increased over the controls and was within the range
of the most recent reported historical data of Tanigawa
et al. (1987). Therefore, the mid-dose group is also considered
to be a no-effect dose for this tumor type and contrasts with
the results of the prior study (Johnson et al., 1986). This
tumor, peculiar to the rat, was the only malignant tumor
with an increased incidence among the treated animals in
this study.

Mesothelium extends over the entire surface of the sero-
sal cavities. With few exceptions, this tissue is a monolayer
with little morphological variation from site to site. The vis-
ceral and parietal components of the tunica vaginalis con-
taining mesothelial tissue cover the testes (and epididy-
mides) and the inner surface of the scrotal sac, respectively.
These tunics are the primary site of mesothelioma in the
Fischer rat. Even when methyl(acetoxy methyl)nitrosamine
was injected into the peritoneal cavity, mesotheliomas
arose from the scrotal mesothelium rather than that of the
more directly exposed peritoneal mesothelium (Berman
and Rice, 1979). It has been reported that areas of the mes-
ethelium on the tunics display rates of cell division which are
approximately 10 times the rate in the mesothelium of
other areas of the serosa (Whitaker et al., 1982). Further
evidence that the mesotheliomas arise in the testicular tunic
comes from the data of Solleveld et al. (1984), who reported
an incidence of mesotheliomas of the peritoneal cavity in
female rats of less than 0.1% in a total of 2370 female con-
tral rats in NTP studies. Additionally, there are some indi-
cations that there may be a hormonal component to the
induction of these mesotheliomas. The Fischer 344 strain
of rat has the highest incidence of scrotal mesotheliomas
among the various strains of rats used for chronic studies.
The Fischer 344 rat developed a very high incidence of Ley-
dig cell tumors (greater than 90% incidence in control rats
at the end of 2 years) resulting in dramatic hormonal imbal-
cances in aging rats carrying these tumors (Turek and Des-
jardins, 1979). An increased incidence of mesotheliomas in
the Fischer 344 rat occurs coincident with the increased inci-
dence of Leydig cell tumors and hormonal changes.
Thyroid gland follicular cell tumors are an uncommon tumor in the Fischer rat. The incidences of adenomas in the control groups in this study are similar to those reported in NTP studies (Solleveld et al., 1984; Haseman, 1983). NTP has reported a mean control incidence in males of 1.0% with a range of 0-5% while that in females is 0.4% with a range of 0-4% in individual studies (Solleveld et al., 1984; Haseman, 1983). The incidence of adenomas in the male low- and mid-dose groups does not exceed the historical control incidences, nor are they statistically increased over the concurrent control groups. Follicular cell carcinoma incidences in the Fischer rat generally parallel the incidence of adenomas with the NTP, reporting a mean incidence of 0.8% (range 0-7%) in males and 0.4% (range 0-2%) in females. Aside from the high-dose female group, the incidence of follicular cell carcinomas does not exceed the historical control range and in no case are they statistically increased over the concurrent control.

The increased incidence of follicular cell adenomas, in the absence of a definite increase in incidence of follicular cell carcinomas, is a response which is very typical of a very weak goitrogen. Data are not yet available to assess the goitrogenic potential of acrylamide in these animals.

Seven rats in this study were diagnosed as having a rare or perhaps infrequently reported neoplasm of the brain (malignant reticulosis). The cells have nuclei that are more pleomorphic than those of most glial neoplasms, often having elongated, indented, or reniform nuclei. The most striking differential microscopic features were the pronounced perivascular infiltration by neoplastic cells and the frequent meningeal involvement. The cells also infiltrated the neuropil to varying degrees.

Garman (1989) reported three instances of malignant reticulosis in a population of 3703 treated and control Fischer 344 rats aged 18 months and older, while Goodman (Goodman et al., 1979) reported a total of 2 in a population of 1754 control rats in NCI studies. The largest number of these neoplasms reported was 19 of 89 primary brain tumors in a population of 4480 male and 4480 female Sprague-Dawley-derived rats (Krinke et al., 1985).

The relatively infrequent historical occurrence of this neoplasm in rats makes interpretation of its significance difficult. While the increased incidence in the high-dose female rats is not statistically significant, it appeared only in dosed rats in the female groups. However, in the males, one was found in a control group while the other was in the low-dose group.

A variety of other tumor types observed with increased incidence in the former study (Johnson et al., 1986), including papillomas of the oral cavity in female rats, adenomas of the clitoral gland, and uterine adenocarcinomas, were not present at increased incidences in the treated animals of this study.

Acrylamide produces peripheral nerve degeneration visible by light microscopy at doses of approximately 2 to 5 mg/kg/day and, at daily doses of 5 mg/kg/day and higher, clinical signs of ataxia and limb weakness (Burek et al., 1980; Fullerton and Barnes, 1966; Pryor et al., 1983). A prior 2-year study reported a slightly increased incidence in peripheral nerve degeneration, as determined by light microscopy, in the high-dose group (2 mg/kg/day) at termination of the study. The results of this study are similar to those observed in prior studies.

Acrylamide has shown no carcinogenic potential in humans in two epidemiological studies. American Cyanamid has completed the largest epidemiological study on acrylamide to date (Collins et al., 1989). This study examined the mortality patterns of 2293 males exposed to acrylamide in four plant locations. Death rates due to all causes were below expected rates based on national mortality rates for the U.S. males (1925–1980). There were no statistically significant excesses of cancer among workers exposed to acrylamide. In addition, no trends were observed with increasing exposure. Results were adjusted for race, age, and calendar time. Death rates due to all causes were also at or below expected levels. The conclusion drawn from this study is that acrylamide is not carcinogenic to humans and confirms the findings of a smaller prior epidemiology study on employees of the Dow Chemical Co. (Sobel et al., 1986).

Review of the toxicological data on acrylamide indicates that its neurotoxic mechanism of action may be through reaction with cytoskeletal proteins, microtubules, and microfilaments (Tanii and Hashimoto, 1983; Howland and Alli, 1986). In studies designed to examine binding of $[{14C}]$-acrylamide to the neuronal cytoskeleton in vitro and in vivo, specific binding to microfilament- and microtubule-associated proteins occurred in a time- and dose-dependent manner (Lapadula et al., 1989a, b). In vitro studies with whole cells have also shown a specific interaction of acrylamide with vimentin filaments, which are similar to the intermediate filaments of neurofilaments (Sager, 1989). Acrylamide treatment resulted in a separation of the vimentin filaments from their attachment to the microtubules within the cell, indicating an effect on the function of the cytoskeletal proteins in a whole-cell system. Since these proteins have functional roles in a wide variety of cellular processes, such as cell division, axoplasmic transport, and maintenance of cell shape, interference with their function could be the basis of a variety of toxic effects of acrylamide which all occur at the same dose.

Chromosomal events can also be related to specific protein reaction rather than direct reaction with the DNA. In vitro studies have shown a very weak reaction of acrylamide with DNA (Solomon et al., 1985), requiring 40 days of incubation. While Carlson et al. (1986) have shown that the $^{14}$C from $^{14}$C]acrylamide may appear in nucleic acids, they
did not identify specific adducts with acrylamide or its metabolites. On the other hand, Sega et al. (1988) have shown that dominant lethal effects and chromosomal breakage in mouse postmeiotic sperm are correlated with the level of protein modification. Sperm DNA was not modified in their studies. Extensive modification of the chromosomal proteins during chromosomal condensation can cause steric strains on the DNA strand which can result in breakage of that DNA strand. This mechanism of action may explain why acrylamide can produce chromosomal effects but not point mutations in genetic toxicological tests. Protein modifications which result in chromosomal events require multiple hits to produce a genetic event. The protein-based mechanism of toxicity may also explain why acrylamide produces a variety of toxic effects at similar doses.

Under the conditions of this study, chronic intake of acrylamide in the drinking water produced slight peripheral neuropathy at the high dose of 2 mg/kg/day in the male and at 3 mg/kg/day in the female Fischer 344 rat. In addition, there was a statistically increased incidence of only one malignant neoplasm, scrotal mesothelioma, at the high dose of 2 mg/kg/day in males and of benign thyroid follicular cell neoplasms of both males and females at the high dose of 2 and 3 mg/kg/day, respectively, and at the low dose in females (1 mg/kg/day). The data from this study, even when combined with the data from the prior study of Johnson et al. (1986), have shown that the no-observed-effect doses for both sexes subsequent to chronic administration of acrylamide to rats for both neurotoxicity and malignant tumor are similar.

The significance of the mesotheliomas was discussed by Johnson et al. (1986) and little additional information has appeared since that publication. A tumor which is virtually unheard of in man is produced most commonly in Fischer rats by only a few chemicals.

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