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# Amended Final Report on the Safety Assessment of Polyacrylamide and Acrylamide Residues in Cosmetics[1]

***Polyacrylamide*** **is a polymer of controllable molecular weight formed by the polymerization of acrylamide monomers available in one of three forms: solid (powder or micro beads), aqueous solution, or inverse emulsions (in water droplets coated with surfactant and suspended in mineral oil). Residual acrylamide monomer is likely an impurity in most Polyacrylamide preparations, ranging from <1 ppm to 600 ppm. Higher levels of acrylamide monomers are present in the solid form compared to the other two forms. Polyacrylamide is reportedly used in 110 cosmetic formulations, at concentrations ranging from 0.05% to 2.8%. Residual levels of acrylamide in Polyacrylamide can range from <.01% to 0.1%, although representative levels were reported at 0.02% to 0.03%. Because of the large sizes of Polyacrylamide polymers, they do not penetrate the skin. Polyacrylamide itself is not significantly toxic. For example, an acute oral toxicity study of Polyacrylamide in rats reported that a single maximum oral dose of 4.0 g/kg body weight was tolerated. In subchronic oral toxicity studies, rats and dogs treated with Polyacrylamide at doses up to 464 mg/kg body weight showed no signs of toxicity. Several 2-year chronic oral toxicity studies in rats and dogs fed diets containing up to 5% Polyacrylamide had no significant adverse effects. Polyacrylamide was not an ocular irritant in animal tests. No compound-related lesions were noted in a three-generation reproductive study in which rats were fed 500 or 2000 ppm Polyacrylamide in their diet. Polyacrylamide was not carcinogenic in several chronic animal studies. Human cutaneous tolerance tests performed to evaluate the irritation of 5% (*w/w*) Polyacrylamide indicated that the compound was well tolerated.** ***Acrylamide*** **monomer residues do penetrate the skin. Acrylamide tested in a two-generation reproductive study at concentrations up to 5 mg/kg day$^{-1}$ in drinking water, was associated with prenatal lethality at the highest dose, with evidence of parental toxicity. The no adverse effects level was close to the 0.5 mg/kg day$^{-1}$ dose. Acrylamide tested in a National Toxicology Program (NTP) reproductive and neurotoxicity study at 3, 10, and 30 ppm produced no developmental or female reproductive toxicity. However, impaired fertility in males was observed, as well as minimal neurotoxic effects. Acrylamide neurotoxicity occurs in both the central and peripheral nervous systems, likely through microtubule disruption, which has been suggested as a possible mechanism for genotoxic effects of acrylamide in mammalian systems. Acrylamide was genotoxic in mammalian in vitro and in vivo assays. Acrylamide was a tumor initiator, but not an initiator/promoter, in two different mouse strains at a total dose of 300 mg/kg (6 doses over 2 weeks) resulting in increased lung adenomas and carcinomas without promotion. Acrylamide was tested in two chronic bioassays using rats. In one study, increased incidence of mammary gland tumors, glial cell tumors, thyroid gland follicular tumors, oral tissue tumors, uterine tumors and clitoral gland tumors were noted in female rats. In male rats, the number of tumors in the central nervous system (CNS), thyroid gland, and scrotum were increased with acrylamide exposure. In the second study, using higher doses and a larger number of female rats, glial cell tumors were not increased, nor was there an increase in mammary gland, oral tissue, clitoral gland, or uterine tumors. Tumors of the scrotum in male rats were confirmed, as were the thyroid gland follicular tumors in males and females. Taken together, there was a dose-dependent, but not statistically significant, increase in the number of astrocytomas. Different human lifetime cancer risk predictions have resulted, varying over three orders of magnitude from $2 \times 10^{-3}$ to $1.9 \times 10^{-6}$. In the European Union, acrylamide has been limited to 0.1 ppm for leave-on cosmetic products and 0.5 ppm for other cosmetic products. An Australian risk assessment suggested negligable health risks from acrylamide in cosmetics. The Cosmetic Ingredient Review (CIR) Expert Panel acknowledged that acrylamide is a demonstrated neurotoxin in humans and a carcinogen in animal tests, but that neurotoxic levels could not be attained by use of cosmetics. Although there are mechanisms of action of acrylamide that have been proposed for tumor types seen in rat studies that suggest they may be unique to the rat, the Panel was not convinced that these results could be disregarded as a species-specific finding with no relevance to human health and safety. Based on the genotoxicity and carcinogenicity data, the Panel does not believe that acrylamide is a genotoxic carcinogen in the usual manner and that several of the risk assessment approaches have overestimated the human cancer risk. The Panel did conclude, however, that it was appropriate to limit acrylamide levels to 5 ppm in cosmetic formulations.**

## INTRODUCTION

In 1990, the Cosmetic Ingredient Review (CIR) Expert Panel concluded that Polyacrylamide, with less than 0.01% acrylamide monomer content, is safe as a cosmetic ingredient as currently used (Elder 1991). Since that safety assessment was completed, additional studies have been performed, many assessing the carcinogenic potential of acrylamide monomer. New risk assessments were prepared and made available to the Panel. This amended safety assessment combines the data in the original report with the newly available information as a basis for making a determination of the levels of acrylamide residues from the use of Polyacrylamide in cosmetic formulations that may be considered safe. Where multiple sources of the same new

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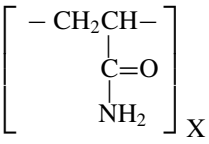
[1]Reviewed by the Cosmetic Ingredient Review Expert Panel.

information were available, the most recent published study was cited.

## CHEMISTRY

### Definition and Chemical Structure

Polyacrylamide is the homopolymer formed from the polymerization of acrylamide monomers that generally conforms to the formula shown below (Pepe et al. 2002).



Synonyms for Polyacrylamide (Pepe et al. 2002) include 2-Propenamide, Homopolymer, and Acrylamide Homopolymer.

### Chemical and Physical Properties

Polyacrylamide is soluble in water, slightly soluble in glycerol, ethylene glycol, and lactic acid, but is insoluble in ethanol, methanol, and ether (McCollister 1965).

When Polyacrylamide is prepared by polymerization of acrylamide at pH 9 or above, the polymers generally are water soluble. The amide groups give Polyacrylamide a highly hydrophilic character in this state. When polymerization takes place at pH 2.5 or below, the resulting Polyacrylamide is not water soluble. In its cross-linked form, Polyacrylamide is defined as "water-swollen" rather than water soluble (Isacoff and Zeigler 1978; Molyneux 1984; Hills 1985).

SNF (2000) stated that Polyacrylamide is available in one of three forms: white solid, aqueous solution, and inverse emulsion. The solid form may be powder or microbeads, 90% of which is Polyacrylamide, with the remainder being water content. Aqueous solutions are self-explanatory. Inverse emulsions (aka dispersions) contain Polyacrylamide in water droplets coated with surfactant molecules and suspended in mineral oil. Regardless of form, the Polyacrylamide is nonionic.

The molecular weight of Polyacrylamide is controllable; experimental conditions can produce specific desired weights. Isacoff (1973) reported that the molecular weight for documented cosmetic preparations of Polyacrylamide is 30,000 to 12 million. SNF (2000) stated that the molecular weights range from 8 to 20 million.

When using small quantities of Polyacrylamide, high viscosities may be obtained in aqueous systems (Isacoff 1973; Isacoff and Zeigler 1978). Aqueous solutions of Polyacrylamide are stable over a pH range of 3 to 10, with little effect on viscosity (Isacoff and Zeigler 1978).

The chemical and physical properties of Polyacrylamide are summarized in Table 1.

### Analytical Methods

In the infrared absorption spectra of Polyacrylamide, specific absorptions are exhibited at wave numbers 3310 $cm^{-1}$, 3140 $cm^{-1}$, 1610 $cm^{-1}$, and 1420 $cm^{-1}$ using the potassium bromide disk method (The Comprehensive Licensing Standards of Cosmetics by Category 1986).

### Method of Manufacture

Hills (1985) stated that acrylamide polymerization reactions normally reach 98% completion and can occur in aqueous solutions as well as oil emulsions, noting that, in the presence of free

**TABLE 1**
Chemical and physical properties of Polyacrylamide

| Property | Description | Reference |
|---|---|---|
| Physical form | Three forms:<br>Solid—powder or microbeads (require dissolution before use)<br>Aqueous solutions (ready to use)<br>Inverse emulsions (dispersions) | SNF 2000 |
| Molecular weight (cosmetic grade) | 30,000–12,000,000 | Isacoff 1973 |
| | 8 to 20 million | SNF 2000 |
| Density | 1.122 g/ml at 30°C | |
| Viscosity (dynes/cm) | | |
| 2% solution | $3.1 \times 10^{-4}$ | Isacoff 1973 |
| 5% solution | $2.6 \times 10^{-3}$ | Isacoff 1973 |
| 8% solution | $7.7 \times 10^{-3}$ | Isacoff 1973 |
| pH | 6.5–6.8 (1% solution) | Isacoff and Zeigler 1978 |
| | Nonionic | SNF 2000 |
| Solubility | | |
| Polymerized at pH 9 or above | Polymers water soluble | Molyneux 1984; Hills 1985 |
| Polymerized at pH 2.5 or below | Polymers not water soluble | Molyneux 1984; Hills 1985 |
| Cross-linked form | Polymers not water soluble, but water swollen | Molyneux 1984; Hills 1985 |

radicals, acrylamide is readily polymerized. According to SNF (2000), free radical polymerization is currently used to produce Polyacrylamide.

### Residual Acrylamide

In studies on Polyacrylamide (Dow 1954), the average concentrations of acrylamide were found to be less than 0.01%. In data provided to the Cosmetic, Toiletry, and Fragrance Association (CTFA) in 1990, then current manufacturers of Polyacrylamide listed a maximum of 0.1% for residual acrylamide monomer content (CTFA 1990).

SNF (2000) stated that it is practically impossible to react 100% of acrylamide in a free-radical polymerization. Post treatment (so-called "burn-out") to remove residual acrylamide is effective for aqueous solutions and inverse emulsions, resulting in levels <100 ppm (<0.01%). This technique cannot be used with the solid form. Postsynthesis treatment of the solid form must be done carefully to avoid reducing the molecular weight of the polymer, with the result that residual acrylamide concentrations of less than 100 ppm are difficult to achieve. Representative residual acrylamide levels would be 200 to 300 ppm (0.02% to 0.03%). Residual acrylamide monomer in a cosmetic formulation, of course, depends on the amount of Polyacrylamide used in that formulation and, per the discussion above, the form which is used.

## USE

### Cosmetic Use

Polyacrylamide functions in cosmetics as a binder, film former, and hair fixative, as listed in the *International Cosmetic Ingredient Dictionary and Handbook* (Pepe et al. 2002).

Other reports describe Polyacrylamide used as a foam builder and stabilizer for shampoos and foam baths and to impart lubricity and emolliency to many cosmetics and soaps, including moisturizing lotions, hand and body lotions, shaving creams and soaps (Davis 1972; Davis et al. 1972; Ono 1972; Bunting and Laidler 1973; Epton et al. 1973; Nadler and Updike 1974; Jones and Haskill 1976; Molyneux 1984; Taylor and Penhallow 1986).

Available use and concentration data are given in Table 2. In a voluntary reporting program, the Food and Drug Administration (FDA) receives information from industry on the uses of cosmetic ingredients. In 1989, reports to FDA included both frequency and concentration of use of Polyacrylamide in cosmetics (FDA 1989). These historical data are given in Table 2, along with frequency of Polyacrylamide use in each product category and the total number of products in that category from data collected in 2002 (FDA 2002). For example, 8 of the 71 indoor tanning preparations reported to FDA in 2002 contained Polyacrylamide.

SNF (2000) described the use of Polyacrylamide as film-forming/conditioning/fixative agents and as thickeners. Because the desired properties may be undone if concentrations are too high, film-forming/conditioning/fixative agents are used at concentrations ranging from 0.05% to 2% based on active polymer. Because Polyacrylamide used as a thickener is usually present with other ingredients that affect viscosity, lower concentrations may be used. One estimate described use of an inverse emulsion at 1% as a thickener, even though the emulsion actually contained only 60% Polyacrylamide.

Information available directly from industry (CTFA 2001) describes current Polyacrylamide concentration of use and provides estimates of the acrylamide residue concentration as shown in Table 2. Acrylamide residues were calculated by multiplying the concentration of Polyacrylamide raw material in the product (from the manufacturer) by the percent acrylamide in the raw material (from the supplier). In some cases, Polyacrylamide concentration of use and acrylamide residue estimates were provided in product categories not reported to FDA.

According to the European Commission's Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP), use of Polyacrylamide in cosmetic products is restricted to (a) body-care leave-on products—maximum residual acrylamide content 0.1 mg/kg or 0.1 ppm; and (b) other cosmetic products—maximum residual acrylamide content of 0.5 mg/kg or 0.5 ppm (European Commission 1999). The Ministry of Health, Labor, and Welfare (MHLW) of Japan has not included Polyacrylamide on its negative list or its restricted list (MHLW 2000).

### Noncosmetic Use

Polyacrylamide has a wide range of product uses as well as research uses. It is used as a thickening and suspending agent in waste treatment and soil stabilization, and also is used as a strengthener in production of paper. Biomedical uses include ophthalmic drug inserts, tissue implant materials, tissue models, body fluid models, detectors of penicillin antibodies and hypersensitivity, carriers of hormones and drugs in animal studies, and as an environment for the growth of tumor cells in culture. Polyacrylamide also is used in the research laboratory for separation and purification of biomaterials (Hawley 1971; Cervenka et al. 1972; Maichuk 1975; Ratner and Hoffman 1975–1976; Jones and Haskill 1976; Couvreur et al. 1977; Bissett 1980; Edman and Sjoholm 1981; Fujiki et al. 1985; Wielopolski 1985; Saettone et al. 1986).

Polyacrylamide is also used in sunscreen preparations to aid in retaining sunscreen on the skin after immersion in water (Molyneux 1984).

As given in the Code of Federal Regulations (CFR), FDA has approved the use of Polyacrylamide as a film former in the imprinting of soft-shell gelatin capsules when the concentration does not exceed the minimum required to produce the intended effect and the polymer does not contain more than 0.2% acrylamide monomer (21 CFR 172.255).

Modified Polyacrylamide resin in the category of polymer substances and adjuvants for food treatment has been approved

**TABLE 2**
Product formulation data for Polyacrylamide and estimated Acrylamide concentration

| Product category (Total no. of formulations in category in 2002) (FDA 2002) | Historical | | Current | | |
|---|---|---|---|---|---|
| | Total no. containing Polyacrylamide (FDA 1989) | Concentration of use (FDA 1989) (%) | Total no. containing Polyacrylamide (FDA 2002) | Concentration of use (CTFA 2001) (%) | Estimated acrylamide concentration (CTFA 2001) (ppm) |
| Eye lotion | — | — | — | 1.6–2.5 | <0.1–<1.3 |
| Other eye makeup preparations (152) | — | — | 2 | 0.05 | 0.003 |
| Hair conditioners (651) | — | — | 1 | 0.7–1 | 0.04–<0.05 |
| Tonics, dressings, and other hair-grooming aids (598) | — | — | 4 | 2 | 0.08 |
| Hair colors, rinses, and conditioners | 41 | >0 - 1 | — | — | — |
| Other noncoloring hair preparations | — | — | — | 0.9–1.4 | 0.04–0.06 |
| Foundations (324) | — | — | 4 | 0.2–1.3 | 0.01–0.2 |
| Other makeup preparations (201) | — | — | 1 | — | — |
| Nail and skin care cosmetics[a] | 7 | >0–1 | — | — | — |
| Nail creams and lotions | — | — | — | 0.6 | <0.03 |
| Underarm deodorants (247) | — | — | 1 | — | — |
| Other personal cleanliness products (247) | — | — | 2 | — | — |
| Aftershave lotion (231) | — | — | 2 | 2 | 0.2 |
| Skin cleansing products (775) | — | — | 4 | — | — |
| Face and neck lotions, powders, and creams (310) | — | — | 17 | 0.3–1.6 | 0.02–<1.2 |
| Body and hand lotions, powders, and creams (840) | — | — | 16 | 0.2–2.8 | 0.02–<1.2 |
| Moisturizers (905) | — | — | 24 | 0.3–1.5 | 0.01–<0.75 |
| Night creams, lotions, powders, and sprays (200) | — | — | 6 | 0.3–0.8 | 0.01–0.03 |
| Paste masks/mud packs (271) | — | — | 6 | 0.3–0.7 | 0.04 |
| Skin fresheners (184) | — | — | 1 | — | — |
| Other skin care preparations (725) | — | — | 9 | 0.2–2.5 | 0.01–<0.1 |
| Suntan gels, creams, and liquids (131) | — | — | 2 | 0.5–1 | 0.06–0.1 |
| Indoor tanning preparations (71) | — | — | 8 | — | — |
| Total uses and concentration ranges for Polyacrylamide | 48 | >0–1 | 110 | 0.05–2.8 | |

by FDA for use as a flocculent in clarifying beet or cane sugar juice not exceeding 5 ppm by weight of the juice if it is produced (1) by the copolymerization of acrylamide with not more than 5 mole-percent methacrylyloxyethyl-trimethylammonium methyl sulfate; and (2) contains not more than 0.05% residual acrylamide (21 CFR 173.10).

FDA has approved Polyacrylamide as a chemical used in washing or to assist in the peeling of fruits and vegetables at concentrations not to exceed 10 ppm in wash water and with not more that 0.2% acrylamide monomer (21 CFR 173.315).

FDA has also approved acrylamide–acrylic acid resin (hydrolyzed Polyacrylamide) for human and animal consumption. Acrylamide–acrylic acid resin (hydrolyzed Polyacrylamide) is approved for use as a flocculent in the clarification of beet sugar juice and liquor or cane sugar juice and liquor or corn starch hydrolyzate not exceeding 5 ppm of the juice or 10 ppm of the

hydrolyzate if it is produced (1) by the polymerization of acrylamide with partial hydrolysis or by copolymerization of acrylamide and acrylic acid, with the greater part of the polymer being acrylamide units; and (2) contains not more than 0.05% residual monomer calculated as acrylamide (21 CFR 173.5).

As a thickener and suspending agent in nonmedicated aqueous suspensions intended for additions to animal feeds, FDA has approved acrylamide–acrylic acid resin (hydrolyzed Polyacrylamide) if it is a minimum molecular weight of 3 million, in the viscosity range of 3,000 to 6,000 centipoise at 77°F in a 1% solution, and does not contain >0.05% residual acrylamide (21 CFR 573.120).

FDA has placed carboxymethylcellulose sodium and cationic Polyacrylamide polymer denture adhesive in medical device class III, requiring premarket approval (21 CFR 872.3420). Likewise, Polyacrylamide polymer (modified cationic) denture adhesive is a class III medical device requiring premarket approval (21 CFR 872.3480).

Described as a homopolymer of acrylamide, Polyacrylamide is approved by FDA as an adhesive for use in packaging, transporting, or holding food (21 CFR 175.105) and as a component of the coated or uncoated food-contact surface of paper and peperboard used in producing, manufacturing, packing, processing, preparing, treating, packaging transporting, or holding dry food (21 CFR 176.180).

As reported on the FDA Web site (FDA 2003), in 2002 researchers in Sweden reported finding acrylamide in a variety of fried and oven-baked foods, particularly those with traditional high-temperature cooking processes for certain carbohydrate-rich foods. Since the Swedish report, similar findings have been reported by other countries, and the US results appeared to be in basic agreement with these findings. Acrylamide appears to form as a byproduct of high-temperature cooking processes (greater than 120°C or 248°F). FDA reported that acrylamide levels in 39 samples of potato chips ranged from less than 1.4 $\mu$g to 100 $\mu$g per ounce.

## ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

### Oral Administration

*Polyacrylamide*

Dow (1958) gave three female albino rats aqueous [$^{14}$C]Polyacrylamide by gavage in doses of 10 mg/2 ml $H_2O$, 37.5 mg/3 ml $H_2O$, and 75 mg/4 ml $H_2O$. The animals were then observed for 24 h. The results of the radioactivity analyses were presented as percent of total dose fed to each animal. No radioactivity was observed in any of the three rats. However, the validity of the detection of a trace of radioactivity found in the urine of the rat fed the highest dose was questioned by the investigator, and determined to be a result of possible contamination. When the amount of radioactivity found in the feces and gastrointestinal tracts of the two animals fed the lower doses was taken into account, there was a 97.6% recovery of the total dose in those two animals. [$^{14}$C]Polyacrylamide (molecular weight not stated) was not absorbed and, therefore, was not broken down into a lower molecular weight compound. The Polyacrylamide polymer was too large to pass through the walls of the gastrointestinal tract, and the molecule was not split in any manner allowing absorption.

In a similar study by McCollister et al. (1965), in which an unspecified number of rats were fed 250 mg/kg or 500 mg/kg [$^{14}$C]Polyacrylamide by intubation, no radioactivity was observed in any of the animals. The total amount of radioactivity recovered from the feces and gastrointestinal tract of the animals was 98.2% of the original dose.

*Acrylamide*

West (1959) described the fate of orally administered [$^{14}$C] acrylamide in four male albino rats. Animals were housed in glass metabolism cages and provided with food and water ad libidum. One rat was killed at 72 h after administration and one at 96 h, and their tissues were analyzed for radioactivity.

Excreta from animal 1 were monitored for 6 h. The percent radiolabel excreted was 25% in the urine, with only a small percentage exhaled. Animal 2 was tracked for 18 h. At that time, 3.8% of the radiolabel was exhaled, 42.8 % was in the urine, and 1.0% was in the feces. Animals 3 and 4 were tracked over a longer period of time, producing the data in Table 3.

At 72 h after administration, the tissue distribution of the radiolabel in animal 3 was liver, 0.34%; kidney, 0.03%; spleen, 0.02%; cerebellum, 0.02%; cerebral cortex, 0.07%; and cervical spinal cord, 0.01%. At 96 h after administration, the tissue distribution of radiolabel in animal 4 was liver, 1.19%; kidney, 0.17%; spleen, 0.04%; cerebellum, 0.04%; cerebral cortex, 0.13%; and cervical spinal cord, 0.01%. The author noted that the urine is the primary route of excretion and that radiolabel that remained

**TABLE 3**
Excretion of radiolabel in rats given oral [C$^{14}$]acrylamide (West 1959)

| | Percent excretion | | | |
|---|---|---|---|---|
| | Exhaled air | Urine | Feces | Total |
| Animal 3 | | | | |
| 24 hours | 7.1 | 55.8 | 2.8 | 65.7 |
| 24 to 48 hours | 6.5 | 10.6 | 0.1 | 17.2 |
| 48 to 72 hours | 3.2 | 2.4 | 0.1 | 5.7 |
| | | | Cumulative total | 88.6 |
| Animal 4 | | | | |
| 24 hours | 7.7 | 56.5 | — | 64.2 |
| 24 to 48 hours | 5.1 | 4.9 | 0.3 | 10.3 |
| 48 to 72 hours | 2.5 | 2.0 | 0.3 | 4.8 |
| 72 to 96 hours | 1.4 | 1.2 | 0.7 | 3.3 |
| | | | Cumulative total | 82.6 |

in the body appeared to be evenly distributed, except for a higher accumulation in the liver (West 1959).

## Dermal Administration

*Acrylamide*

Franz et al. (1995) determined the in vitro rat (male Fischer 344) skin penetration of residual acrylamide monomer in three SEPARAN polymer matrices and compared the results with acrylamide monomer alone. These data were compared to the dermal absorption of acrylamide monomer in vivo in the rat. Uniformly labeled [$^{14}$C]acrylamide as a 1% water solution with a specific activity of 0.41 mCi/mmol was added to each polymer. The three SEPARAN polymers were (1) an anionic flake polymer of $6 \times 10^6$ molecular weight and a residual acrylamide content of 0.041% (410 ± 36 ppm); (2) a nonionic flake polymer of $1.5 \times 10^6$ molecular weight with 0.094% residual acrylamide (942 ± 79 ppm); and (3) a slightly anionic flake polymer of $4 \times 10^6$ molecular weight with 0.133% residual acrylamide (1333 ± 38 ppm). The Polyacrylamide flake materials were dissolved in water at a final concentration ≤1% to avoid gel formation.

In vitro skin penetration of 0.5% [$^{14}$C]acrylamide over a 24-h period was 54.15% ± 10.65% in the medium plus 12.97% ± 3.77% in the excised rat skin, for a total of ~67% that is considered to have penetrated the skin. The total recovery of radioactive label was 92.5% ± 16.1%.

In vitro skin penetration of [$^{14}$C]acrylamide from the three Polyacrylamide flake materials each exhibited a lag period from 1 to 8 h followed by a linear increase in penetration. At 24 h, absorption from the anionic and slightly anionic polymer matrices exhibited a shoulder with final absorption of the acrylamide monomer at around 50% of that of the monomer alone, whereas the nonionic matrix did not have a shoulder and did not appear to inhibit skin penetration. The authors speculated that the penetration of acrylamide monomer from the polymer matrix related to the physicochemical properties of the polymer.

In vivo rat skin penetration of 0.5% [$^{14}$C]acrylamide over a 24-h period yielded 30.10% ± 5.67% recovered in tissues and excreta, 31.03% ± 14.02% was associated with the skin itself and was considered to have penetrated the skin, for a total of ~61%. The total recovery of radioactive label was 85.1% ± 7.1%.

The authors concluded that acrylamide monomer extensively penetrates rat skin in vitro and in vivo, but that the in vitro data may overestimate the systemic dose available in the first 24 h. (Franz et al. 1995).

Diembeck et al. (1998) examined the dermal absorption and percutaneous penetration of acrylamide in an ex vivo and in vitro porcine skin assay. [$^{14}$C]Acrylamide was applied in aqueous solutions of 50% acrylamide and 2% Polyacrylamide, and in various cosmetic preparations (water/oil cream, water/oil lotion, oil/water cream, and hydrogel). Except for the 50% acrylamide which was applied at 2000 $\mu$g/cm$^2$, the amount of acrylamide applied was 1.3 $\mu$g/cm$^2$. Table 4 presents the results.

The authors noted the differences in the distribution of the [$^{14}$C]acrylamide from the 50% acrylamide solution compared to the other vehicles, but attributed those differences to the higher amount of applied acrylamide. Independent of vehicle, however, significant levels of acrylamide either penetrated the skin or were found in the skin (Diembeck et al. 1998).

Marty and Vincent (1998) studied the in vitro percutaneous absorption of acrylamide across excised abdominal human skin (450-$\mu$ thickness) obtained from plastic surgery. The skin was mounted in a Franz type diffusion cell. Acrylamide was added to a 2% Polyacrylamide aqueous gel at concentrations of 1.28 and 2.00 ppm. [2,3-$^{14}$C]acrylamide (specific activity of 185 Mbq/mmol) was added to each gel without dilution. The Polyacrylamide gel (15 mg) was applied to the surface of the epidermis. The complete volume of the receptor compartment was collected at 6, 12, and 24 h and replaced by the same volume of fresh medium. Radioactivity on the skin surface and in the dermis and whole epidermis (stratum corneum and viable epidermis) were determined at 24 h. Table 5 presents the results of the study.

The authors concluded there was no specific accumulation in the whole epidermis or dermis. Based on the kinetics of appearance of the radiolabel in the receptor fluid, the authors concluded that there was no effect of the acrylamide concentration on the kinetics of absorption (Marty and Vincent 1998).

Kraeling and Bronaugh (2001) measured the in vitro percutaneous absorption of $^{14}$C-labeled acrylamide (specific activity

**TABLE 4**

Dermal absorption and percutaneous penetration of acrylamide using porcine skin (Diembeck et al. 1998)

| Percent recovered radiolabel | Vehicle | | | | | |
|---|---|---|---|---|---|---|
| | Acrylamide (%) | Polyacrylamide (%) | *w/o* cream (%) | *w/o* lotion (%) | *o/w* cream (%) | Hydrogel (%) |
| On skin surface | 6 | 1.5 | 4.6 | 4.6 | 2.3 | 2.3 |
| In horny layer | 17.5 | 15.4 | 13.1 | 10.8 | 15.4 | 17.7 |
| In epidermis | 2 | 6.2 | 8.5 | 8.5 | 6.9 | 6.2 |
| In dermis | 52.5 | 44.6 | 43 | 44.6 | 40.8 | 39.2 |
| In receptor fluid | 22 | 32.3 | 30.8 | 31.5 | 34.6 | 34.6 |

**TABLE 5**
Percutaneous absorption of acrylamide across human skin in vitro (Marty and Vincent 1998)

| Percent applied dose in | Acrylamide level | |
|---|---|---|
| | 1.28 ppm | 2.00 ppm |
| Receptor fluid | 28.18% ± 10.56% | 21.86% ± 5.69% |
| Whole epidermis | 2.72% ± 1.21% | 3.35% ± 2.02% |
| Dermis | 2.33% ± 0.89% | 1.56% ± 0.15% |
| Total absorbed | 33.24% ± 10.67% | 26.77% ± 4.76% |

of 5 mCi/mmol, 98% radiochemical purity) from an oil-in-water emulsion in female fuzzy rat (strain Hsd:Fuzzy-fz) and human skin. Skin discs were prepared with a punch and placed in flow-through diffusion cells. Fuzzy rat skin in some diffusion cells was occluded by placing a teflon plug in the diffusion cell cap to prevent evaporation of the applied acrylamide. Two levels of radiolabel were applied, 0.1 $\mu$Ci and 0.6 $\mu$Ci. Table 6 shows the penetration in nonoccluded conditions, which appeared to be independent of the dose applied. Penetration was higher under occluded conditions, with 60% in the receptor fluid and 8% remaining in the skin; recovery under occluded conditions rose to 97.7% ± 7.9%. The authors concluded that radiolabeled acrylamide is rapidly and extensively absorbed in rat and human skin when applied in an *o/w* emulsion vehicle.

### Comparison of Routes of Delivery

Ramsey et al. (1984) compared the fate of [$^{14}$C]acrylamide by intravenous, gavage, or dermal administration to male Fischer 344 rats (200 to 250 g). To determine the effect of dose level, a single intravenous dose of 2 mg/kg or 100 mg/kg (in sterile 0.9% saline) was given through an indwelling jugular cannula. The urine was the primary route of excretion (>60%); the percent distribution of radioactivity in the skin, carcass, and cage wash was independent of the dose.

Another group of rats received intravenous [$^{14}$C]acrylamide and were killed in groups of three at 0, 6, 12, 18, and 48 h following the dose to determine the kinetics. Radiolabel reached a maximum in red blood cells (RBCs) between 6 and 12 h after the dose and maintained that level through the end of the experiment; label in the carcass and plasma cleared rapidly—at 48 h the RBC/plasma ratio of radiolabel was 90:1.

An aqueous solution of [$^{14}$C]acrylamide containing 1% Triton X-450 (a nonionic detergent) was applied to clipped skin on the back to achieve dose levels of 2 mg/kg or 50 mg/kg. Blood samples were obtained at frequent intervals. The level of radiolabel in blood plasma increased slightly in the first few hours, then cleared rapidly, following the same kinetics as with the intravenous delivery. Subsequent gas chromatography–mass spectrometry analysis indicated that acrylamide cleared from blood with a half-life of 2 h and that its metabolite, *N*-acetyl-*S*-(3-amino-3-oxopropyl)cysteine, cleared with a half-life of 10 h.

Two groups of four rats each were given bolus doses of 0.05 mg/kg or 30 mg/kg by gavage each day for 13 days. The concentration of radiolabel in RBCs followed the same pattern as with intravenous dosing. The daily excretion of radiolabel in the urine followed a similar clearance pattern for the two dose levels; at day 13, 59.3% ± 11.2% of the high dose label and 63.1% ± 10.9% of the low dose label had been excreted. The authors concluded that acrylamide is cleared in rats mainly by metabolism, and that the metabolites are excreted as a constant fraction of the dose regardless of route of administration (Ramsey et al. 1984).

In an attempt to determine the basis for a reported higher induction of skin papillomas with oral administration compared with topical application, Carlson and Weaver (1985) studied the distribution of [$^{14}$C]acrylamide in SENCAR and BALB/c mice following oral and topical administration. The binding of [$^{14}$C]acrylamide to DNA, RNA, and protein was also measured. The induction of skin papillomas in SENCAR mice by acrylamide was more potent with oral administration compared to topical dosing. There were no reported skin papillomas in BALB/c mice.

**TABLE 6**
Penetration of [$^{14}$C]Acrylamide in nonoccluded rat and human skin (Kraeling and Bronaugh 2001)

| Percent of applied dose found in | Dose | | | |
|---|---|---|---|---|
| | Fuzzy Rat | | Human | |
| | 0.1 $\mu$Ci (4 tests in each of 3 animals) | 0.6 $\mu$Ci (4 tests in each of 2 animals) | 0.1 $\mu$Ci (4 tests in 1 subject) | 0.6 $\mu$Ci (4 tests in each of 2 subjects) |
| Receptor fluid | 47.22 ± 0.25 | 45.53 ± 2.35 | 46.51 ± 3.60 | 53.43 ± 10.54 |
| Total in skin | 5.68 ± 1.05 | 4.15 ± 0.41 | 8.45 ± 1.30 | 7.20 ± 1.56 |
| Total absorption | 52.89 ± 1.30 | 49.68 ± 2.35 | 54.96 ± 4.31 | 60.63 ± 12.10 |
| Wash recovery | 12.20 ± 2.56 | 12.92 ± 1.54 | 6.63 ± 1.13 | 6.68 ± 1.30 |
| | 65.45 ± 3.85 | 62.97 ± 3.41 | 62.30 ± 4.94 | 67.77 ± 13.41 |

In both strains, [$^{14}$C]acrylamide applied topically was readily absorbed and distributed to internal organs and there was 100× more [$^{14}$C]acrylamide in the skin as a result of topical dosing compared to oral administration. From the highest concentration to the lowest concentration of radiolabel, the organs were: skin, stomach, liver, lung, and testes for SENCAR mice. The same rank for BALB/c mice was skin, stomach, lung, testes, and liver. They also found that the distribution resulting from oral administration and topical dosing was similar in both mouse strains. Likewise, the binding of [$^{14}$C]acrylamide to DNA, RNA, and protein extracted from organs and tissues was similar in both strains.

Comparing oral administration to topical dosing, they found that binding to DNA (the likely target for the skin papilloma endpoint) was 4 × less with oral administration compared to topical dosing. The authors concluded that neither the distribution nor the binding to DNA could provide an explanation for the reported skin papilloma induction differences by strain and route of administration (Carlson and Weaver 1985).

Sumner et al. (1999) characterized the urinary metabolites of acrylamide in male Fischer 344 rats following dermal application or intraperitoneal (i.p.) injection. Four animals were given a 50 mg/kg i.p. injection dose or an ∼137 mg/kg dermal dose of [1,2,3-$^{13}$C]acrylamide. Injection was done at ∼1 ml/kg body weight. Dermal exposure was done using Hilltop Chambers and the volume delivered was ∼3 ml/kg body weight. Rats were placed in all-glass metabolism cages. Urine was collected over dry ice for a 24-hour period. $^{13}$C-nuclear magnetic resonance (NMR) spectra were obtained and signals were matched with signals of acrylamide metabolites previously described. Metabolites included:

1. *N*-acetyl-*S*-(3-amino-3-oxopropyl)cysteine,
2. *S*-(3-amino-3-oxopropyl)cysteine,
3. *N*-acetyl-S-(3-amino-2-hydroxy-3oxopropyl)cysteine,
4. diastereomers of N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)-cysteine, and
5. glycidamide.

The authors stated that the first two metabolites were derived from direct conjugation of acrylamide with glutathione, while the others are epoxidation products.

Following the i.p. administration, around 62% of the dose was excreted in the urine; the two glutathione conjugates comprised 69% of the urine excretion. These results were stated to be similar to those obtained by gavage exposure.

Following the dermal application, urine excretion radiolabel ranged from 0.43% to 2.8%; the two glutathione conjugates comprised around 52% of the relatively low urine excretion. The authors noted a higher proportion of glycidamide as a metabolite following dermal application (17%) compared to that produced after i.p. injection (6% to 7%). Extrapolating back from the urine excretion levels, and assuming a similar elimination as that following i.p. administration, the authors suggested that a dermal dose of 137 mg/kg would correspond to a 3.3 mg/kg taken up from the skin over the 24-h duration of the exposure (Sumner et al. 1999).

Sumner et al. (2001) compared the absorption, distribution, metabolism and excretion of acrylamide delivered via inhalation exposure with dermal application. Male Fischer 344 rats and male B6C3F$_1$ mice were exposed to a mixture of [1,2,3-$^{13}$C]acrylamide and [2,3-$^{14}$C]acrylamide (∼3 ppm) for 6 h via nose only inhalation. Dermal application of [2,3-$^{14}$C]acrylamide (in distilled water at a dose of ∼162 mg/kg) was made to the shaved backs of male Fischer 344 rats for 24 h. Following the 6-h inhalation exposure, animals in one group were killed and the retained dose determined. The remaining (inhalation exposure and dermal application) animals' urine, feces, and expired air were monitored for 24 h, at which point all animals were killed and blood and tissues collected and analyzed.

The dermal application led to the absorption of 22% of the applied dose. Of that, 44% was found in excreta and 53% in tissue, whereas 3% was expired. A similar pattern of radioactivity in excreta and tissue was seen for mice and rats following the inhalation exposure. The total radioactivity per body weight recovered in mice was 2.8 times that recovered from rats following inhalation exposure. Of the 2.9 ppm of acrylamide in the air breathed, 19 $\mu$mol was recovered in expired air, urine, feces, and tissues of rats, whereas 11 $\mu$mol was recovered from mice (Sumner 2001).

## ANIMAL TOXICOLOGY

### Acute Oral Toxicity

*Polyacrylamide*

In a 2-week study, groups of two to six female Dow-Wistar rats were fed preparations of nonionic and anionic Polyacrylamide by intubation. The single oral doses were 0.25, 0.5, 1.0, 2.0, and 4.0 g/kg body weight, either as a 10% or a 20% suspension in water or corn oil. All animals survived a maximum single oral dose of 4.0 g/kg body weight. The $LD_{50}$ was not reached (McCollister et al. 1965).

### Acute Dermal Toxicity

*Acrylamide*

Mukhtar et al. (1981) reported the effects in mice of a single topical application of acrylamide on glutathione (GSH) levels and on glutathione *S*-transferase (GST) and aryl hydrocarbon hydroxylase (AHH) activity in the skin and liver. Acrylamide was applied at a dose of 100 mg/kg in 0.2 ml of acetone to 2 × 2-cm shaved skin areas of 32 Swiss male albino mice. Acetone alone served as the control. For each test group, four animals were killed at 2 and 4 h after application of acrylamide.

GSH levels and the activities of GST and AHH were reduced. Table 7 presents these data. The authors speculated that depletion of glutathione may cause membrane damage in skin cells, thereby increasing interactions of active metabolites with,

**TABLE 7**
Reduction in GSH levels and GST and AHH activity as a function of time after topical exposure to acrylamide in the mouse skin and liver (Mukhtar et al. 1981).

| | 2 hours post exposure[a] | | 4 hours post exposure[a] | |
|---|---|---|---|---|
| Reduction in | Skin (%) | Liver (%) | Skin (%) | Liver (%) |
| GSH level | 63 | 38 | 41 | 20 |
| GST activity | 89 | 102 | 65 | 79 |
| AHH activity | 40 | 90 | 27 | 42.5 |

[a]Control value set at 100%.

for example, DNA. They also suggested that depletion of tissue glutathione levels may be partly the cause of acrylamide induced dermatitis and irritation seen in occupational settings (Mukhtar et al. 1981).

## Acute Dermal Irritation

*Polyacrylamide*

Guillot et al. (1982) performed cutaneous tolerance tests on male white albino rabbits to evaluate the irritation of synthetic polymers, including Polyacrylamide, in cosmetic formulations. After 24 h, a 5% (*w/w*) preparation of Polyacrylamide was relatively well tolerated.

Guillot et al. (1983) applied a 0.5-ml suspension of Polyacrylamide (0.5% to 2% *w/w*) in TEA-Stearate, an ionic oil and water emulsion, at concentrations close to those found in actual cosmetic preparations, to intact and scarified clipped skin of 6 male New Zealand white rabbits for 24 h. Of the 17 gelling agents, polymers, and thickeners tested, Polyacrylamide was the least irritating at a 2% concentration.

## Subchronic Oral Toxicity

*Polyacrylamide*

Christofano et al. (1969) reported the toxicity of modified Polyacrylamide resins in feeding studies using both rats and dogs. The number of animals in the studies was not indicated. The animals were given a maximum dose of 464 mg/kg body weight without any signs of toxicity in either species. Concentrations of 500, 2000, 10,000, and 50,000 ppm were fed to rats for 90 days without alterations in growth rate, mortality, urinalysis, hematological findings, gross lesions, and microscopic findings. Dogs fed 500 or 2000 ppm Polyacrylamide for 90 days did not have any abnormal findings.

## Chronic Oral Toxicity

*Polyacrylamide*

*Rats.* The American Cyanamid Company (1960a) reported no toxic effects or significant lesions in 20 female and 20 male rats fed a diet containing either 1.0% or 5.0% Polyacrylamide for 2 years. According to the authors, the only effect of any significance was a slight decrease in growth rate observed in both male and female rats receiving a 5.0% dietary formulation of Polyacrylamide. In an additional feeding study, groups of rats were fed either 2.5%, 5.0%, or 10.0% Polyacrylamide for 2 years. No compound-related lesions were found.

McCollister (1965) fed groups of 20 female and 20 male rats dies containing either 1.0% or a 5.0% Polyacrylamide formulation for a period of 2 years. Throughout the 2 years of observation, there were no significant changes in the appearance or behavior of any of the rats.

Christofano et al. (1969) reported the results of a study in which rats were fed 500, 2000, or 10,000 ppm Polyacrylamide in their diet in a 2-year feeding study. No significant adverse effects were reported. The number of animals tested was not indicated.

*Dogs.* Dow (1957) reported that three groups of Beagle dogs, four per group, were fed diets containing either 1.0% or 5.0% Polyacrylamide, which contained 0.01% acrylamide monomer, in a 2-year feeding study. No adverse effects were found when compared to the untreated controls.

The American Cyanamid Company (1960b) reported the results of a study in which two groups of Beagle dogs, four per group, were fed dietary concentrations of either 2.5 or 5.0% Polyacrylamide for 2 years. No compound-related lesions were detected.

McCollister (1965) fed diet containing either 1.0% or a 5.0% Polyacrylamide to groups of 4 beagle dogs in 1- and 2-year feeding studies. During these studies, all dogs were normal in appearance and behavior.

Christofano et al. (1969) reported the results of a study in which dogs were fed 500, 2000, or 10,000 ppm Polyacrylamide in their diet in a 2-year feeding study. The results demonstrated no adverse effects at the two lower doses. The number of animals tested was not indicated.

## Ocular Irritation

*Polyacrylamide*

Dow (1954–1955) reported a study in which a small amount of an undiluted formulation of solid Polyacrylamide (molecular weight not stated) was placed in the right conjunctival sac of one rabbit, and the eye was washed 30 s later. The left conjunctival sac of the same rabbit was treated with the same amount of solid Polyacrylamide without subsequent rinsing. There was no indication of corneal or conjunctival irritation when each eye was observed immediately after treatment, and then again within 2 or 3 min. A very slight conjunctival response, indicated by prominent capillaries, was observed in the unwashed eye after 1 h. At 24 h after treatment, both eyes were normal.

Guillot et al. (1982) performed ocular irritation tests on male white albino rabbits; the number of animals was not indicated. A 2% aqueous solution of fluorescein was used to help evaluate the extent of surface damage and corneal opacity. Ulceration

and granulation also were measured. The results were scored at 1 h, 24 h, and 2, 3, 4, and 7 days. Polyacrylamide, at a concentration of 5% *w/w*, did not provoke significant injury to ocular membranes.

## ACRYLAMIDE NEUROTOXICITY

Smith and Oehme (1991) reviewed the neurotoxicity of acrylamide. They stated that acrylamide can cause axonopathy by transection of neurons; the portion of the axon that is separated simply degenerates. Both the central nervous system (CNS) and the peripheral nervous system (PNS) are targets, although axons may regenerate in the PNS. They noted that, according to McCollister et al. (1965), Polyacrylamide was not neurotoxic, but *N*,*N*′-methylene-*bis*-acrylamide was marginally neurotoxic. The authors suggest that axonopathy may result from inactivation of microtubules, neurofilaments, or microtubule-associated proteins.

On the premise that acrylamide neurotoxicity is related to neuronal intracellular transport and involves microtubules, Sickles et al. (1995), examined the disruption of mitosis, another microtubule-based intracellular transport process, by acrylamide. The authors compared the effects of acrylamide and methylene *bis*-acrylamide (a non-neurotoxic acrylamide derivative with two acrylamide residues) on human fibrosarcoma HT 1800 cells. Cultures were grown overnight; acrylamide (in distilled water) was added to a final concentration of 0, 1.0, 2.5, 5.0, or 10 mM. Methylene *bis*-acrylamide was added at equimolar levels. As a point of reference, the authors noted that a typical i.p. injection of acrylamide in rats produces an acrylamide concentration of 0.7 mM in the rat sciatic nerve.

Control cultures had 2.12% ± 0.16% of cells in mitosis. Colchicine, a standard chemical used to arrest cells in mitosis and used here as a positive control, substantially increased that percentage. The number of cells in mitosis increased as a function of acrylamide concentration, reaching statistical significance compared to controls at concentrations of 5 and 10 mM. Methylene *bis*-acrylamide had no effect. They reported that the formation of the mitotic spindle, separation, and chromosome alignment were similar to controls, but that chromosome migration was blocked. The authors implicated inhibition of mitotic motors as the mechanism of action, an effect that would be similar to the effect on motor proteins in axonal cells to produce neurotoxicity (Sickles et al. 1995).

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

*Polyacrylamide*

Christofano et al. (1969) performed a three-generation reproduction study on rats fed 500 or 2000 ppm Polyacrylamide. The number of animals in the study was not indicated. Normal reproductive functions, such as mating index, incidence of pregnancy and parturition, and gestation time, were recorded for the first and second generations. Body weight, growth rate, behavioral reactions, skeletal structure, and mortality rates were normal for both the parent rats and their progeny. No compound-related lesions were noted.

*Acrylamide*

Union Carbide (1987) reported the results of a combined two-generation reproduction study and dominant lethal assay in Fischer 344 rats given acrylamide in drinking water performed at their Bushy Run Research Center. Male and female weanling rats ($F_0$ generation) were exposed to acrylamide at 0.5, 2.0, or 5.0 mg/kg day$^{-1}$. A control group received only drinking water. Each group consisted of 30 animals of each sex. Exposure lasted 10 weeks. After a 2-week mating period, exposure of the $F_0$ females continued through gestation, paturition, and lactation. Male $F_0$ rats were mated to untreated females for 3 weeks; at gestational day 14, untreated females were killed and examined. The $F_0$ males were killed after this assay and necropsied.

After weaning the $F_1$ litters, $F_0$ females were killed and necropsied. Ten or 20 $F_1$ weanlings per group were selected at random, killed, and necropsied; selected tissues were examined histologically. Thirty $F_1$ weanlings per sex per group, randomly selected to produce the $F_2$ generation, were exposed to acrylamide using the same regimen as their parents. The same examinations done on the $F_0$ parents and $F_1$ progeny above were done on the $F_1$ parents and $F_2$ progeny.

Initial effects observed included reduced body weights for $F_0$ males (at 2 and 5 mg/kg doses at 4 weeks and 0.5 mg/kg at 6 weeks) and females (at 2 and 5 mg/kg doses throughout and at 0.5 mg/kg late) and reduced weight gain for $F_0$ males (at all dose levels) and females (at 2 and 5 mg/kg doses throughout and at 0.5 mg/kg late). Head tilt and leg splay were observed in $F_0$ males and leg splay in $F_0$ females.

Survival of $F_1$ progeny was unaffected by treatment. No treatment related lesions were seen at necropsy or in histologic examination of $F_0$ males or females, or selected $F_1$ weanlings.

No dominant lethal effects in the $F_0$ males were seen at 0.5 or 2.0 mg/kg day$^{-1}$, but there was an effect on pregnancy parameters in untreated females mated with males from the 5 mg/kg group; total and viable implantations per litter was reduced, resorptions were increased, and pre- and postimplantation losses were increased. No effects on pregnancy parameters were observed in untreated females mated to males from the lower dose groups.

The $F_1$ breeding animals exhibited the same weight and weight gain effects seen in the $F_0$ animals. $F_2$ progeny survival was unaffected although litter sizes were reduced at the high dose level. Necropsy of $F_1$ adults and $F_2$ weanlings found no treatment-related lesions. The same was true for histopathology using conventional tissue staining; staining of peripheral nerve sections with Bodian's method, however, revealed some axonal fragmentation and/or swelling in randomly selected high-dose $F_1$ males, but not females.

The authors concluded that there was evidence of parental toxicity; the 0.5 mg/kg dose group being close to the no observable effect level. There was prenatal lethality at the 5 mg/kg

dose level, but not at the other dose levels (Union Carbide 1987).

Chapin et al. (1995) published the results of a National Toxicology Program (NTP) study of the reproductive and neural toxicities of acrylamide and three analogues in Swiss mice using the NTP's reproductive assessment by continuous breeding (RACB) protocol. Treatment agents were acrylamide at 3, 10, and 30 ppm; *N*,*N*′-methylene *bis*-acrylamide (MBA) at 10, 30, and 60 ppm; *N*-(hydroxymethyl)acrylamide (HMA) at 60, 180, and 360 ppm; and methacrylamide (MACR) at 24, 80, and 240 ppm.

Key features of this protocol include dosing cohabitating male and female mice ($F_0$) with the treatment agent in drinking water over a 98-day dosing period, during which the animals produced multiple litters. At the end of that period, the animals were separated, with continued dosing for 6-weeks, until delivery of the last litter, the $F_1$ mice. During this 6-week holding period, crossover matings were done male control with female control, male high dose with female control, and male control with female high dose in order to determine the sex affected by the treatment and the presence of dominant lethal events. The $F_1$ mice were mated with nonsibling partners of the same dose group. End points include clinical signs, grip strength, body weights, food and water consumption, number of litters, pups per litter, pup weight, resorptions, gross necropsy findings, and histopathology of reproductive organs and nerve tissue.

The authors reported that acrylamide did not affect body weight of the $F_0$ males or females. Overall there was no progressive reproductive toxicity with acrylamide dose increases. Grip strength was not adversely affected by acrylamide, the percentage change from initial values was lower at week 17 in the high-dose group for male forelimbs and hindlimbs and for female forelimbs.

The assessment of dominant lethal events in male mice showed an increase in resorptions and postimplantation loss and a decrease in the number of live fetuses for the high-dose group (60 ppm). An 11% decrease in live pups in the $F_0$ mice was contrasted with a 47% decrease in the $F_1$ mice (again, the high dose group). No explanation for the increased effect in the second generation was demonstrated by the data.

The crossover matings produced no statistically significant differences between the groups. No organ weight changes were found at any dose level. There were no effects of acrylamide on sperm concentration, motility, or abnormalities; the estrous cycle was unaffected. There was no effect on postnatal survival of $F_2$ progency. Histologic examination of tissues from $F_1$ males revealed no dose-related abnormal findings, other than testicular degeneration in 1 of 10 animals in the middle and high dose groups (Chapin et al. 1995).

Results of this NTP study are shown in Table 8.

## GENOTOXICITY

### Bacteria and Yeast

*Acrylamide*

Pharmakon Research International, Inc. (1982) conducted an Ames plate test using *Salmonella* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 treated with acrylamide at concentrations up to 10 mg/plate with and without metabolic activation. Strains TA 1537, TA 1538, TA 98, and TA 100 were negative, but an increase in reversion frequency in strain TA 1535 with metabolic activation was noted.

Bull et al. (1984) tested acrylamide for mutagenic activity in the Ames test. Acrylamide was negative in strains TA 1535, TA 1537, TA 98, and TA 100, with or without aroclor induced rat liver fraction S9 activation, even up to 30 mg of the test substance added to each plate. The authors also used a preincubation exposure in which the bacteria were exposed in suspension for 30 min prior to addition of agar, with the same absence of effect.

Arany (1985) tested acrylamide in *Salmonella* strains TA 1535, TA 1537, TA 98, and TA 100. Acrylamide (in distilled water) was added at concentrations from 10 to 5000 $\mu$g per plate, with or without metabolic activation with aroclor induced rat liver fraction, S9. Positive and negative controls gave the expected results; there was no increase in mutation frequency with any acrylamide concentration.

Hendricxx (1985) reported the results of treating *Saccharomyces cerevisiae* with acrylamide. Cell viability was slightly decreased by acrylamide at concentration levels from 1 to 500 $\mu$g/ml, but there was no gene conversion (mitotic recombination of trp 5-12 with trp 5-27 to produce trp+) with or without metabolic activation with aroclor induced rat liver fraction, S9. Positive and negative controls gave the expected results.

**TABLE 8**
Subjective comparison of the effects of Acrylamide, MBA, HMA, and MACR in $F_0$ mice (Chapin et al. 1995)

| Treatment | Dose (mmol/kg day$^{-1}$) | Development and female reproduction | Male reproduction | Dominant lethality | Neurotoxicity |
|---|---|---|---|---|---|
| Acrylamide | 0.094 | No toxicity | No toxicity | Observable toxicity | Minimal toxicity |
| MBA | 0.06 | Minimal toxicity | Observable toxicity | Observable toxicity | No toxicity |
| HMA | 0.83 | Observable toxicity | Observable toxicity | Moderate toxicity | Minimal toxicity |
| MACR | 0.57 | No toxicity | No toxicity | No toxicity | No toxicity |

## Drosophila

*Acrylamide*

Valencia (1984) performed a sex-linked recessive assay in fruit flies treated with acrylamide. Acrylamide (ppm weight/volume) in 5% sucrose was fed to adult males (8 to 30 h of age, deprived of food for previous 4 h). The control group received the sucrose solution only and a positive control using dimethylnitrosamine at 25 ppm was also done. Acrylamide was highly lethal at 2500 ppm and above, whereas concentrations of 500 ppm and lower had little mortality. In the genetic testing, a concentration of 100 ppm was used. There was no difference reported in the sex-linked recessive lethals in fruit flies by the feeding route of administration. The positive control yielded the expected results.

Tripathy et al. (1991) investigated the genotoxicity of acrylamide in both somatic and germ cells of *Drosophila melanogaster*. Fruit fly larvae were exposed to acrylamide at concentrations of 0, 0.25, 0.50, 1.0, 2.5, and 5.0 mM. About 100 larvae were washed in 20% saline and transferred into vials containing 1.5 g instant medium to which was added 5 ml of acrylamide in distilled water at the appropriate concentration. The concentration at which 50% of the larvae failed to hatch was 5.0 mM acrylamide.

Small single spots, large single spots, and twin spots in the wing were indicative of mosaic somatic mutations in wing primordial cells. At 0.25 and 0.50 mM acrylamide, mutation induction was not conclusive. At both 1.0 and 2.5 mM, the induction of small and large single spots was increased, but twin spots were not conclusive. At 5.0 mM, induction of all three spot configurations was seen. The number of sex-linked lethal mutations was increased at 0.25 and 0.5 mM, but was not significantly different from the control. At 1.0, 2.5, and 5.0 mM acrylamide, there was a statistically significant increase in lethal events. The authors concluded that acrylamide is genotoxic to both somatic and germ cells of *D. melanogaster* (Tripathy et al. 1991).

## Mammalian Assays

*Acrylamide*

The earliest reported findings are in studies by Pharmakon Research International, Inc. (1982). In a forward gene mutation assay, acrylamide was mixed with the medium used to culture Chinese hamster ovary (CHO) $HGRPT^-$ cells at concentrations of 37.5, 75, 150, 300, 600, 900, and 1200 $\mu$g/ml, with and without metabolic activation by rat liver fraction S9. An increase in the number of $HGRPT^+$ cells was seen at 300 $\mu$g/ml with metabolic activation and at 37.5 $\mu$g/ml without metabolic activation. In a DNA repair test, rat hepatocyte cells in primary culture were treated with acrylamide at 1.0, 3.3, 10, 33.3, and 100 mg/ml in distilled water. A positive control used 2-acetoamidofluorene at $10^{-4}$ M. Radioactivity incorporated into DNA was measured. An increase in DNA repair synthesis was seen at all dose levels.

Shelby et al. (1987) used the mouse heritable translocation test to further elucidate what the authors referred to as a known dominant lethal effect in male rodents. Mice were given i.p. injections on five consecutive days at either 40 or 50 mg/kg. There were 120 male mice per group. Each male was caged with two females on days 7 to 10 following the last injection. After this time, the animals were individually caged. Male progeny were weaned and mated. Litter size determined whether the males were considered fully fertile. Males suspected of being partially fertile were further mated and the uterine contents were examined to assess viable and dead implants. Randomly chosen males from the partially fertile group were analyzed cytogenetically for reciprocal translocations.

In the heritable translocation assay, the number of sterile and semisterile males was 13 and 36, respectively, out of 125 male progeny (total of 39% translocations) for the 50 mg/kg group. For the 40 mg/kg group, there were 12 sterile males and 27 semisterile males out of 162 male progeny (total of 24% translocations). These figures were compared to 16 sterile males and 1 semisterile male in the historical control population of 8,095 males (0.2% translocations). All 10 of the semisterile males analyzed cytogenetically were reciprocal translocation carriers. The authors concluded that acrylamide is an effective mammalian germ cell mutagen (Shelby et al. 1987).

Adler et al. (1988) reported that acrylamide can induce chromosome aberrations in mouse bone marrow cells. Male and female (101/E1 $\times$ C3H/E1)$F_1$ mice were given acrylamide (in buffered saline) by i.p. injection. Buffered saline was used as a control and cisplatin as a positive control in all studies. Both a chromosome analysis and a micronucleus test were done. For the chromosome analysis, bone marrow was obtained at 12, 18, 24, 30, and 36 h after a dose of 100 mg/kg.

A dose-response study of chromosome damage was done at 24 h after i.p. injection of 0, 50, 100, or 150 mg/kg acrylamide. In addition, differentiating spermatogonia were scored for chromatid aberrations in a subset of five males per dose at the 24-h interval. For the micronucleus assay, polychromatic cells in bone marrow samples were assayed in samples taken 18, 24, and 36 h after a dose of 100 mg/kg. A dose response study of polychromatic cells was done using samples taken at 24 h after doses of 50, 75, or 125 mg/kg.

As a function of time after acrylamide injection, chromosome damage (gaps, breaks, and exchanges) was significantly increased over the control at all times. In the dose-response data, the frequency of gaps did increase with dose, and there was a upward trend in aberrant cells as dose increased. The overall chromosome aberration rates were of the same order of magnitude as cisplatin at 0.5 mg/kg.

The number of micronuclei per polychromatic cells was significantly increased over the control and was maximal at 24 hours after the single dose of acrylamide and there was an increase in micronuclei as a function of increased dose. The overall induction of micronuclei was of the same order of magnitude as cisplatin at 0.5 mg/kg.

The dose-response assay of chromatid aberrations in spermatogonia did not yield an increase at any dose compared to

the control; cisplatin at 2.5 mg/kg did produce the expected increase. There was a statistically significant reduction in mitotic index at the 150 mg/kg acrylamide dose comparable to that for cisplatin. The authors concluded that acrylamide is a clastogen in bone marrow cells (Adler 1988).

Čihák and Vontorková (1988) also studied the effects of acrylamide in the bone marrow of mice. Acrylamide diluted with distilled water was given to adult male ICR-SPF mice. There were five animals in each treatment group. A control group received only distilled water injections. No positive control was reported. In a time-after-treatment study, acrylamide was given at a dose of 100 mg/kg, and assays were performed at 6, 18, 24 and 48 h after i.p. injection. In a dose-response study, animals were given two i.p. injections; one at 24 and one at 6 h prior to sacrifice. Doses used were 2 × 25, 2 × 50, and 2 × 100 mg/kg.

Chromosome aberrations were significantly increased over the control at all times after dosing with 100 mg/kg acrylamide and was maximal at 24 h. Micronuclei were not significantly different from the control except at 24 h after dosing with 100 mg/kg acrylamide. In the dose-response study, micronuclei were significantly increased at all dose levels. The authors concluded that acrylamide is clastogenic in somatic cells (Čihák and Vontorková 1988).

Sega et al. (1989) examined the binding of acrylamide to DNA and protamine in spermatozoa of mice. Male (C3H × 101)$F_1$ mice were given i.p. injections of [1-$^{14}$C]acrylamide (specific activity of 1.0 mCi/mmol) at a dose of 125 mg/kg. At 4 h and then daily for 23 days, six males were killed, the vasa deferentia and the caudal epididymides were recovered and pooled.

Assuming that the effect of acrylamide is via alkylation of nucleophiles, the authors measured the extent of alkylation in sperm heads. For sperm heads recovered from the vasa deferentia, at day 4, the number of alkylations per sperm began to increase to a maximum at day 9 followed by a decrease to day 1 levels at day 17. Alkylation of sperm DNA did not increase over the same time course; it remained flat.

For sperm heads recovered from the caudal epididymides, the number of alkylations per sperm head increased late on day 1 to a maximum at day 8 followed by a decrease to early day 1 levels at day 14. Alkylation of sperm protamine followed the identical time course. The authors concluded that acrylamide alkylation of protamine suggests that protamine is a likely target for acrylamide genetic damage (Sega et al. 1989).

Sega and Generoso (1990) extended the above study by measuring DNA breakage in spermatozoa of mice treated with acrylamide. Male (C3H × C57BL/10)$F_1$ mice were given testicular injections of $^3$H- or $^{14}$C-labeled thymidine. Acrylamide at a dose of 100 mg/kg was given by i.p. injection to the $^3$H-labeled mice and a control injection to the $^{14}$C-labeled mice. Sperm were recovered from the vasa deferentia from 1 to 21 days after treatment. A dose-response study was also done using 25, 50, 75, 100, and 125 mg/kg doses; sperm were recovered at 10 days.

Alkylation elution profiles of DNA were obtained at each time/dose. Spermatozoan DNA elution increased at day 1 to a maximum at day 10 followed by a decrease to control levels at around day 17. The shape of this curve closely approximated the shape of the alkylations per sperm head and the alkylation of protamine reported in the previous study.

The authors noted that the mechanism by which DNA breaks occurs at some sperm developmental stages has to be different from the acrylamide binding to protamine, because protamine is not found in pachytene spermatocytes or early spermatids, only in late spermatids and spermatozoa (Sega and Generoso 1990).

Sega et al. (1990) reported that unscheduled DNA synthesis at different sperm development stages correlated with DNA alkylation (adduct formation) in mice treated with acrylamide. Male (C3H × 101)$F_1$ and (C3H × BL10)$F_1$ mice were given i.p. injections of acrylamide in Hanks' balanced salt solution at 0, 7.8, 15.6, 31.2, 62.5, and 125 mg/kg. Mice were given testicular injections of $^3$H-labeled thymidine at the time of treatment or at various times after treatment. Sperm were recovered from the caudal epididymides at day 16 (early spermatid stage).

The unscheduled DNA synthesis (UDS) as a function of time after treatment with the $^3$H-labeled thymidine increased as a function of testicular injection time. UDS was maximal at 6 h and decreased at later testicular injection times. Adduct formation exhibited a similar pattern as a function of time after acrylamide treatment (46 mg/kg), with a maximum at 6 h.

Using a 6-h radiolabel injection time, UDS as a function of time after acrylamide treatment began to increase above control levels at day 9 to a maximum at day 15 followed by a decrease. The increase in UDS was linear with acrylamide dose. The authors noted that the time course of UDS as a function of testicular injection of radiolabel was unexpected; UDS was expected to reach a maximum with little appreciable time between acrylamide treatment and testicular injection of radiolabel.

The authors stated that the only other times such a delay was seen, the treatment agent required metabolic activation. The authors concluded that acrylamide or an acrylamide metabolite can cause a UDS response at early sperm development stages, but not at later stages (Sega et al. 1990).

Russell et al. (1991) reported the induction of specific-locus mutations in male mouse germ cells by acrylamide. Male (C3H/R1 × 101/R1)$F_1$ mice were given i.p. injections of acrylamide at 50 mg/kg on each of 5 successive days. In all of the treatment and control groups, 638 males were used. Treated males were caged with untreated females at different times after exposure (different times in sperm development).

For exposed spermatogonial stem cells, there were no specific-locus mutations or mutations at other loci in 17,112 offspring. For exposed poststem-cell stages, there were five specific-locus mutations and three mutations at other loci in 28,971 offspring. In concurrent controls in the same laboratory, there were no specific-locus mutations and one mutation at another loci in 13,709 offspring.

The authors concluded that the data demonstrate the mutagenic potential of acrylamide, but that the absence of mutations

in spermatogonial stem cells reduces the concern for paternally contributed mutations (Russell et al. 1991).

Gutierrez-Espeleta et al. (1992) demonstrated that dermal application of acrylamide can produce damage in male mouse germ cells. Male (C3H/R1 × 101/R1)$F_1$ mice were shaved with a fine clipper and and 100 $\mu$l of an acrylamide solution (acrylamide in 70% ethanol and 30% water) was applied using a micropipette daily for five days; smaller amounts were used with smaller animals. The doses were 0, 25, 50, 75, 100, and 125 mg/kg day$^{-1}$. Each treatment group consisted of 30 males. Treated males were mated with untreated females. Successfully mated females were separated from the males and uterine analyses were carried out 12 to 14 days later.

The authors characterized dominant lethal mutations in parental germ cells as leading to death of first generation carriers, which in mice generally occurs around the time of implantation, resulting in a resorption in the uterus. A positive effect is seen when there is a reduction in the average number of living embryos, and a significant increase in either the average number of dead implantations or the number of females with one or more dead implantations. All groups except the 25 mg/kg dose group were positive, and the magnitude of the responses increased as a function of dose over the 50 to 125 mg/kg dose range. The authors concluded that the results are consistent with acrylamide absorption through the skin, distribution to early spermatozoa in seminiferous tubules, causing dominant lethal mutations (Gutierrez-Espeleta et al. 1992).

Ehling and Neuhäuser-Klaus (1992) reported results different from Russell et al. (1991) described above. To determine dominant lethal mutations, male (102/E1 × C3H/E1)$F_1$ mice, 13 to 14 weeks of age, were given i.p. injections of acrylamide in distilled water at doses ranging from 50 to 125 mg/kg. Treated males were mated with untreated females in a sequential mating procedure. For the specific-locus test, male (102/E1 × C3H/E1)$F_1$ mice, 10 to 13 weeks of age, were given i.p. injections as above. Each male was caged immediately with an untreated virgin female for 4 days. After 4 days a new virgin female was placed in the cage. Offspring were classified according to those derived from treated spermatocytes and differentiating spermatogonia, and those from $A_s$ spermatogonia.

The results confirm the work of others that acrylamide produces dominant lethal mutations. In the specific-locus mutation assay, however, these results found a positive effect in both post-spermatogonial germ cell stages (consistent with Russell et al. 1991) and spermatogonia (contrasts with Russell et al. 1991 report of no effect). The authors speculated that the finding may relate to the higher dose level in their study which may not be detoxified and that spermatogonial cells cannot repair the pre-mutational damage (Ehling and Neuhäuser-Klaus 1992).

In an attempt to further clarify the genotoxic effects of acrylamide, Tsuda et al. (1993) undertook a series of tests: (1) mutagenicity in the *Escherichia coli* WP2 *uvrA*$^-$/microsome system, the *Salmonella*/microsome system, and the Chinese hamster V79H3/HPRT system; (2) a *Bacillus subtilis* spore rec assay; (3) induction of sister-chromatid exchanges (SCEs), chromosomal structural changes, and polyploidy in Chinese hamster cells; and (4) cell transformation in the BALB/c 3T3 cell assay. All experiments were performed simultaneously using acrylamide from a single source.

The authors concluded that acrylamide does not produce gene mutations, but does induce DNA damage in the *B. subtilis* spore rec assay. In the other assays, acrylamide did induce polyploidy, chromosomal structural changes, and SCEs, and was capable of transforming BALB/c 3T3 cells into malignant cells capable of causing tumors when transplanted in mice (Tsuda et al. 1993).

Russo et al. (1994) reported results of the EEC/STEP project "Detection of Germ Cell Mutagens" using mice injected i.p. with acrylamide. The endpoints included (1) micronuclei in early spermatids, meiotically dividing or premeiotic S phase; (2) SCEs in differentiating spermatogonia; and (3) micronuclei in peripheral blood reticulocytes. They concluded that acrylamide was a genotoxic agent in both germ and somatic mouse cells in vivo, but that its effects on premeiotic cells are weak compared to late sperm development stages.

Using the PAINT/DAPI analysis of male germ cells in first-cleavage metaphases, Marchetti et al. (1997) claimed to be able to assay multiple cytogenetic endpoints simultaneously. In a demonstration of the methodology, 50 mg/kg acrylamide was given to male mice by i.p. injection daily for 5 days. Using the PAINT/DAPI assay the authors found that acrylamide induced clastogenic effects as a function of the stage of germ cell development, in agreement with data discussed above.

In another study of a test system, Krebs and Favor (1997) used the transgenic Muta Mouse in vivo mutagenesis assay to compare acrylamide and ethylnitrosourea effects in germ and somatic cells. The authors reported that as conducted the test system did not yield sufficient DNA from germ cells to make an analysis. Analysis of liver DNA did produce acceptable results which demonstrated that acrylamide and ethylnitrosourea are mutagenic.

## Mammalian Assays

*Glycidamide*

Generoso et al. (1996) examined the hypothesis that acrylamide is genotoxic as a result of its conversion to the reactive epoxide, glycidamide. They hypothesized that glycidamide should exhibit the same pattern of genotoxicity as acrylamide if glycidamide is the active metabolite and conducted the following tests: (1) dominant lethal; (2) heritable translocation; and (3) UDS. Glycidamide used in these tests was dissolved in Hanks' balanced salt solution.

In the dominant lethal test, male (C3H/RL × 101/RL)$F_1$ mice were given a single i.p. injection of glycidamide at a dose of 125 mg/kg. They were mated with untreated females and the females were killed 13 to 17 days after mating for uterine analysis. The heritable translocation test was performed with glycidamide at 100 mg/kg. Treated males were mated to untreated females 3.5

to 7.5 days post treatment to produce the male progeny that were tested for translocations. For the UDS assay, male (C3H/RL × C57BL)$F_1$ mice were given a single i.p. injection of glycidamide at 150 mg/kg and testicular injections of [methyl-$^3$H]thymidine at different times after treatment.

Based on a range-finding 30-day toxicity study, the authors had selected a dose of 175 mg/kg for the dominant lethal test because that was the highest dose that did not affect survival or body weight. This dose reduced the mating ability of treated males to the point that it was not practical to do the study. The 125 mg/kg dose had no effect on mating ability. Glycidamide was an effective enhancer of dominant lethal mutations, with a maximum during the 4.5- to 9.5-day window, which corresponds to the late spermatid and earyl spermatozoa phases of sperm development in the treated males. In the translocation assay, treatment with 100 mg/kg produced a reduction in litter size per female to 5.8; this corresponds to an estimated 40% dominant lethal rate when compared to the expected litter size of 9.5 derived from past experience. The heritable translocation rate of 20.18% in treated males was around two orders of magnitude higher than the spontaneous rate seen with past experience. UDS was maximal in the early spermatid stage of sperm development in the treated males (i.e., at 2 h after exposure) and decreased thereafter.

The authors noted that acrylamide and glycidamide induces similar patterns of dominant lethal mutations in male mice, but glycidamide was more effective. Induction of heritable translocations was generally similar for the two chemicals. Glycidamide exposure produced a maximum UDS at 2 h compared to previous reports described above where acrylamide produced a maximum UDS at 6 h. The authors did compare the actual levels of radiolabeled thymidine incorporated, adjusting to equimolar injections and for the specific activity of the radiolabel, and stated that the agreement was good. They suggested that the time delay could be explained by the time required to convert acrylamide to glycidamide (Generoso et al. 1996).

## Genotoxicity Reviews

Dearfield et al. (1995) published a review article describing the state of knowledge regarding acrylamide genotoxicity

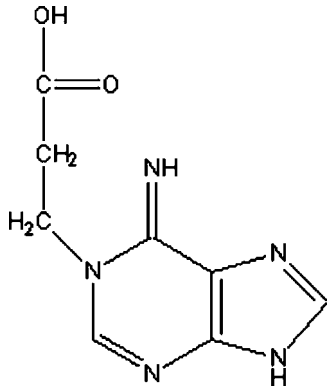



**FIGURE 2**
Example of Michael-type reaction product, 1-carboxyethyl adenine, of acrylamide with a DNA base (Dearfield et al. 1995).

at that time. They describe three chemical reactivities of acrylamide that may be relevant to acrylamide genotoxicity. First, acrylamide can undergo free radical polymerization to Polyacrylamide as shown in Figure 1.

The authors stated that the role of this reaction in the genotoxicity of acrylamide is unknown. Second, acrylamide is an $\alpha,\beta$-unsaturated chemical subject to carbanion addition in a Michael-type reaction in which a DNA base is the donor instead of the classic thiol, hydroxy, or amino groups. An example, 1-carboxyethyl adenine, is shown in Figure 2, but the authors characterize the binding to DNA as weak and that the more likely reaction is with -SH groups in proteins. They noted that the primary urinary metabolites of acrylamide are glutathione adducts. The authors also described the enzyme mediated oxidation of acrylamide to glycidamide as shown in Figure 3.

The authors presented arguments pro and con for acrylamide itself and its epoxide, glycidamide being the active chemical responsible for acrylamide genotoxicity. Without resolving this question, and using the then available data on gene mutations and chromosomal alterations, an estimate was made of the number of induced human genetic diseases per million offspring using risk estimates based on the doubling dose in male mouse studies.

They considered that dermal exposure to acrylamide usually occurs during preparation of Polyacrylamide solutions, but did

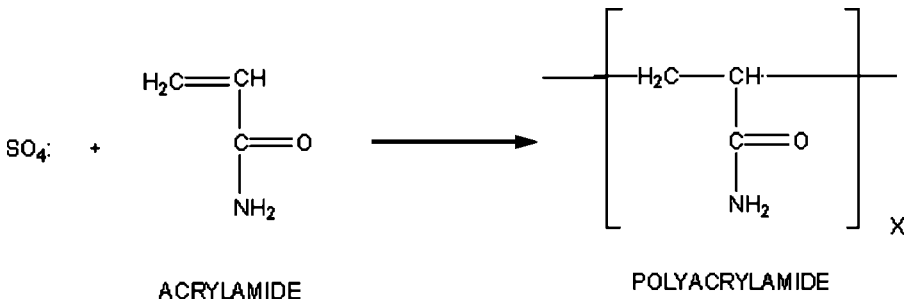



**FIGURE 1**
Radical-mediated polymerization of acrylamide (Dearfield et al. 1995).

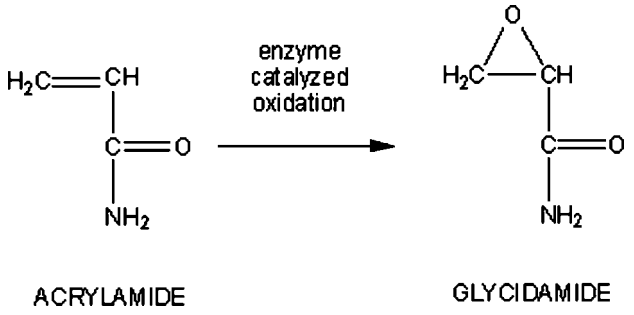



**FIGURE 3**
Enzyme-mediated oxidation of acrylamide to glycidamide (Dearfield et al. 1995).

not address acrylamide residues in cosmetic products. They cite the example of sewer line maintenance workers mixing acrylamide to produce Polyacrylamide solutions for use as grout to seal sewer lines. They derived an upper value of daily exposure of 0.13 mg/kg day$^{-1}$. For an adult male dermal exposure to that level, they stated that the contribution of gene mutations was estimated to be 0.73 diseases per million offspring and the contribution of chromosomal aberrations was estimated to be 2.2 diseases per million offspring. The authors stated that it was not clear how the gene mutation and chromosomal aberration risks should be combined, nor did these estimates include any risks from female exposure (Dearfield et al. 1995).

## ACRYLAMIDE CARCINOGENICITY

Bull et al. (1984a) studied the effects of acrylamide in an initiation/promotion assay in Sencar mice and a lung ademoma bioassay in A/J mice. For the skin initiation/promotion study, female Sencar mice were treated by gastric intubation, intravenous (i.v.) injection, and topical application. The vehicle for oral or i.v. administration was vegetable oil and acetone for topical application. Dose levels were 12.5, 25.0, and 50.0 mg/kg at each of six applications. Two weeks after the end of the tumor initiation phase, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) in acetone was applied to the shaved back of each animal 3 times per week for 20 weeks. Controls received acetone alone.

The authors reported a dose-response relationship for time to first tumor and for mice with multiple tumors with acrylamide, regardless of route of administration, although oral was the most effective and topical the least. Acrylamide did not increase the tumor yield in the absence of TPA promotion.

In the mouse lung adenoma bioassay, two colonies of male and female A/J mice were separately tested. One group of animals received oral doses of 6.25, 12.5, or 25 mg/kg acrylamide in distilled water three times per week for 8 weeks. The other group of animals received i.p. injections of 1, 3, 10, 30, or 60 mg/kg acrylamide in distilled water using the same dosing regimen. At 8 months (i.p. group) or 9 months (oral group), the animals were killed, their lungs were fixed and the surface adenomas were counted.

Animals at the one colony given oral treatment did have a dose-dependent increase in lung adenomas in both sexes. The animals in the colony receiving i.p. injections at the 60 mg/kg dose exhibited frank peripheral neuropathy after just 11 injections and that study group was terminated. A dose-dependent increase in lung adenomas was seen in the other treated groups. The authors concluded that the i.p. route was slightly more potent (Bull et al. 1984a).

Bull et al. (1984b) extended this work with oral administration of acrylamide (dissolved in water) to female ICR-Swiss mice for 3 days a week for 2 weeks. Total doses were 0, 75, 150, and 300 mg/kg. At 4 weeks, TPA in acetone was applied to the shaved backs of treated animals three times a week for 20 weeks. Skin tumors were tallied weekly over the course of a year and lung adenomas were determined at the end of the year. The appearance of papillomas was determined on the skin. At 52 weeks the survivors were killed and the lungs and skin were evaluated histologically.

Acrylamide inductions with TPA promotion produced a dose-dependent increase in the number of animals with skin tumors. The combined number of lung adenomas and carcinomas was increased significantly as a function of acrylamide dose and TPA promotion. At the high acrylamide dose, there was an increase in lung adenomas and carcinomas without TPA promotion. The authors commented that ICR-Swiss mice appear to be less susceptible to acrylamide-induced skin tumors compared to Sencar mice, but the same as A/J mice as regards lung tumors (Bull et al. 1984b).

Johnson et al. (1986) conducted a chronic toxicity and carcinogenicity study in which Fischer 344 rats were given drinking water containing acrylamide. Male and female rats were provided treated drinking water which yielded doses of 0, 0.01, 0.1, 0.5, or 2.0 mg/kg day$^{-1}$ for 2 years. Each treatment group consisted of 90 animals per sex. Rats were observed twice daily and examined monthly for palpable masses. Ten animals were selected at 6, 12, and 18 month interim necropsies. Blood for routine hematologic and clinical chemistry analysis was drawn at 3, 6, 12, 18, and 24 months (study termination); urine was collected at these times as well. All surviving animals were necropsied at 24 months.

Minimal decreased body weights in the 0.5 and 2.0 mg/kg day$^{-1}$ were recorded. Increased mortality was seen in rats receiving the highest dose, especially females, from the 21st month. Increased mortality was statistically significant for both sexes at the highest dose at study termination. Hematology parameters, urinalysis, and blood chemistry results were unremarkable. Clinical signs of peripheral neuropathy were not seen, but microscopic examination of peripheral nerves detected degenerative lesions. While male rats had more degenerative lesions in the tibial nerve compared to controls, those same male controls had more lesions than female controls.

The authors reported an increased incidence in acrylamide-treated female rats of (1) mammary gland tumors, (2) CNS tumors, (3) thyroid gland/follicular epithelium tumors, (4) oral

tissue tumors, (5) uterine tumors, and (6) clitoral gland tumors. In male rats, tumor increases were seen in (1) central nervous system, (2) thyroid gland, and (3) scrotum.

Among the mammary tumors, fibromas, benign mammary tumors, and mammary adenocarcinomas were increased in the high-dose group. Increases in benign tumors were seen at the next lower dose, but was not significant. A linear trend as a function of dose was seen with adenocarcinomas, but none reached statistical significance except the high dose. The CNS glial cell tumors were high in the controls, but higher still in the highest-dose group.

Male rats had an increased incidence of mesotheliomas of the scrotum at the two highest dose levels, although this result was complicated by the high historical control incidence of these tumors in the colony. The only tumor for which there was a statistically significant increase in both sexes was the thyroid gland/follicular epithelium.

The authors remarked about the diverse types of tumors whose incidence was increased with acrylamide exposure and that, except for scrotal mesotheliomas, the increase was seen only at the highest dose (Johnson et al. 1986).

Friedman et al. (1995) reported the results of a lifetime carcinogenicity study in rats exposed to acrylamide. This study was undertaken to address several ambiguities in the Johnson et al. (1986) study described above. Ultrapure (electrophoresis grade) acrylamide mixed with filtered tap water was provided to test animals as drinking water to produce doses of 0, 0.1, 0.5, or 2.0 mg/kg day$^{-1}$ for males or 0, 1.0, or 3.0 mg/kg day$^{-1}$ for females. Water consumption was measured weekly and no differences were observed between control and treated animals throughout the study period (44 to 45 days of age to 106 to 108 weeks). All animals were observed for mortality, morbidity, or other signs of toxicity twice daily. Physical examinations for signs of toxicity were conducted weekly for the first 16 weeks, then monthly for 24 weeks, and biweekly for the remainder of the study. After 24 weeks, examinations included palpation for tumors. A complete necropsy was performed on all study animals. The brain, liver, kidneys, and testes were weighed. The adrenal glands, aorta, bone and bone marrow, brain, epididymides, esophagus, heart, kidneys, large intestine, liver, lungs, mesenteric lymph nodes, ovaries, pancreas, sciatic nerve, pituitary gland, prostate gland, salivary glands, seminal vesicles, skeletal muscle, small intestine, skin/mammary gland, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid gland, tongue, trachea, ureters, urinary bladder uterus, vagina, and eyes were examined for gross lesions. Histology was performed on selected tissues. In all female rats, microscopic examination of the stained sections of the thyroid, brain and mammary glands was done. Thyroid and testes were examined in male rats. All male or female rats found dead or killed moribund were further examined.

Mortality rates were low through the first 60 weeks. Mortality in the high-dose male group increased after that time and continued to be higher than the controls and other dose groups. At study termination, the mortality in the high dose males was 75%, compared to 53% and 44% in each of two control groups. At study termination, female mortality was 28% and 40% in the two control groups and 35% and 49% in the low- and high-dose treatment groups, respectively.

Mean body weights were decreased in the high-dose male group from week 8 until termination, but not in other dose groups. Treated female body weight decreases appeared sooner and were more pronounced than in males. The authors noted an absence of any signs of neurotoxicity in either male or female animals.

The microscopic examination demonstrated a statistically significant increase in mesotheliomas of the tunica vaginalis testis in male rats in the high dose-group. There was an increase in the mid-dose group, but it was not statistically significant. No attempt was made to differentiate benign from malignant mesotheliomas. In female animals, the incidence of mammary gland adenocarcinoma was not different between the two treatment groups and the controls, but there was an increased incidence of mammary gland fibroadenomas in both treatment groups compared to the two control groups.

Male and female rats in the high dose-groups, and female rats in the lower dose-group, had a statistically significant increase in the combined thyroid follicular cell adenomas and adenocarcinomas. Taken alone, adenocarcinomas were increased in the high-dose female group, but the difference was not statistically significant.

In contrast to the visual findings of no signs of neurotoxicity, sciatic nerve degeneration was seen in both male and female high dose groups at a higher frequency than in the controls.

The authors compared and contrasted their findings with those of Johnson et al. (1986) described above. The previous finding of increased incidence of glial cell tumors in female rats was not confirmed. The incidence of astrocytomas, however, was increased in the highest-dose male group in this study. Combining all neurological neoplasms in this study, however, there is not a statistically significant increase. In the current study, there was no increase in mammary tumors; in fact, the incidence of fibroadenoma is unusually low. The highest dose given female rats in this study was higher than the highest dose in the Johnson et al. (1986) study in order to pinpoint the nature of the effect on mammary tissue. The hypothesis was that some increases would be seen compared to the previous study and the pattern used for risk assessment. At the higher dose in this study, however, there was not an increase in adenocarcinomas.

The authors confirmed previous findings that acrylamide exposure increases the incidence of mesotheliomas in the male rat in a dose-dependent manner. They stated that the likely no-effect dose for mesotheliomas in the male rat was the mid-dose, 0.5 mg/kg day$^{-1}$. The authors also remarked on the finding of a rare malignant reticulosis in the brain seen in seven rats in this study that appear different from glial neoplasms. Although the incidence of malignant reticulosis in the treated group compared to the control did not reach statistical significance, five appeared in treated rats and none in the control female groups. Both the

control and low-dose male group had one each (Friedman et al. 1995).

Damjanov and Friedman (1998) undertook a light and electron microscopy study to examine mesotheliomas of the tunica vaginalis testis in male rats exposed to acrylamide. In the previous study (Friedman et al. 1995), the authors found an increase in the frequency of mesotheliomas. According to these authors, mesotheliomas occur spontaneously in rats at rates ranging from 1% to 4%, primarily in the tunica vaginalis testis, an extension of the abdominal mesothelium lining the inside of the scrotum.

In this study, the authors examined tumor material from the previous study in an attempt to determine whether acrylamide induces these tumors or simply potentiates the development of these, already frequent, rat tumors. They compared the morphology of mesotheliomas from untreated controls with those from acrylamide-treated groups. No differences were found.

The authors also noted that these mesotheliomas are best characterized as having limited growth potential and appear most like human benign mesotheliomas of the tunica vaginalis testis or adenomatoid tumors, even though current veterinary pathology practice is to consider all rat mesotheliomas as potentially malignant (Damjanov and Friedman 1998).

## CLINICAL ASSESSMENT OF SAFETY

### Skin Irritation

*Acrylamide*

Hollander (1952) reported the results of applying acrylamide in 25 persons at 1%, 5%, 10%, and 25% strength of the material as supplied by the manufacturer. Both the number and degree of irritant responses (1 week after application) are given in Table 9. He concluded that acrylamide is a skin irritant.

### Occupational Exposures

*Polyacrylamide*

McCollister et al. (1965) carried out chronic environmental studies in Polyacrylamide production plants over a period of 5 years indicated that workers were being exposed to polymer dust through inhalation. Average airborne concentrations of the polymer were reported to be 1 mg/m$^3$. Approximately 5 mg/day of the dust, with a diameter greater than 50 $\mu$m per particle, had the potential to be trapped in the upper respiratory passages and swallowed.

**TABLE 9**
Human skin irritation produced by Acrylamide (Hollander 1952)

| | Percent dilution of the acrylamide supplied by manufacturer | | | |
|---|---|---|---|---|
| Reactions in 25 subjects | 1% | 5% | 10% | 25% |
| Subjects not irritated | 14 | 12 | 10 | 4 |
| Subjects irritated | 11 | 13 | 15 | 21 |
| + irritation | 9 | 6 | 5 | 7 |
| + + irritation | 2 | 7 | 8 | 8 |
| + + + irritation | — | — | 2 | 4 |
| + + + + irritation | — | — | — | 2 |

The data from physical examinations conducted from 1952 through 1959 and in 1962 had no indication of unusual pathological response. The number of men examined was not indicated. The results of these medical examinations showed no indication of adverse effects caused by exposure to Polyacrylamide dust (McCollister et al. 1965).

Dow (1955) examined research employees from a development and pilot plant, and employees who worked on full-scale production of Polyacrylamide periodically in tests designed to include any diagnostic test or procedure necessary to indicate any possible effects of Polyacrylamide exposure and to evaluate the general health of the study participants. The data from 162 examinations of 106 men who worked with Polyacrylamide did not indicate any abnormal effects from exposure to Polyacrylamide encountered in the research, development, or manufacturing processes. There was no indication of more disease than one might expect to find in a group of similar men in the general population.

*Acrylamide*

Bergmark et al. (1993) used measurements of adducts of hemoglobin (Hb) and acrylamide to monitor occupational exposure to acrylamide over the preceeding 4 months (the life-span of the human erythrocyte). Blood was collected from 41 workers in a chemical factory in China synthesizing acrylamide from acrylonitrile and polymerizing acrylamide to form polyacrylamide. This study addresses primarily the methodology of detecting adducts to different amino acid groups in Hb.

The authors compared the Hb adduct data to both the acrylamide levels in the location in the factory to which each worker was assigned and to the job description. The acrylamide air concentration measured in the polymerization facility was 1.52 mg/m$^3$ (0.19–8.8) and in the synthesis facility was 0.73 mg/m$^3$ (0.11–3.01). Both the location to which the worker was assigned and the job description were related to the measured Hb-adduct level. The authors estimated that the Hb-adduct level in high acrylamide concentration areas was 10 to 30 times higher than would be expected with inhalation and elimination, and postulated that dermal absorption may be the predominant route of uptake (Bergmark et al. 1993).

Calleman et al. (1994) reported the relationship of Hb adducts as a measure of exposure to acrylamide as described above to neurological effects in the 41 workers at a chemical factory in China. The workers underwent a complete medical and neurological examination. Signs and symptoms indicating peripheral neuropathy were increased (statistically significant) in these factory workers compared to a group of non-factory workers from the same city. The authors developed a neurotoxicity index (NIn)

to include the multiple neurological parameters measured, including (1) numbness of extremities; (2) cramping pain; (3) loss of position sensation, pain sensation, touch sensation, and vibration sensation; (4) clumsiness of hands; (5) difficulty grasping; (6) unsteady gait; (7) decrease or loss of ankle reflexes; (8) muscular atrophy; and (9) electroneuromyography measurements. A total score of 50 was possible with each endpoint at the maximum severity for peripheral neuropathy. The authors concluded that the NIn was significantly correlated with Hb adducts of acrylamide, cumulative in vivo doses of acrylamide, and employment time.

Marsh et al. (1999) reported the most recent data on mortality patterns among a cohort of 8508 workers at various manufacturing facilities at which some of the workers were exposed to acrylamide ($>0.001$ mg/m$^2$ year$^{-1}$) and others were unexposed ($<0.001$ mg/m$^2$ year$^{-1}$). For the 1925 to 1994 study period, there were no significant increased overall risks or manufacturing facility-specific risks of CNS cancer, thyroid or other endocrine cancer, or testicular cancer.

A significant 2.26× risk of pancreatic cancer mortality was found among workers with exposure to $>0.3$ mg/m$^2$ year$^{-1}$. In this case the study had the statistical power to detect an increase in risk of 2× or higher. Because of the small numbers, however, except for cancer of the respiratory system, the study had insufficient statistical power to detect small, but important, increases in mortality from other cancers. The authors did note a nonsignificant increased mortality from cancer of the respiratory system in these workers (7% in the exposed and 4% in unexposed).

Although not statistically significant, the authors did note increased standard mortality ratios (SMRs) for thyroid cancer and thyroid plus other endocrine cancers among workers exposed to acrylamide (4× risk) and workers not exposed to acrylamide (2 × risk). Given the small numbers, again, it was not possible to provide any explanation other than to speculate that some other unmeasured occupational factor may be at play. As with any mortality study, this work did not measure cancer incidence and may be confounded by successful treatment of cancer and/or death from other causes (Marsh et al. 1999).

## ACRYLAMIDE RISK ASSESSMENTS

Several risk assessments have been conducted based on the available data. Hazard identification is usually considered a subset of risk assessment, with the guide being "no hazard, no risk," even if there is significant exposure. Therefore, that available hazard assessments are presented first.

### Hazard Identification

Shipp et al. (2001a) considered the results of the two chronic bioassays that have been conducted in rats: the Johnson et al. (1986) and the Friedman et al. (1995) studies. The Shipp et al. (2001a) analysis of those data are presented below.

*Tunica Vaginalis Mesotheliomas (TVMs)*

Shipp et al. (2001a) noted that TVMs were increased in both studies, but the data were not identical. In the Johnson et al. (1986) study, TVMs were increased at both the 0.5 and 2.0 mg/kg day$^{-1}$ dose levels compared to the control group. Only the 2.0 mg/kg day$^{-1}$ dose group was increased in the Friedman et al. (1995) study compared to the controls. In the lower-dose groups in both studies, TVMs were similar in frequency to the controls. This suggested low-dose nonlinearity and the possibility of a threshold effect.

*Mammary Tumors*

Shipp et al. (2001a) stated that both studies identified increases in mammary tumors of two types: adenomas/adenocarcinomas and fibromas/fibroadenomas. Although adenocarcinomas were considered malignant cells arising from glandular epithelial cells, fibroadenomas were described as benign neoplasms derived from the stromal matrix/fibrous connective tissue). These differences were used as the basis for suggesting that these two tumor types be considered separately and not combined. When adenocarcinomas were considered alone in the Friedman et al. (1995) study, there was no increase in any dose group compared to controls, and although there was a suggestion of a dose-response relationship in the Johnson et al. (1986) study, the incidence rate for each dose level was comparable to historical control rates. The authors concluded that no increase in adenocarcinomas could be attributed to acrylamide exposure.

Using the incidence data for the remaining three mammary tumor types in the two studies, the authors went on to suggest that (1) the incidence of adenomas alone was not increased in either study, so there is no hazard identified; (2) the increased incidence of fibromas only in the 2.0 mg/kg day$^{-1}$ group in the Johnson et al. (1986) study was not confirmed in the 3.0 mg/kg day$^{-1}$ dose group in the Friedman et al. (1995) study, so it was considered unlikely that the finding represents a hazard associated with acrylamide exposure; and (3) the increased incidence of fibroadenomas in the two highest dose groups in the Friedman et al. (1995) study, but not in the Johnson et al. (1986) study, could not be dismissed, even though all of the frequencies were within historical controls. Of all the mammary tumor types, therefore, only fibroadenomas were considered treatment related (Shipp et al. 2001a).

*Glial Cell Tumors*

According to Shipp et al. (2001a), there was an increase in glial cell lesions in female rats at the highest dose in the Johnson et al. (1986) study when tumors and glial cell proliferation (potentially precancerous lesions) were combined, but not in the Friedman et al. (1995) study, which used more female rats. There was no increase in glial cell tumors in either study in male rats. Because glial cell proliferation is not clearly a precancerous state, was not found in both studies, and when considered alone was not increased in the one study, there is no demonstrated effect. Although not statistically significant, the astrocytomas

observed in control and treated male and female rats appeared to be increased as a function of dose when the two studies are looked at together (astrocytes are a type of glial cell). A re-read of the histopathology data from the two studies identified that there was regional localization of the astrocytomas common to controls and treated animals. The authors suggested that this similarity may be traced to a common cause.

*Thyroid Follicular Cell Adenomas*

Shipp et al. (2001a) noted that, in male rats at the high dose in both studies, there was an increase in thyroid follicular cell adenomas, but no effect at any other dose level. No increases in follicular cell adenocarcinomas were seen in any treatment group in either study. The authors note that follicular cell tumors are not common in rats and concluded that the incidence of thyroid follicular cell adenomas was related to treatment.

*Oral Cavity Papillomas and Uterine Adenocarcinomas*

Shipp et al. (2001) stated that Johnson et al. (1986) found an increase in the incidence of oral cavity papillomas and uterine adenocarcinomas in female rats at the 2.0 mg/kg day$^{-1}$ dose level, but that, at a higher dose (3.0 mg/kg day$^{-1}$) using more animals, Friedman et al. (1995) did not see an increase in either. The authors suggested there was no treatment-related effect.

Shipp et al. (2001a) went on to examine the possible mode of action of acrylamide that may be responsible for the acrylamide-related TVMs, mammary fibroadenomas, astrocytomas, and thyroid follicular cell adenomas. Two competing modes of action were considered: (1) genotoxicity, and (2) neurohormonal alterations.

*Genotoxic Mode of Action*

Recognizing that acrylamide can be genotoxic in mammalian in vitro and in vivo test systems, Shipp et al. (2001a) attempted to relate the exposure levels in those studies to the dose levels in the chronic bioassays—concluding that genotoxicity was found usually only at exposures that would be equivalent to doses significantly higher than used in the chronic bioassays. Because there were some increases in tumors in those studies, the authors concluded that it was unlikely that a genotoxic mechanism was responsible. In addition, these authors cited the work of Bull et al. (1984a, 1984b), which demonstrates the need for tumor promotion to cause acrylamide-produced tumor initiation events to proceed to tumor formation.

*Neurohormonal Alterations—TVMs*

Shipp et al. (2001a) considered that the absence of tumors in tissue of similar embryonic type in areas other than the scrotum or in females suggests that TVMs are unique to the male at a specific site. These authors also suggested that the rarity or absence of TVMs in other rat strains or in mouse species further narrows the endpoint to the Fischer 344 strain of rats.

Although a hormonal imbalance was stated to be one possible mechanism, these authors considered that the appearance of Leydig cell tumors (LCTs), which may produce a physical stimulus on the surrounding mesothelium, not unlike that seen with solid state carcinogenesis. The finding that the degree of progression of TVMs in the Friedman et al. (1995) study correlated with the size of LCTs in the testes, that malignant TVMs were found only in animals where LCTs occupied over 75% of the testes, and that TVMs that were likely only localized hyperplasia were found in animals where LCTs occupied 25% or less of the testes, all combined to suggest to these authors the possibility that LCT formation may play a key role in TVM induction. They went on to describe the possible cascade of events beginning with decreases in serum prolactin and testosterone levels associated with acrylamide exposures. Compensatory increases in leutinizing hormone (LH) to maintain testosterone levels are a feature of the aging male rat and could be at play when acrylamide-induced testosterone decreases occur. LH increases, in turn, can stimulate Leydig cells directly through LH receptors on Leydig cells in the rat (but absent in humans), leading to hyperplasia and LCTs, according to these authors.

These authors considered aspects of male rats (other than Leydig cell LH receptors) that may make this animal sensitive to acrylamide, including the fact that blood levels of testosterone are subject to rapid change in the rat (relatively stable and bound in human blood) and that the rat has a larger Leydig cell mass to blood volume than humans.

Taken together, all of these factors suggested to Shipp et al. (2001a) that it was unlikely that the increase of TVMs in male Fischer 344 rats associated with acrylamide has any relevance to humans and should not be considered in any risk assessment.

*Neurohormonal Alterations—Mammary Fibroadenomas*

Shipp et al. (2001a) state that mammary fibroadenomas in female Fischer 344 rats form spontaneously in response to age-related increases in prolactin levels and associated increases in progesterone and decreases in estrogen, leading to a sustained pseudopregnancy. These hormonal changes are said to produce a cell proliferative response in fibroblast cells in the mammary gland, which, if sustained as in pseudopregnancy, can lead to fibroadenomas. Although acrylamide was associated with a decrease in prolactin in male rats, these authors proposed that modification of dopamine levels at the level of the ovary by acrylamide could actually enhance progesterone levels—with subsequent enhancement of the spontaneous level of mammary fibroadenomas. These authors went on to note that fibroadenomas in human females result from increases in estrogen or decreases in progesterone—the opposite of the hormonal signals in rats. As a result, they suggested that the increase in mammary fibroadenomas in female Fischer 344 rats associated with acrylamide has no relevance to humans and should not be considered in any risk assessment.

*Neurohormonal Alterations—Astrocytomas*

Shipp et al. (2001a) stated that spontaneous astrocytomas may arise from nerve damage or neurotoxicity, hormone

stimulation (e.g., thyroid-stimulating hormone [TSH]), or be a response to neurotransmitters (e.g. dopamine). Although acrylamide is known to cause neuropathy, the two chronic bioassays in which a dose-related increase in astrocytomas was suggested did not find any neuropathy beyond that expected in the aging Fischer 344 rat. No evidence was cited to support acrylamide stimulated thyroid hormones. These authors stated that acrylamide does not alter brain dopamine levels, but that acrylamide may react with certain dopamine receptors. No information was cited to suggest that human astrocytes would have different dopamine receptors than the rat. The authors concluded that the incidence of astrocytomas should be considered in a human risk assessment.

*Neurohormonal Alterations—Thyroid Follicular Cell Tumors*

Several mechanism were considered by Shipp et al. (2001a) to explain the increase in thyroid follicular cell adenomas and adenocarcinomas in rats, including interfering with production of thyroid hormones or increasing their elimination. In turn, either reducing production or increasing elimination of thyroid hormones would stimulate the pituitary to produce more TSH and continuously stimulate the thyroid gland, leading to hyperplasia and tumor formation. Regardless of the mechanism, these authors suggested that the effect in humans would not be the same because of high affinity binding proteins which stabilize thyroid hormones in human blood. Rats do not have high-affinity binding proteins as evidenced by the half-life of thyroid hormone $T_4$, which is 1 day in the rat compared to 5 to 9 days in the human. The authors speculate on other mechanisms of action of acrylamide that may have relevance for humans, including enhancement of intracellular cyclic adenosine monophosphate (cAMP) levels. They note that such action would represent a likely threshold phenomenon, but that it could not be ruled out in humans. The authors concluded that the incidence of thyroid follicular cell tumors should be considered in a human risk assessment.

## Exposure Assessment

Dybing and Sanner (1997) assessed the exposure of humans to acrylamide from cosmetics. They assumed a level of Polyacrylamide in cosmetics of 2% and a maximum monomer level of 0.01% in the polymer. They addressed only dermal exposure, estimating daily exposure to acrylamide of 65 $\mu$g from leave-on products and 1.4 $\mu$g from rinse-off products.

To make this determination for leave-on products, twice-daily application of a cream applied at 1 mg/cm$^2$ over a body area of 19,400 cm$^2$ was assumed. The total of 38.8 g of cream was estimated to contain 2% of Polyacryamide, of which 0.01% would be acrylamide monomer, for a total daily exposure of 78 $\mu$g of acrylamide. Citing the unlikely application of cream to the total body surface twice daily and the unlikely use of a cream and lotion simultaneously, the authors assert that a reasonable total daily exposure to acrylamide would be 38 $\mu$g from creams and lotions.

Without equivalent analysis, the authors state that a hair setting product would mean exposure to 24 $\mu$g of acrylamide (on the day of use) and nail products would add a further 0.5 $\mu$g, for a grand total of 65 $\mu$g.

For rinse-off products such as shaving cream and soap, total daily uses of 2 g and 4.8 g, respectively, were used, with the assumption that only 10% would be left on the skin. Exposure to monomer done as above yielded a daily exposure of 1.4 $\mu$g acrylamide. Combining exposures from leave-on and rinse-off cosmetic products yielded a total of 66.4 $\mu$g/day acrylamide (Dybing and Sanner 1997).

For comparison purposes, for a 60-kg person, this estimated acrylamide exposure would be 1.1 $\mu$g/kg/day, or for a 70 kg-person, 0.95 $\mu$g/kg/day.

The European Cosmetic Toiletry and Perfumary Association (COLIPA) developed a different estimate of exposure (COLIPA 1999). Assuming that over 90% of products containing Polyacrylamide have concentrations of acrylamide of less than 1 ppm, and more than 75% have concentrations less than 0.4 ppm, the worst-case exposure to acrylamide was established as 0.33 $\mu$g/kg day$^{-1}$.

Shipp et al. (2001b) estimated the lifetime average daily dose (LADD) of acrylamide from use of nine specific personal care and grooming product categories containing Polyacrylamide. Based on the product categories for which use data were available, these nine categories were created from the FDA product categories as given in Table 10.

Shampoos were included because the original CIR Safety Assessment included a statement that Polyacrylamide was used as a foam builder and stabilizer in shampoos. Products in the "other eye makeup category" were considered unlikely to include products in the frequently used and separately identified categories of eye liner, mascara, eyebrow pencil, eye shadow, eye lotion, or eye makeup remover. The authors stated, therefore, that products in that FDA category are likely not frequently used and were not included. No information was available on the use of indoor tanning preparations, so these were not included.

The authors stated that products applied to the skin have a LADD, which is the fraction comprised of the percent of Polyacrylamide in the product, amount of acrylamide in the Polyacrylamide, amount of product used in 1 year, absorption fraction for the product, deposition fraction for the product, and years of exposure, divided by 75 $\times$ 365 days in a lifetime, and body weight. For all products applied directly to the skin, the deposition fraction is unity; for hair styling products, it may be less than unity. For products applied to the skin and rinsed off, a residue fraction was added to the numerator in place of the deposition fraction.

Because each of the variables above was not best represented by a single number, but rather by a distribution, Shipp et al. (2001b) used a Monte Carlo approach. For example, the amount of acrylamide residue in Polyacrylamide varies from a low of $<$1 ppm to 600 ppm. A lognormal distribution was developed that used a mean of 0.3 mg acrylamide per gram Polyacrylamide

**TABLE 10**
Product groups considered in the Shipp et al. (2001b) analysis compared to FDA categories reported as containing Polyacrylamide

| Product groups considered in the Monte Carlo analysis | FDA product categories reported to contain Polyacrylamide |
|---|---|
| 1. Hair conditioners | Hair conditioners |
| 2. Hair styling products | Tonic, dressings, and other hair-grooming aids |
| 3. Foundations | Foundations |
| 4. Deodorants | Deodorants (underarm) |
| 5. Cleansing products | Other personal cleanliness products |
| | Paste masks (mud packs) |
| | Other skin care preparations |
| | Skin cleansing creams, lotions, liquids, and pads |
| 6. After shave | After shave lotions |
| 7. Body lotions | Body and hand lotions, creams, etc. (excluding shaving preparations) |
| 8. Special purpose creams | Face and neck lotions, creams, etc. (excluding shaving preparations) |
| | Moisturizing creams, lotions, etc. |
| | Night Creams |
| 9. Shampoos | None reported |
| Not included | Other eye makeup preparations |
| Not included | Indoor tanning preparations |

(equivalent to 300 ppm or 0.03%), a minimum of 0.01 mg/g (10 ppm or 0.001%), and a maximum value of 1 mg/g (1000 ppm or 0.1%). The most current data reported by industry to FDA (FDA 1989) and the maximum value of 2.5% reported by COLIPA (SCCNFP 1999) were used to estimate the percentage of Polyacrylamide in a product. A lower boundary of 0.01% was chosen. This range of 0.01% to 2.5% is consistent with recent data (CTFA 2001) on concentration of use which ranged from 0.05% to 2.8%. The values were log-transformed for ease in handling the two order of magnitude range. Each of the nine products had an independent distribution of Polyacrylamide in the product.

Shipp et al. (2001b) collected information on the amount of product used per year was obtained from the AC Nielsen Household Panel market research survey.

According to Greenberg (2002), the following AC Nielsen household panel data are collected after each panelist shopping trip: date of purchase; for each Universal Product Code (UPC), the number of units, price paid, and deals used; age and sex of primary and secondary shopper; dealing—specified by the panelist as manufacturer coupon, store coupon, store sale, or other; store name; source of the coupon—at home, at the register, elsewhere in the store; usage of frequent shopper cards; total shopping trip purchase amount; complete item description through UPC dictionary; and method of payment—cash, check, credit card, or debit card.

These data are linked to the demographics of the household. The household panel is geographically dispersed and demographically balanced so that the sample profile matches the U.S. population. According to Greenberg (2002), the household panel data has a long-standing reputation in the marketplace and its use of hand-held bar code scanning has revolutionized household panel data collection.

In the market survey data provided by Shipp et al. (2001b), ~40,000 households scanned the UPC codes of all products purchased each week with an in-home scanner. The product types evaluated were matched with the nine product types and the following specific data collected: amount of product purchased, in 1-ounce intervals, over 2 years and the percentage of households at that interval; and for each product category, the number of ounces purchased over 2 years as a function of household size.

Shipp et al. (2001b) based the fraction of acrylamide absorbed on absorption studies of acrylamide in an aqueous solution (Sumner 1999) to avoid the possibility that studies of acrylamide in another solvent might overestimate the skin absorption. Because Sumner (1999) measured urinary metabolites, data from Sumner (2001) were used to back calculate the range of absorbed acrylamide from an aqueous solution: 1.16% to 7.56%.

Shipp et al. (2001b) based the residue fraction for rinse-off products on several pieces of data including residue of triclocarban from antimicrobial soap of 1.5%, triclosan from bar soap of 1%, and zinc pyridinethione from shampoo of 1% to 2%. An overall residue fraction was assumed to be a uniform distribution over the range from 1% to 2%.

The deposition fraction, as noted earlier, was unity for products applied directly to the skin. For hair styling products the amount of product left on the hands and scalp. It was assumed that the amount available for absorption would be in direct proportion of the surface area of the hair, hands and scalp. Hair surface area was determined based on the number of hair strands (90,000 to 140,000), an average hair diameter of 60 microns, and hair length (1 to 25 cm for males and 2 to 50 cm for females). Sex-specific deposition fractions distributions were developed.

Specific data on the number of years an individual might use a product over a lifetime were not available, although use in baby product categories has not been reported, suggesting that newborn is not the lower boundary. It was stated that most product types (deodorants, makeup, special purpose lotions, facial cleansing products, hair styling products, and after shaves) were consistent with usage beginning at teenage years and extending over some or all of life. Other product types (shampoos, hair conditioners, and body lotions) were consistent with usage beginning at age 3. Polyacrylamide is present in only a certain portion of products in each of the product categories, so even if the product type was used from age 3 onward, that product type would not always be one containing Polyacrylamide. A uniform

distribution was assumed with minimums of 0.01 years of use to either 63 or 73 years as above. A variation was used in which the use of shampoos was fixed at 73 years and the use of deodorants was fixed at 63 years.

Based on the distribution of possibilities for each of the factors, a Monte Carlo analysis was done and the total LADD median (50th percentile) of $62 \times 10^{-9}$ mg/kg day$^{-1}$, mean of $260 \times 10^{-9}$ mg/kg day$^{-1}$, and 90th percentile of $430 \times 10^{-9}$ mg/kg day$^{-1}$ for females was determined; separate total LADD values were determined for males (Shipp et al. 2001b). The LADD values determined for each sex and for each product type are given in Table 11.

## Dose-Response Analysis

Dybing and Sanner (1997) prepared a risk assessment of cosmetics containing acrylamide. They assumed a level of Polyacrylamide in cosmetics of 2% and a maximum monomer level of 0.01% in the polymer. They addressed only dermal exposure, estimating daily exposure to acrylamide of 65 $\mu$g from leave-on products and 1.4 $\mu$g from rinse-off products as described earlier.

**TABLE 11**
Absorbed LADD in mg/kg day $^{-1} \times 10^{-9}$ (Shipp et al. 2001b)

| Product | Median (50th percentile) | Mean | 90th percentile |
|---|---|---|---|
| | *Female* | | |
| Deodorants | 7.6 | 94 | 110 |
| | 19[a] | 190[a] | 230[a] |
| Shampoos | 0.41 | 3.8 | 5.2 |
| | 1.0[a] | 7.6[a] | 11[a] |
| Hair conditoners | 0.26 | 2.9 | 3.7 |
| Hair styling products | 0.15 | 2.1 | 2.4 |
| Cleansing products | 0.10 | 1.1 | 1.4 |
| Body lotions | 8.1 | 110 | 140 |
| Special purpose creams | 3.7 | 33 | 46 |
| Foundations | 0.87 | 8.3 | 11 |
| Total LADD for females | 62 | 260 | 430 |
| | 85[a] | 350[a] | 590[a] |
| | *Male* | | |
| Deodorants | 5.8 | 49 | 67 |
| | 15[a] | 97[a] | 140[a] |
| Shampoos | 0.39 | 3.5 | 4.8 |
| | 0.99[a] | 7.0[a] | 10[a] |
| Hair conditoners | 0.25 | 2.6 | 3.5 |
| Hair styling products | 0.23 | 2.4 | 3.1 |
| Cleansing products | 0.095 | 1.0 | 1.3 |
| Body lotions | 7.7 | 100 | 130 |
| After shave lotions | 4.3 | 39 | 53 |
| Total LADD for males | 50 | 200 | 340 |
| | 68[a] | 250[a] | 440[a] |

[a] Years of exposure fixed at 73 for shampoos and at 63 for deodorants.

Assuming that acrylamide is rapidly absorbed through the skin, 75% of applied acrylamide was estimated to be absorbed. For a 70-kg person, the absorbed dose would be 0.7 $\mu$g/kg day$^{-1}$. They note that this level is 500× lower than the no-observed-adverse-effect level (NOAEL) for neurotoxic effects and 5000× lower than the NOAEL for reproductive and developmental toxicity. The cancer risk included all cancers found in the two chronic bioassays in rats, and did not exclude those that may result from hormonal action.

These authors stated that a genotoxic mechanism cannot be ruled out and it may be that there is no threshold effect. Therefore, they used the Environmental Protection Agency (EPA) linear multistage model estimate of cancer risk of $2.8 \times 10^{-3}$ for a lifetime daily acrylamide dose of 1.0 $\mu$g/kg (EPA 1990). A lifelong risk of cancer from cosmetics containing Polyacrylamides, that would expose an individual to 0.7 $\mu$g/kg day$^{-1}$ acrylamide, was estimated to be $2 \times 10^{-3}$. This is 200× higher than the $1 \times 10^{-5}$ lifetime risk of cancer risk that the World Health Organization (WHO 1996) recommends that should not be exceeded for genotoxic agents (Dybing and Sanner 1997).

The COLIPA developed a different estimate of cancer risk (COLIPA 1999) using a worst-case exposure to acrylamide of 0.33 $\mu$g/kg day$^{-1}$ described earlier. These authors chose a 30% absorption of acrylamide through the skin, based on their analysis of the available data, leading to a final worst-case exposure of 0.1 $\mu$g/kg day$^{-1}$. Using benchmark-dose methodology applied to the two existing chronic bioassays (Johnson et al. 1986; Friedman et al. 1995), a $T_{25}$ of 3.6 mg/kg day$^{-1}$ was determined. As above, the $T_{25}$ is the chronic dose at which there would be a 25% increase of a specific tumor type (above the background rate) caused by a chemical. On the assumption that the stable intermediate, glycidamide, is responsible for any carcinogenic effect, the authors also scaled the cancer effect as a function of the lower metabolic rate in the human compared to the rat, yielding a factor of 0.28.

The risk calculation, therefore, becomes the daily exposure to acrylamide (0.1 $\mu$g/kg day$^{-1}$) times 0.25 increase in cancer above background divided by the $T_{25}$ dose (3.6 mg/kg day$^{-1}$), times a 0.28 scaling factor. The units cancel to yield an increase in cancer above background of $1.9 \times 10^{-6}$. The authors compared this with the $1 \times 10^{-5}$ lifetime cancer risk that the WHO (1996) recommends that should not be exceeded for genotoxic agents, concluding that even the worst-case scenario presents a risk lower than that acceptable level (COLIPA 1999).

The opinion of the SCCNFP was adopted in 1999 with the conclusion that the residual acrylamide monomer should be limited to less than 0.1 ppm in leave-on cosmetic products and less than 0.5 ppm in other cosmetic products (SCCNFP 1999).

In its evaluation of acrylamide residues in cosmetics, the SCCNFP cited the EPA estimate of cancer risk of $2.8 \times 10^{-3}$ for a lifelong daily dose of 1.0 $\mu$g/kg. Using an estimate of a daily absorbed dose of 0.1 $\mu$g/kg day$^{-1}$, they calculated the lifetime risk using this methodology would be $2.8 \times 10^{-4}$.

These authors expressed a preference for a newer EPA approach of establishing a benchmark dose ($LED_{10}$) which is the 95% lower confidence interval of the dose causing tumors in 10% of the animals as a starting point for linear extrapolation (EPA 1996). They also calculated the $T_{25}$ (the chronic dose at which there would be a 25% increase of a specific tumor type (above the background rate) caused by a chemical). A scaling factor was used to estimate human risk based on animal studies in both cases. Using the $T_{25}$ approach, a lifetime risk of cancer resulting from a daily exposure to 1.0 $\mu$g/kg was $1.3 \times 10^{-4}$. Using the $LED_{10}$ approach, the equivalent value is $0.83 \times 10^{-4}$.

They argued that there is a degree of consistency between all of the cancer risks (old EPA, new EPA, $T_{25}$) and concluded that the best estimate of worst-case lifetime cancer risk from acrylamide in Polyacrylamide-containing cosmetics was $1.0 \times 10^{-4}$. They compared this to the WHO acceptable risk level of $1.0 \times 10^{-5}$, and concluded that this would represent an unacceptably high risk. Therefore a limitation of residual acrylamide monomer to less than 0.1 ppm in leave-on cosmetic products and less than 0.5 ppm in other cosmetic products was suggested (SCCNFP 1999).

The CTFA (2000) provided an evaluation of the toxicology of Polyacrylamides for use in leave-on cosmetics with special reference to acrylamide monomer. Although noting that there were no data available on the use of such products in the United States, they cite the COLIPA data that estimated the upper limit of Polyacrylamide exposure to be 26.8 g per day. This was compared to a no-effect dose for humans of neurotoxicity of between 1.0 and 3.0 mg/kg $day^{-1}$ and to an absence of identifiable site-specific increase in cancer in workers exposed to a median exposure (in the high exposure group) of $\sim$5 $mg/m^3$ $years^{-1}$.

This evaluation went on to discuss the pharmacokinetics of acrylamide, its metabolism to glycidamide, and argued that the mutagenic action of acrylamide could be attributed to glycidamide. According to this analysis, skin penetration studies using excised animal or human skin overestimate the potential risk of acrylamide; first, because in vivo studies (Sumner et al. 1992, 1999) suggested that an upper bound of 14% of the dermally applied dose of acrylamide is absorbed and second because excised skin studies do not assess the biologically active dose which may only be 3%. Based on this consideration, it was suggested that the COLIPA (1999) estimate of worst case exposure of 0.1 $\mu$g/kg $day^{-1}$ should more properly be 0.01 $\mu$g/kg $day^{-1}$ (CTFA 2000).

Shipp et al. (2001a) considered three approaches: FDA method, EPA method, and $T_{25}$ method. As currently done by the FDA, a unit risk is created from a single dose in a chronic bioassay with an endpoint incidence that is significantly higher than the background rate. The level of the treatment agent that would produce a 1 in 1,000,000 risk level is then calculated. The unit risk for acrylamide based on follicular cell adenomas and adenocarcinomas from the Friedman et al. (1995) study was $\sim 1 \times 10^{-1}$ $(mg/kg/day)^{-1}$. The dose that would present a 1 in 1,000,000 risk of that end point was 0.01 $\mu$g/kg/day.

As recommended by the US EPA for chemicals that have a threshold or nonlinear mode of action, an MOE approach to cancer risk estimation should be used. A lower bound on dose at the specified level of risk, in this case 10% ($LED_{10}$), was developed from dose modeling of the thyroid follicular cell tumors and determined to be 0.19 mg/kg $day^{-1}$. The margin of exposure approach then considers the key event in the chemicals mode of action, extrapolation across species, the shape of the dose-response curve, and human variability.

Because the mode of action by which acrylamide may cause thyroid follicular cell tumors is unknown and only postulated to relate to intracellular cAMP levels, it may be that this key event occurs at levels lower than the doses at which a significant increase in tumors was seen. A factor of 10 lower was considered appropriate.

Because humans were considered less sensitive than rats for thyroid follicular cell tumors a conservative approach of unity in extrapolating from rats to humans was used. The dose-response curve is sufficiently shallow for this end point that it is not possible to accurately pinpoint the dose at which a threshold may exist. To reflect this uncertainty, another factor of 10 was applied. Human variability was not directly suggested, but it was noted that there are differences in thyroid hormone function among the population. An additional factor of 3 was applied to accommodate the most sensitive in the population. Overall a dose reduction of 300 was determined, which reduced the $LED_{10}$ from 0.19 mg/kg $day^{-1}$ to 0.63 $\mu$g/kg $day^{-1}$.

These authors also considered the approach used in the European Union in which the chronic dose ($T_{25}$) at which there would be a 25% increase of a specific tumor type (above the background rate) caused by a chemical. The $T_{25}$ for thyroid follicular cell tumors was estimated to be 2.46 mg/kg day (Shipp et al. 2001a).

Shipp et al. (2001b) used the LADD data developed from the Monte Carlo analysis of acrylamide intake from the use of personal products containing Polyacrylamide to estimate lifetime cancer risk. In this analysis, incidence data for astrocytomas or thyroid follicular cell adenomas and adenocarcinomas in male and female F344 rats.

Using the FDA method of calculating a unit risk, the authors stated, a departure dose is defined which is the lowest dose in the bioassay for which there is a significant increase in the incidence of the tumor compared to the control. The unit risk is simply the incidence rate minus the background rate divided by the departure dose (expressed in mg/kg $day^{-1}$).

The highest unit risk was for thyroid follicular cell adenomas and adenocarcinomas in males at 0.1 per mg/kg $day^{-1}$. The authors state that the dose corresponding to an extra lifetime risk of $1 \times 10^{-6}$ (one in a million) would be 0.01 $\mu$g/kg $day^{-1}$. Were a $1 \times 10^{-5}$ risk level acceptable, as the WHO recommends should not be exceeded for genotoxic agents, the corresponding dose would be 0.1 $\mu$g/kg $day^{-1}$. Using the unit risk developed via the FDA method, the lifetime cancer risks based on the absorbed

LADD data were calculated. All values were below the $1 \times 10^{-6}$ criteria.

Using the EPA MOE approach applied to a $LED_{10}$ of 0.2 mg/kg day$^{-1}$, the MOE was determined to be 300 and the dose to be 0.67 $\mu$g/kg day$^{-1}$. The ratios of the absorbed LADD values to the MOE dose were also calculated and were below unity, interpreted by the authors that all LADD values were below the target risk level. In addition to the MOE approach, the authors also made a linear extrapolation from the $LED_{10}$ to a target risk of $1 \times 10^{-5}$ (value of $2 \times 10^{-5}$ mg/kg day$^{-1}$) and again calculated the ratio of the absorbed LADD values to that dose. All ratios were below unity, again interpreted by the authors that all LADD values were below the target risk level.

The authors also used the European Union $T_{25}$ method to calculate the smallest dose that would result in a 25% increase in a specific tumor, determining that this would be 2.46 mg/kg day$^{-1}$ for thyroid follicular adenomas and adenocarcinomas. Using that value as the point of departure and doing a linear extrapolation, a lifetime dose of $2.98 \times 10^{-5}$ mg/kg day$^{-1}$ was determined to correspond to a $1 \times 10^{-5}$ lifetime cancer risk. Again calculating the ratio of the absorbed LADD values to this dose, all ratios were below unity, again interpreted by the authors that all LADD values were below the target risk level.

Potential sources of error in the development of the absorbed LADD values were discussed. Because there are no uses of Polyacrylamide in shampoos, currently reported by industry, including shampoos, could be in error causing the LADD values to be an overestimate. In determining a distribution of acrylamide monomer levels in Polyacrylamide, the authors used an upper bound of 1000 ppm. The most recent data collected indicate an upper bound of 600 ppm. Use of the 1000 ppm value, if in error, would overestimate the LADD values. The assumption was made that an individual would use a product type that contained Polyacrylamide at some point in their life.

Because the proportion of products in a product type that contain Polyacrylamide is small, it may be that an individual could use that product type over a lifetime and never use one containing Polyacrylamide. If assumption of sometime use of product with Polyacrylamide is in error, the LADD values would be overestimated. The Nielsen survey data included data for households of five or more individuals. For purposes of the calculation, this number was assumed to always be five. If the number were actually seven for a given household and there was a corresponding higher purchasing of relevant products, then dividing by five would erroneously overestimate the individual use and consequently the LADD values. The authors also commented that the absorption of acrylamide monomer is based on assumptions that are not testable at this time and therefore may lead to either an over- or underestimate of LADD.

Finally, the authors commented on the interpretation of the 90th percentile LADD values. The 90th percentile represents a frequent use of products. That high usage would have to increase by another factor of 100 to reach the target risk level of $1 \times 10^{-5}$. Purchase and use of 100× more that that high use was considered unlikely (Shipp et al. 2001b).

On the basis of the hazard and exposure assessment, SNF (2001b) concluded that the safety of Polyacrylamide in cosmetics could be adequately assured if this ingredient was used at concentrations in the product that did not result in acrylamide levels >2 ppm in leave-on products or >5 ppm for rinse-off products.

### Other Safety Assessments

The European Commission's Scientific Committee for Toxicity, Exotoxicity, and the Environment (CSTEE) calculated the excess cancer risk to be $4.5 \times 10^{-3}$. Given the uncertainties of the risk estimates, however, they stated that the carcinogenic risk for consumers may be lower. They concluded that, with regard to the inherent toxic properties of acrylamide (neurotoxicity, genotoxicity to both somatic and germ cells, carcinogenicity, and reproductive toxicity) the exposure to humans should be kept as low as possible (CSTEE 2001).

The European Commission's Scientific Committee on Food (SCF) dismissed a risk assessment approach citing that (1) it is rarely known which model correctly reflects a chemical's mode of action; (2) there is no agreement among regulatory bodies regarding which model should be used; and (3) the risk estimate depends critically on which model and can vary significantly. This group concluded that acrylamide levels in food should be as low as reasonably achievable (SCF 2002).

The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) of Australia prepared an assessment of acrylamide (NICNAS 2002). Public health risks from ingestion of acrylamide in water and food and from dermal absorption from cosmetic products were considered to be negligible. They cite a 0.0003 mg/kg day$^{-1}$ dermally absorbed level of acrylamide from cosmetic products containing Polyacrylamide and note that no tumors of any sort were found at an oral dose of 0.1 mg/kg day$^{-1}$ in chronic bioassays in rats, which is a 300-fold margin of safety (NICNAS 2002).

## SUMMARY

Polyacrylamide is a polymer of controllable molecular weight formed from the polymerization of acrylamide monomers. Polyacrylamide is available in one of three forms: solid (powder or microbeads), aqueous solution, or inverse emulsions (Polyacrylamide in water droplets coated with surfactant and suspended in mineral oil). Residual acrylamide monomer is likely an impurity in most Polyacrylamide preparations, but there is a wide range from <1 ppm to 600 ppm with higher levels in the solid form compared to the other two forms.

The functions of Polyacrylamide in cosmetics listed in the *International Cosmetic Ingredient Dictionary and Handbook* include binder, film former, and hair fixative. Other sources have stated that it is used as a foam builder and stabilizer in shampoo products and as a vehicle in sunscreen preparations and to

impart lubricity and emolliency to soaps, moisturizing lotions, hand and body lotions, and shaving cream. Polyacrylamide is reportedly used in 110 cosmetic formulations, at concentrations ranging from 0.05% to 2.8%. Residual levels of acrylamide in Polyacrylamide can range from <0.01% to 0.1%, although representative levels were reported between 0.02% to 0.03%.

Other uses of Polyacrylamide include use as a biomedical polymer, as a flocculent in waste water treatment and food processing, as a gel material for separating biomolecules, and in imprinting drug capsules. Use in foods, drugs, and devices is regulated by the FDA, with restrictions on the amount of Polyacrylamide that can be used, and the acrylamide residue in either the polymer or in the final product is restricted.

Because of the large sizes of Polyacrylamide polymers, absorption of the compound does not occur. Acrylamide monomer residues, however, may penetrate the skin. The extent of skin penetration in studies of excised skin or in vivo using radioactive tracers varied more than 10-fold.

Polyacrylamide itself is not significantly toxic. For example, an acute oral toxicity study of Polyacrylamide in rats reported that a single maximum oral dose of 4.0 g/kg body weight was tolerated. In a subchronic oral toxicity study in both rats and dogs, animals were given Polyacrylamide at a maximum dose of 464 mg/kg body weight, with no signs of toxicity in any animals. Several 2-year chronic oral toxicity studies in rats and dogs fed diets containing up to 5% Polyacrylamide had no significant adverse effects, including behavior or appearance.

Cutaneous tolerance tests performed to evaluate the irritation of 5% (*w/w*) Polyacrylamide indicated that the compound was well tolerated. Skin irritation tests of Polyacrylamide applied to the intact and scarified skin of rabbits showed that the compound was the least irritating of all the compounds tested and tolerance to the compound was rated as good to very good.

Undiluted Polyacrylamide applied to the conjunctival sac of the rabbit caused a very slight conjunctival response, indicated by prominent capillaries. At 24 h post treatment, the conjunctival sac was normal. In an ocular irritation test of Polyacrylamide on rabbits, using fluorescein to evaluate the extent of surface damage and corneal opacity, 5% (*w/w*) Polyacrylamide did not provoke significant injury to ocular mucous membranes.

No compound-related lesions were noted in a three-generation reproductive study in which rats were fed 500 or 2000 ppm Polyacrylamide in their diet. Acrylamide tested in a two-generation reproductive study at concentrations up to 5 mg/kg day$^{-1}$ in their drinking water was associated with prenatal lethality at the highest dose, with evidence of parental toxicity. The no adverse effects level was close to the 0.5 mg/kg day$^{-1}$ dose. Acrylamide tested in an NTP reproductive and neurotoxicity study at 3, 10, and 30 ppm in a continuous breeding protocol in mice produced no toxicity in development and female reproduction, but did produce significant toxicity as measured by dominant lethality in male reproduction and minimal neurotoxicity.

Acrylamide neurotoxicity occurs in both the central and peripheral nervous systems. Studies of mechanism have focused on microtubule disruption, which can affect neuronal intracellular transport. Microtubule disruption has been suggested as a possible mechanism for genotoxic effects of acrylamide in mammalian systems.

Polyacrylamide was not tested in any genotoxicity test systems, but acrylamide was tested in bacterial and yeast test systems (with almost uniformly finding no genotoxicity), fruit flies (mixed results), mammalian in vitro assays (genotoxicity found), and mammalian in vivo assays (genotoxicity found as a function of sperm cycle). Studies attempting to elucidate the mechanism of acrylamide genotoxicity in mammalian cells have focused on glycidamide, a metabolic intermediate, as the active agent.

Polyacrylamide in several chronic animal studies did not produce any evidence of carcinogenesis.

Acrylamide was a tumor initiator, but not an initiator/promoter, in three different mouse strains, except for one strain at a total dose of 300 mg/kg (six doses over 2 weeks) in which acrylamide alone increased lung adenomas and carcinomas without promotion. Acrylamide was tested in two chronic bioassays using rats. Increases in mammary gland tumors, glial cell tumors, thyroid gland follicular tumors, oral tissue tumors, uterine tumors, and clitoral gland tumors were associated with acrylamide exposure in female rats. In male rats, tumor increases were seen in the CNS, thyroid gland, and scrotum, associated with acrylamide exposure. In the second study, using higher doses and a larger number of female rats, glial cell tumors were not increased, nor was there an increase in mammary gland, oral tissue, clitoral gland, or uterine tumors. Tumors of the scrotum in male rats were confirmed, as were the thyroid gland follicular tumors in males and females. Taken together, there was a dose-dependent, but not statistically significant, increase in the number of astrocytomas.

Various risk assessments have examined the human risk that may be suggested by the chronic bioassays. In the hazard assessment phase, some assume all the tumors represent a health risk and combine incidences, others evaluated, and eliminated, some of the rat tumors as not relevant to humans. In the exposure assessment phase, some approaches assumed application to a large portion of the body at a single quantity of cosmetic per unit area with a single Polyacrylamide concentration and a single acrylamide residue level. Another treated each of these factors as best represented by a distribution and combined them using a Monte Carlo analysis. Dose-response analysis was done using linear extrapolations in some analyses, but most presented the result of using linear extrapolations and nonlinear or threshold of action extrapolations. In no case was there a suggestion that cosmetics containing Polyacrylamide present any risk of reproductive toxicity or neurotoxicity as a result of acrylamide residues. Different human cancer risk predictions have resulted, however, including a lifetime risk of cancer of $2 \times 10^{-3}$, $2.8 \times 10^{-4}$, $1.3 \times 10^{-4}$, $1.0 \times 10^{-4}$, $1.9 \times 10^{-6}$. In another approach, the absorbed levels of acyrlamide resulting from the Monte Carlo calculation were compared to the dose extrapolated (linear and nonlinear) to produce a target risk level of $1 \times 10^{-5}$ and found

all modeled exposures to be well below any of the extrapolated doses.

The most extensive epidemiological assessment of workers exposed to acrylamide at Polyacrylamide production chemical plants involved 8508 workers over the time period of 1925 to 1994, with the findings that there was no increase in mortality that could be attributed to CNS tumors, thyroid or other endocrine cancer, or testicular cancer. There was a nonsignificant increase in mortality from cancer of the respiratory system. The statistical power of the study, as extensive as it was, was insufficient to detect small, but important increases in these tumors.

In addition to the safety assessment done by the scientific committee responsible for cosmetic ingredient assessment in Europe, the scientific committee on toxicology, ecotoxicology, and environmental toxicity and the scientific committee on food have each reached conclusions—all different.

For cosmetics there was a concern about increased cancer and a limitation of 0.1 ppm for leave-on cosmetic products and 0.5 ppm for other cosmetic products was recommended. For the environment, recognizing neurotoxicity, genotoxicity, reproductive toxicty, and carcinogenicity associated with acrylamide, the exposure to humans was recommended as low as possible. For food, the recommendation was as low as reasonably achievable.

In Australia, a risk assessment suggested negligable health risks from acrylamide in cosmetics.

## DISCUSSION

In 1990, the CIR Expert Panel completed a safety assessment of Polyacrylamide, with the conclusion that Polyacrylamide, with less than 0.01% acrylamide monomer content, is safe as a cosmetic ingredient as currently used. Since that time, the results of a large number of studies of acrylamide have become available.

The Panel noted that Polyacrylamide, as currently supplied to cosmetic product manufacturers, has a molecular weight range from 8 to 20 million. Such large molecules do not penetrate the skin. This is confirmed in absorption studies in which cosmetic formulations containing Polyacrylamide were not readily absorbed. The Panel considered that in the available safety test studies, Polyacrylamide itself is not toxic in acute, subchronic or chronic oral studies; in dermal and ocular irritation studies, in a three-generation reproductive study, or in occupational studies of workers exposed to Polyacrylamide dust. Therefore, Polyacrylamide itself is considered safe as used in cosmetic formulations.

The Panel recognized, however, that acrylamide monomer, a residual of the polymerization that results in the formation of Polyacrylamide, is present in any Polyacrylamide material used in cosmetics. The Panel also noted that the level of acrylamide as a percentage of Polyacrylamide is not a constant. It varies as a function of the form in which Polyacrylamide is supplied and ranges from <0.01% to 0.1%, with representative levels at 0.02% to 0.03%.

In its consideration of the available safety data, the Panel acknowledged that acrylamide is a demonstrated neurotoxin in humans and a possible carcinogen in animal tests. The Panel considers that the neurotoxicity of acrylamide is at high exposure levels and that such levels could not be attained by use of cosmetics. The results of two animal carcinogenesis studies have been considered by the Panel in detail, along with several risk assessment analyses based primarily on these two studies.

The Panel considered that the incidences of four tumor types were increased in rats exposed to acrylamide in the two studies. Although there are mechanisms of action of acrylamide that have been proposed for these tumor types that suggest they may be unique to the rat, the Panel was not convinced that these results could be disregarded as a species-specific finding with no relevance to human health and safety. The Panel did note that the postulated mechanisms for these different tumor types are consistent with a nongenotoxic mechanism.

Most of the genotoxicity produced by acrylamide is chromosomal damage at high dose levels. This effect is more consistent with a mechanism of microtubule damage resulting from covalent binding, as has been postulated for neurotoxic effects of acrylamide. It was acknowledged that the epoxide derivative of acrylamide is capable of reacting with DNA. For such a mechanism to be relevant, it would require cellular metabolism of acrylamide to the epoxide derivative. Yet there are no reports of tumors in liver or kidney tissue capable of producing the metabolite. In addition, on reviewing the available genotoxicity data, the Panel commented that forward and reverse mutations, typically seen with a genotoxic chemical, were not found. Based on the genotoxicity and carcinogenicity data, the Panel does not believe that acrylamide is a genotoxic carcinogen in the usual manner.

The consequence of not considering acrylamide as a typical genotoxic carcinogen impacted on the Panel's consideration of the various carcinogenicity risk assessment approaches that have been proposed. All such approaches make assumptions that the mechanism of action is that of a classic genotoxic carcinogen in order to extrapolate from the doses used in animal studies to low doses appropriate to human exposures. The Panel considers it likely, therefore, that the approaches used have overestimated the carcinogenicity risk of low doses.

The Panel also considered that data are now available on the presence of acrylamide in certain foods cooked at high temperatures. These levels appear to result in human exposures to acrylamide that are higher than those that could occur as a result of cosmetic use. If the increase in cancer risk suggested by several of the risk assessments were correct, the consequences of acrylamide in food should be easily linked to a rise in cancer incidence but no such evidence appears to exist.

Although risk assessment approaches were not feasible given the factors discussed above, the Panel did conclude that it was appropriate to limit the acrylamide levels in cosmetics. The

available chronic animal study that used 5% Polyacrylamide with 0.01% acrylamide residue was considered the highest dose at which no carcinogenic effect was seen. The resulting 5 ppm of acrylamide was established as the upper limit of acrylamide residue in cosmetic formulations.

## CONCLUSION

The CIR Expert Panel concluded that Polyacrylamide is safe as a cosmetic ingredient in the practices of use and concentrations described in this safety assessment, if the level of acrylamide monomer in formulation is not greater than 5 ppm.

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