NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN

ACRYLAMIDE

March 2005

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

PREFACE

On January 1, 1990, acrylamide was listed as a known carcinogen under Proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986, Health and Safety Code section 25249.5 *et seq.*) The No Significant Risk Level (NSRL) for acrylamide of 0.2 μg/day was also established in regulation in 1990 (Title 22, California Code of Regs. section 12705(c))[1].

Historically, toxicity concerns over acrylamide centered on worker health and safety, primarily for neurological, male reproductive and cancer effects. However, in 2002 it was discovered that acrylamide can form during the cooking of starchy foods at high temperatures. This unexpected discovery shifted the concern for health risks to the public from acrylamide in the diet. Since 2002, acrylamide has been discovered in many plant-based foods that have been baked or fried at high temperatures.

Cancer now occurs in nearly one out of every four individuals. While the underlying cause of many cancer cases is unclear, numerous epidemiological studies have shown that dietary factors affect an individual's cancer risk. The World Health Organization has estimated that about 30 percent of cancer cases worldwide are associated with dietary factors. Characterization of carcinogens in the diet is complicated by the complex and varied nature of the food humans consume, and is far from complete. Some dietary factors that have been associated with increased cancer risk include high caloric intake and increased consumption of processed meats and red meat. Other dietary factors have been associated with decreased cancer risk; these include increased consumption of fruits and vegetables and increased consumption of dietary fiber. In addition, some specific carcinogenic compounds present in the diet have been identified, such as those formed during the high temperature cooking of meats (e.g., benzo[a]pyrene and PhIP). Acrylamide is yet another carcinogen recently recognized to be formed as a result of cooking at high temperatures, although in this case, formation occurs in certain plant-based foods. Given the typical daily intake of acrylamide from the diet, it is plausible that dietary acrylamide contributes to the rate of cancer observed in the population.

After the discovery of acrylamide in many foods, the Office of Environmental Health Hazard Assessment (OEHHA), the lead agency for the Proposition 65, was requested to provide additional guidance on the applicability of Proposition 65 regulations to acrylamide in foods. In response to these requests OEHHA held a public workshop on May 12, 2003 to explore regulatory options. Subsequently on August 1, 2003, OEHHA released a draft acrylamide work plan for developing regulations. One of the proposed activities in the draft work plan was to update the NSRL for acrylamide. The draft work plan was released for public comment (August 1 – September 26, 2003), and a consultation on the draft work plan was held with the Carcinogen Identification Committee (CIC) at their October 17, 2003 public meeting. OEHHA considered all the public input and the Committee advice in finalizing the acrylamide work plan, which was released on March 12, 2004.

---

[1] Lifetime exposure at the no significant risk level is calculated to result in one excess cancer in an exposed population of 100,000 (Title 22, California Code of Regulations section 12703(b)).

As the work plan notes, additional scientific data relevant to the cancer dose-response of acrylamide have been published since the adoption of the NSRL in 1990. These data were reviewed for the update of the NSRL. In addition, the CIC and public advised OEHHA to take into account several factors in updating the acrylamide NSRL, such as how people may differ in their susceptibility to cancer from acrylamide. These factors have also been addressed in updating the NSRL, as documented in this report. The proposed NSRL for acrylamide is 1.0 μg/day.

Acrylamide is a carcinogen, producing tumors at multiple sites in rats and mice. In female rats acrylamide produced tumors of the mammary gland, thyroid, central nervous system, oral cavity, uterus and clitoral gland. In male rats acrylamide produced tumors of the thyroid, testis, and central nervous system. In studies of female mice examining only the lung and skin, acrylamide produced lung and skin tumors. In studies of male mice examining only the lung, acrylamide produced lung tumors. The general public is exposed primarily through cigarette smoke and certain foods that have been cooked at high-temperature. Occupational exposures occur mainly from its use as a polymerizing agent in grouts and cements, and to produce polyacrylamide. The ability of acrylamide to produce cancer in animals, and the applicability of animal findings to humans is well recognized by scientists in the United States and throughout the world. The World Health Organization recognizes "the presence of acrylamide in food as a major concern in humans based on the ability to induce cancer and heritable mutations in laboratory animals." The International Agency for Research on Cancer and the U.S. Environmental Protection Agency consider acrylamide to be a probable human carcinogen. The National Toxicology Program considers acrylamide as "reasonably anticipated to be a human carcinogen." The U.S. Food and Drug Administration considers acrylamide to be a potential human carcinogen. The National Institute for Occupational Safety and Health considers acrylamide to be an occupational carcinogen.

Acrylamide is genotoxic. It damages DNA and causes mutations in human cells. Cancer is seen in animals exposed over their lifetimes to acrylamide orally. After eating food containing acrylamide, the chemical is taken up by the body and distributed to tissues in the body. For these reasons dietary exposures are considered to pose a cancer risk to humans.

The available human cancer studies conducted to date are insufficient to determine the level of cancer risk from acrylamide. Consequently, OEHHA, as well as other scientific authorities rely on animal cancer data to estimate risk to humans. In developing the proposed NSRL for acrylamide, OEHHA used cancer data from all the key animal cancer studies available to date. Differences in the way humans and animals absorb, distribute and metabolize acrylamide were also taken into account in developing the proposed NSRL.

Table of Contents

SUMMARY OF FINDINGS

The cancer potency of acrylamide was estimated from dose-response data of multiple acrylamide-responding tumor sites observed in four long-term drinking water studies, two in male rats and two in female rats (Johnson *et al.*, 1986; Friedman *et al.*, 1995). These tumor sites were the mammary gland, thyroid, central nervous system, oral cavity, uterus and clitoral gland in female rats, and thyroid, testis (mesothelioma), and central nervous system in male rats. Epidemiological data are inadequate for characterizing the dose-response relationship for acrylamide, although there is one study (Marsh *et al.,* 1999) that provides a basis for checking the quantitative consistency of cancer potency derived from the animal data.

Acrylamide and its epoxide metabolite glycidamide are genotoxic. While the carcinogenic mode of action of acrylamide is not understood, a genotoxic mechanism is likely. Some data suggest multiple mechanisms might be operative including non-genotoxic mechanisms, although the evidence is fairly limited at this point. Thus, a low-dose linear approach to dose-response characterization was taken. Cancer potency was derived by applying a linearized multistage model to the animal cancer bioassay data. Since acrylamide induced tumors at multiple sites in male and female rats, a combined cancer potency estimate was derived for the acrylamide treatment-related cancer sites observed in each experiment using Monte Carlo analysis. There are species differences in pharmacokinetics, with greater conversion of acrylamide to glycidamide in rats than humans, and longer half lives of acrylamide and glycidamide in humans than in rats. These factors are taken into account in deriving human cancer potency from the animal potency. Species differences in pharmacodynamics are also taken into account in deriving human cancer potency. Sources of interindividual variation in sensitivity to acrylamide-induced cancer, including differences in pharmacokinetics arising from genetic variability in the enzymes involved in activation and detoxification, variability due to age sensitivities, and variability due to co-exposures to other carcinogens or promoters are discussed. Since the rat cancer bioassays did not include early-in-life exposures, variability due to early-life susceptibility is not accounted for by potency estimates based on these studies. No specific adjustments to the acrylamide cancer potency estimates were made to address inter-individual variability. The use of the default inter-species pharmacodynamics factor may or may not be adequate to account for both interspecies differences in pharmacodynamics and inter-individual human variability in response. Thus, the cancer potency derived may not be adequately protective of children and other sensitive groups.

For each of the treatment-related tumor sites observed in the four rat studies, a probability distribution of cancer potency estimates was derived using likelihood theory. The linear term (q_1) of the multistage model fit to dose-response data for a given site represents the cancer potency for that site. For each tumor site judged to be associated with acrylamide treatment, this assessment derived a distribution of estimates corresponding to the 0.1 to 99.9 percentiles of q_1. The goal of the analysis was to determine a composite cancer potency for all sites affected by acrylamide in a given experiment so that the total risk of cancer from acrylamide exposure could be derived. Thus, for each experiment, distributions of q_1 for each tumor site were added using Monte Carlo techniques to obtain a single combined distribution, representing cancer potency for all selected sites affected by acrylamide in each rat study. The four combined (multisite) potency distributions were further analyzed through Monte Carlo techniques to obtain an overall geometric mean of potencies from the four studies. The upper 95 percent confidence bound of the geometric mean distribution of cancer potencies was taken as the cancer potency for

acrylamide. Thus, the cancer potency for acrylamide is based on cancer incidence data from the four available oral cancer studies in rats.

The cancer potency estimate takes into account the difference between humans and rats in the conversion of acrylamide to glycidamide, the primary DNA-reactive metabolite of acrylamide. The cancer potency includes a default interspecies extrapolation factor to account for potential differences in pharmacodynamic responses between rats and humans. Cancer potency estimates based on the rat bioassays were found to be four-fold lower than a cancer potency estimate based on pancreatic tumors observed among acrylamide-exposed workers (Marsh *et al.*, 1999), which provides a measure of the quantitative consistency and plausibility of the rat-derived estimates.

The Proposition 65 "no significant risk level" (NSRL) is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The cancer potency estimate and corresponding NSRL are given in Table 1. This value is more than 10,000 times lower than no-observable-effects levels (NOELs) derived for non-cancer endpoints (i.e., neurotoxicity, reproductive toxicity), according to NOELs reported by other public health institutions. Thus, cancer is believed to be the most sensitive toxic endpoint for acrylamide.

Table 1. Cancer potency and NSRL for acrylamide.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (μg/day)
Acrylamide	0.70	1.0

INTRODUCTION

This report describes the derivation of a cancer potency value and NSRL for acrylamide (CAS number 79-06-1, molecular weight 71.1). "Acrylamide" was listed on January 1, 1990 as known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq.*).

Acrylamide is a multisite carcinogen. In mice, in studies examining only the lung and skin, acrylamide induced lung and/or skin tumors (Bull *et al.*, 1984a,b; Robinson *et al.*, 1986). In female rats acrylamide induced tumors of the mammary gland, thyroid, central nervous system, oral cavity, uterus and clitoral gland and, in male rats, tumors of the thyroid, testis (mesothelioma), and central nervous system (Johnson *et al.*, 1986; Friedman *et al.*, 1995). In addition to inducing cancers of the rat testes, acrylamide caused male reproductive toxicity, affecting male fertility in mice and rats, and causing heritable genetic mutations in male mice (CERHR, 2004a,b). Additionally, the central nervous system is a target not only for acrylamide-induced tumorigenicity, but also neurotoxicity. Human occupational studies and animal experiments have demonstrated acrylamide to be a potent neurotoxicant (CERHR, 2004; U.S. EPA, 1991). This document addresses in detail the dose response relationship for cancer effects, but not for the other toxicity endpoints. It presents a cancer dose-response assessment, characterizing the carcinogenic potential of acrylamide and deriving an exposure level below which significant carcinogenic risk is considered by OEHHA to be minimal.

Acrylamide is used as a polymerizing agent in grouts and cements, and to produce polyacrylamide. Polyacrylamide is used widely as a flocculating agent in water treatment processes and is used commonly in biological laboratories as a chromatographic medium to separate DNA and other macromolecules. Thus, worker exposures have occurred as a result of these uses (Calleman, 1996). The general population is also exposed to acrylamide since it is present in cigarette smoke, and it is present in many human foods as a result of high-temperature cooking (Tareke *et al.*, 2002; U.S. FDA, 2004a; 2004b; Dybing *et al.*, 2005).

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

Acrylamide caused statistically significant increases in the incidence of tumors in male and female rats (Johnson *et al.*, 1986, Friedman *et al.*, 1995) and male and female mice (Bull *et al.*, 1984b, Bull *et al.*, 1984a; Robinson *et al.*, 1986). In rats, acrylamide induced tumors at multiple sites in both males and females (see Tables 2-5). In mice, acrylamide induced tumors of the lung in both sexes (Bull *et al.*, 1984a,b; Robinson *et al.*, 1986). Cancer studies in mice were of specialized design (e.g., skin papilloma/lung adenoma protocol [Robinson *et al.*, 1986]), limited exposure and study duration, and histopathological evaluation (only lung and skin tissue were examined) and therefore were not as reliable as the rat studies for dose-response evaluation. Because of their limited duration and histopathology, it is not known if acrylamide also causes tumors at sites other than the lung and skin in mice. Standard two-year bioassays of acrylamide in mice are needed to address this data gap.[2]

For this NSRL assessment, cancer potency estimates were derived from carcinogenicity studies in rats. The mouse studies were not used because of their more limited study design and the greater uncertainty associated with the extrapolation of studies of short duration and specialized study design. However, the apparent sensitivity of mice in the specialized studies and other factors (e.g., efficient epoxide formation *in vivo* [Paulsson *et al.*, 2002]) suggest that mice may be as, if not more, sensitive than the rat to the carcinogenic effects of acrylamide. Our understanding of the potential for carcinogenesis in humans may evolve with the development of additional cancer bioassay in the mouse, related mechanistic data and epidemiological study.

In rats, the carcinogenicity of acrylamide has been investigated in four drinking water studies, two in male Fischer 344 rats receiving acrylamide in the drinking water for 104 weeks, and two in female F344 rats receiving acrylamide in drinking water for 104 weeks, the first published as Johnson *et al.* (1986), and the second as Friedman *et al.* (1995).

The first set of cancer studies (Johnson *et al.*, 1986) employed multiple dose groups, followed good laboratory practices, and included a full histopathological evaluation of suspected tumorigenic lesions. A review, sponsored by American Cyanamid, of the first set of chronic drinking water studies raised issues regarding the background incidence of central nervous system and oral tumors in male control animals; the dose-response relationships for male testicular mesotheliomas, central nervous system tumors and combined tumors were

[2] The U.S. Food and Drug Administration, in conjunction with the National Toxicology Program, is planning to conduct a series of standard two-year bioassays in the mouse, as well as in the rat (U.S. FDA, 2004b).

characterized as atypical; and the possible confounding by a sialodacryoadenitis virus (Friedman *et al.*, 1995). The industry sponsored a second set of drinking water studies to address some of the issues raised in the industry review and to further characterize dose-response relationships for risk assessment purposes. Thus, a second set of chronic drinking water studies was initiated.

U.S. FDA audited the second set of studies, which was conducted at Tegeris Laboratories in Maryland and later published as Friedman *et al.* (1995). The U.S. FDA auditors found a number of potential deficiencies in study conduct and reporting (U.S. FDA, 1996; 1998; Van Gemert, 2001). These deficiencies included problems with the environmental controls in the facility, problems in data reporting, and indications that the rats may have received less acrylamide than reported. Another deficiency for cancer potency estimation noted by OEHHA was the limited histopathological examination of tissues of the central nervous system (see below). The sponsors of the studies provided responses to the auditor's criticisms, suggesting that the dosing and conduct of the studies were adequate (Van Gemert, 2001). The study sponsors noted that many of the concerns over data reporting arose from the fact that the Tegeris Laboratory had ceased to operate and that U.S. FDA had audited only a portion of the records that had been transferred to another company. OEHHA notes that while some of the limitations may have lowered the study sensitivity, and provide a bias toward underestimation of the cancer potency, overall the study employed larger groups of animals and extended the dose range for female rats (Johnson *et al*. (1986) dosed groups of female rats at 0, 0.01, 0.1, 0.5, or 2 mg/kg-day; Friedman *et al*. (1995) dosed groups of female rats at 0, 1 or 3 mg/kg-day).

Another consideration in study selection is the degree of correspondence between the findings of the two sets of studies. In the females both studies found increases in tumors of the mammary gland, central nervous system (marginal significance in Friedman *et al.*, 1995) and thyroid, with the earlier Johnson *et al.* study more sensitive for mammary and central nervous system and less sensitive for thyroid carcinogenesis. In addition, oral cavity, uterine and clitoral gland tumors were significantly increased in the earlier but not the latter study. With regard to findings in male F344 rats, in both studies the thyroid and testes were target sites for carcinogenesis, with slightly greater sensitivity in the thyroid in the later study and the converse for the testis. In addition, an increase in central nervous system tumors of marginal statistical significance was observed in the later study in males. OEHHA notes the overall consistent findings in terms of tumor type and cancer potency observed between the Johnson *et al.* (1986) and the Friedman *et al.* (1995) studies. OEHHA concludes that, on balance given the strengths and weaknesses noted here and elsewhere, both sets of studies are adequate for potency estimation. Neither study is clearly superior. Since the studies used the same rat strain, and no one sex consistently appeared to be more sensitive than the other, all four studies are used as the basis of potency estimation. (See section "Dose-Response Assessment" below).

In addition to the animal bioassays, occupational and dietary epidemiological studies have been published that provide some quantitative information on acrylamide exposure and cancer response (Mucci *et al.*, 2003; 2004; Bosetti *et al.*, 2002; Pelucchi *et al.*, 2003). Because of methodological deficiencies, the dietary studies are unsuitable for risk assessment (Hagmar and Tornqvist, 2003; Dybing and Sanner, 2003; Erdreich and Friedman, 2004; Koehler, 2004). While the occupational studies are not of sufficient power or reliability (Erdreich and Friedman, 2004; Koehler, 2004), they potentially can be used to derive an upper bound estimate on cancer potency (OEHHA, 2003). The most reliable epidemiological study for this purpose is the study of acrylamide-exposed workers by Marsh *et al.* (1999).

Study Descriptions and Cancer Dose-Response data

Johnson et al. (1986)

In the first set of drinking water studies, Johnson *et al.* (1986) treated male and female F344 rats with acrylamide in the drinking water at doses of 0, 0.01, 0.1, 0.5 or 2.0 mg/kg body weight per day for two years. Over the first 18 months on study, there were no significant dose-related decreases in mortality or body weight among the male or female rats, except for a slight decrease (less than four percent) in the mean body weight of the high-dose males, indicating that the tumor data were not complicated by overt toxicity. Some rats appeared to contract a viral infection starting about day 210 of the study, which affected all dose groups and controls. No mortality resulted from the infection, and the authors concluded that the results of the study were not confounded by the occurrence.

Table 2. Tumors in female F344 rats receiving acrylamide in drinking water for two years in the Johnson *et al.* (1986) study

Tissue/tumor type	Acrylamide, mg/kg-day					
	Control	**0.01**	**0.1**	**0.5**	**2.0**	**trend**[e]
Mammary gland[a]						
adenocarcinoma	2/58	1/58	1/52	2/55	6/57	p=0.0045
adenoma, fibroma or adenocarcinoma	10/60	12/60	10/60	20/58[b]	28/60[b]	p=0.0009
Central nervous system[a]						
glial tumors	1/60	2/59	1/60	0/60	7/60[b]	p=0.0004
Thyroid gland[a]						
follicular cell adenoma or adenocarcinoma	1/54	0/55	1/50	1/54	5/50[b]	p=0.0008
Oral cavity[a]						
squamous cell papilloma or carcinoma	0/60	3/60	2/60	3/60	8/60[b]	p=0.0008
Uterus[a]						
adenocarcinoma	1/56	2/56	1/51	0/55	5/49[c]	p=0.0045
Clitoral gland (gross lesions)[d]						
adenoma or carcinoma	0/60	1/60	3/60	4/60	5/61[b]	p=0.02

[a] Tumor incidences were tabulated from individual animal data by Dearfield *et al.* (1988). The numbers of animals at risk (i.e., the denominators) are based on the number of animals alive at the appearance of first tumor for that tumor type.

[b] Significantly greater than controls, $p \leq 0.05$

[c] Significant after adjustment for early mortality, $p \leq 0.05$ (Johnson *et al.*, 1986).

[d] Only animals having gross lesions were examined histologically. Thus, incidence data reported may underestimate tumor response in this tissue.

[e] Mantel-Haenszel trend test

Johnson *et al.*, 1986 reported dose-related increases in the tumor incidences of mammary gland, central nervous system, thyroid gland, oral cavity, uterus and clitoral gland among acrylamide-treated females (Table 2). Among male rats, treatment-related increases in tumors of the testes and thyroid gland were observed (Table 3). Additionally, an increased incidence of benign pheochromocytoma of the adrenal gland was observed among male rats. However the benign

tumors were not significantly associated with exposure by trend test, and the combined incidence of benign and malignant pheochromocytoma was not statistically significantly different in dosed groups compared to controls. Adrenal tumors were therefore not included in the cancer potency estimations.

Table 3. Tumors in male F344 rats receiving acrylamide in drinking water for two years in the Johnson *et al.* (1986) study

	Acrylamide, mg/kg-day					
Tissue/tumor type	**Control**	**0.01**	**0.1**	**0.5**	**2.0**	**trend**[c]
Thyroid gland[a] follicular cell adenoma	1/57	0/53	2/57	1/53	7/54[b]	p=0.0001
Testis[a] tunic mesothelioma	3/57	0/50	7/57	11/53[b]	10/54[b]	p=0.0057

[a] Tumor incidences were tabulated from individual animal data by Dearfield *et al.* (1988). The numbers of animals at risk (i.e., the denominators) are based on the number of animals alive at the appearance of first tumor for that tumor type.

[b] Significantly greater than controls, $p \leq 0.05$

[c] Mantel-Haenszel trend test

Friedman et al. (1995)

The second set of drinking water studies of acrylamide, also in F344 rats, was designed to extend the earlier findings of Johnson *et al.* (1986) and "to clarify the carcinogenicity of acrylamide and provide adequate information for risk assessments" (Friedman *et al.*, 1995). The authors employed two control groups to assess the variability in reported background rates.

Groups of male rats were administered acrylamide via drinking water for two years at doses of 0 (control 1), 0 (control 2), 0.1, 0.5 or 2.0 mg/kg-d (Friedman *et al.*, 1995). The numbers of male animals per group were 102, 102, 204, 102 and 75, respectively. Groups of female rats were given acrylamide via drinking water for two years at doses of 0, 0, 1.0 or 3.0 mg/kg-d. The numbers of female rats per group were 50, 50, 100 and 100, respectively. The mean body weights of the high-dose male and female rats were slightly lower (about two to nine percent over the course of the experiment) than the control animals; these differences were statistically significant. There were no apparent dose-related differences in survival among the treated groups until after the 14th month in males and the 23rd month in females, well after the appearance of the first tumors at 12 months (Friedman *et al.*, 1995).

In the Friedman *et al.* (1995) studies, tumor incidences in the two control groups were essentially the same and were combined for analyses here (Tables 4 and 5). Specifically, incidences of testicular mesothelioma, glial tumors, and thyroid follicular cell tumors were identical in the two control groups of male rats, and incidences of glial tumors and thyroid follicular cell tumors were

identical in the two control groups in female rats, and the incidences of mammary tumors were similar (8 % versus 14 %) in the two control groups of female rats.

Dose-related increases in tumors of the mammary gland and thyroid were observed among acrylamide-treated female rats (Friedman *et al.*, 1995) (Table 4).

Table 4. Tumors in female F344 rats receiving acrylamide in drinking water for 104 weeks in the Friedman *et al.* (1995) study

	Acrylamide, mg/kg-day			
Tissue/tumor type	**Control[a]**	**1.0**	**3.0**	**trend[d]**
Mammary gland adenocarcinoma or fibroadenoma	11/96	21/94	30/95[b]	p=0005
Central nervous system glial tumors	0/100	2/100[c]	3/100[c]	p=0.06
Thyroid gland follicular cell adenoma or adenocarcinoma	2/100	10/100[b]	23/100[b]	p<0.0001

[a] Two concurrent control groups were combined since tumor rates observed in both control groups were similar.

[b] Significantly greater than controls, $p \leq 0.05$

[c] The number of animals at risk is based on the number of animals examined histopathologically for brain tumors. However, only a fraction of the spinal cord samples were examined microscopically; thus, the tumor incidences may be underestimates.

[d] Mantel-Haenszel trend test

Dose-related increases in tumors of the thyroid and testes were observed among acrylamide-treated male rats (Table 5). Additionally, there were increases in the incidences of glial tumors of the central nervous system, which were marginally significant by trend test (p=0.06) for both the male and female rats (Tables 4 and 5).

Table 5. Tumors in male F344 rats receiving acrylamide in drinking water for 104 weeks (Friedman *et al.*, 1995)

Tissue/tumor type	Acrylamide, mg/kg-day				
	Control[a]	0.1	0.5	2.0	trend[d]
Central nervous system glial tumors	2/204	2/98[b]	1/50[b]	3/75[b]	p=0.06
Thyroid gland follicular cell adenoma or adenocarcinoma	6/202	12/203	5/101	17/75[c]	p<0.0001
Testis tunic mesothelioma	8/204	9/204	8/102	13/75[c]	p<0.0001

[a] Two concurrent control groups were combined, since tumor rates observed in both control groups were similar.

[b] The number of animals at risk is based on the number of animals examined histopathologically for brain tumors; less than half the animals were examined for these tumors in the low- and mid-dose groups. In addition, of the animals examined for brain tumors, only a fraction of the spinal cord samples were examined microscopically; thus, the tumor incidences may be underestimates.

[c] Significantly greater than controls, $p \leq 0.001$

[d] Mantel-Haenszel trend test

Friedman *et al.* (1995) stated that a major objective of their study "was to investigate whether glial tumors in the Johnson *et al.* study were significant." However, in some of the acrylamide-treated male groups, less than half of the brain and spinal cord tissues were examined histopathologically for glial tumors. In the female 1.0 mg/kg-d dose group, only one-fifth of the spinal cord samples were examined microscopically. The incomplete histopathology of the spinal cord is surprising since 10 of the 34 glial cell tumors from the Johnson *et al.* (1986) study occurred in the spinal cord (Shipp *et al.*, 2001). Since central nervous system tumors were observed among female rats in the Johnson *et al.* (1986) studies, and since there was incomplete histopathology performed by Friedman *et al.* (1995), the marginally significant increases in glial tumors among male and female rats in the Friedman *et al.* (1995) studies were taken by OEHHA to be treatment-related.

Damjanov and Friedman (1998) reanalyzed the pathology slides of the testicular mesotheliomas observed in the male rats and concluded that the morphology of the tumors did not differ between the control and treated groups. The authors suggested that the mesotheliomas may be

benign, while noting that standard practice in veterinary pathology is to classify such tumors as malignant.

Friedman *et al.* (1995) and NICNAS (2002) noted that the incidence of the acrylamide-induced mammary tumors in female F344 rats in both sets of studies were within the range of historical background rates observed for control female F344 rats in two-year carcinogenesis studies conducted by the National Toxicology Program. However, no data were presented in Johnson *et al.* (1986) or Friedman *et al.* (1995) on the historical rates for the laboratories where the studies were conducted. The most relevant historical control comparison would consist of studies conducted within the same time frame of the study under consideration, in the same laboratory, by the same route, in the same species, strain and gender, with the same source of animals (Haseman, 1995). The most appropriate control group is the concurrent control group, although the historical controls can provide further insight on the significance of the study findings. The incidence rates for mammary tumors in the control animals in the acrylamide studies in female F344 rats are within the range of historical background rates reported by the National Toxicology Program, but again, a better comparison would be with the more relevant laboratory controls. The consistent findings of significantly elevated rates of mammary tumors in both long-term rat studies (Johnson *et al.*, 1986; Friedman *et al.*, 1995) as well as the consistent background rate of tumors observed in the two control groups employed by Friedman *et al.* (1995), leads to the conclusion that the mammary tumors are treatment related and not incidental.

Cancer studies in humans

The largest epidemiological cohort of acrylamide-exposed workers, that by Marsh *et al.* (1999), provides potentially useful quantitative information for evaluating an upper bound on the potential cancer risks of acrylamide in humans. This can then be compared to the estimates derived from animal bioassays as a "reality check" (OEHHA, 2003). Marsh *et al.* (1999) reported the follow-up mortality experience of 8508 acrylamide exposed workers in the U.S through 1994. An earlier follow-up of this cohort through 1983 had been reported by Collins *et al.* (1989).

Marsh *et al.* (1999) and others (Granath *et al.*, 2001; Erdreich and Friedman, 2004) noted that the study had low statistical power to detect an association at most cancer sites, except the lung. Calleman (1996) had previously calculated that the earlier follow-up study (Collins *et al.*, 1989) likewise had limited power to detect an acrylamide cancer response, assuming rodent tumor responses are predictive of human risk. Thus the lack of findings of increased cancer risk at particular sites in the cohort studied by Marsh *et al*. (1999) and Collins *et al*. (1989) does not indicate a lack of sensitivity at those sites to acrylamide carcinogenesis.

Marsh *et al.* (1999) reported a statistically significant association between cumulative exposure and risk of pancreatic cancer among highly exposed workers relative to workers in the lowest exposure category, but the authors noted that no clear dose-response relationship was observed (Table 6A). Since there were very few cases in the mid-dose groups, providing unstable risk estimates, Schulz *et al.* (2001) noted that using fewer exposure cut-points yielded a monotonically increasing dose-response relationship (Table 6B). In addition to cumulative exposure, pancreatic cancer risk was also significantly associated with mean intensity of exposure, duration of exposure and time-since first exposure in univariate regression analyses (Marsh *et al.*, 1999).

Table 6A. Pancreatic cancer mortality among acrylamide exposed workers in the U.S. (Marsh *et al.*, 1999)

Dose range ($mg\text{-}yr/m^3$)	Dose mean[a] ($mg\text{-}yr/m^3$)	Cases	SMR	Person-years	Ratio[b] ($Dose_{air}/Dose_{food}$)
<0.001	0	30	0.80	196431	--
0.001 - <0.03	0.0126	3	2.77	13286	0.057
0.03 - <0.30	0.119	2	0.73	24449	0.54
≥ 0.30	2.69	9	2.26[c]	22819	12

SMR, standard mortality ratio, based upon local county comparisons.

[a] Estimated assuming a lognormal distribution of occupational exposures

[b] Ratio of acrylamide dose mean in occupational air to acrylamide dose mean in food (estimated by OEHHA) over the same period. Equivalence across inhalation and oral dose routes was assumed. Dietary intake at the estimated mean current U.S. dietary exposure level (30 μg/day; DiNovi and Howard, 2004) for 50 years results in a cumulative exposure of 548 mg acrylamide. Cumulative exposure to workers exposed to acrylamide via inhalation in units of $mg\text{-}yr/m^3$ was converted to mg by assuming an inhalation rate of 10 m^3/workday and 250 workdays per year.

[c] Statistically different from workers in the lowest exposure category, $p<0.05$.

Table 6B. Pancreatic cancer mortality among acrylamide exposed workers in the U.S. Reanalysis of Marsh *et al.* (1999) by Schulz *et al.* (2001)[a]

Dose range ($mg/m^3/yr$)	Dose mean[b] ($mg/m^3/yr$)	Cases	SMR	Person-years
<0.001	0	30	0.80	196431
0.001 - <0.30	0.12	5	1.31	37735
≥ 0.30	2.7	9	2.26[c]	22819

SMR, standard mortality ratio, based upon local county comparisons.

[a] Schulz *et al.* (2001) noted that the number of cases in the two lower exposure groups in the analysis by Marsh *et al.* (1999) were small (two to three cases) (see Table 6A), providing unstable estimates. Schulz *et al.* (2001) therefore combined results from these two groups.

[b] Mean exposure was estimated assuming a lognormal distribution of occupational exposures.

[c] Statistically different from workers in the lowest exposure category, $p<0.05$.

The findings of pancreatic cancer among this cohort of acrylamide-exposed are merely suggestive of an association at best for the following reasons:

1. The statistical power of the study is low, as discussed above.

2. The smoking status of the workers in this cohort could not be adequately ascertained. Cigarette smoking is associated with about a 1.5- to 3-fold increase in pancreatic cancer (Marsh *et al.*, 1999; IARC, 2002; PHS, 2004). Moreover, cigarette smoke contains acrylamide. Indeed, daily intake of acrylamide among smokers is estimated to be more than triple that of non-smokers (Bergmark, 1997). Thus, smoking status is a potential confounder in this study.

   Marsh *et al.* (1999) performed covariate regression analyses where two variables were simultaneously modeled, "cumulative exposure" and "time-since first exposure," or, separately, "mean intensity of exposure" and "time-since first exposure." Inclusion of "time-since first exposure" in the models reduced the relative risk estimates for both "cumulative exposure" and "mean intensity of exposure." The authors stated that these modeling results "suggest potential confounding with time since first exposure to acrylamide and with a history of smoking." However, the authors did not present data that "time-since first exposure" and smoking history were related. Also, as the authors pointed out, the variables "cumulative exposure" and "time-since-first exposure" are likely to be correlated. Their inclusion in the same model almost certainly biases the findings towards non-significance.

3. Since all individuals are exposed to acrylamide, as evident by high background levels in blood (Schettgen *et al.*, 2002; 2003; Hagmar *et al.*, 2005), and acrylamide is wide-spread in food (Robie and DiNovi, 2003; DiNovi and Howard, 2004), the referent workers in the lowest exposure category are not truly unexposed. Indeed, as shown in Table 6A, only workers exposed at the highest exposure category are expected to have had cumulative exposures that significantly exceeded the cumulative exposure via the diet. The workers studied in the Marsh *et al.* (1999) cohort and whose mortality experience is shown in Tables 6A and 6B were hired between 1950 and 1973 and followed through 1994. Assuming a starting age of 18 years and an average date of hire of about 1962, the cohort was roughly 50 years of age on average in 1994. U.S. FDA researchers estimate that people in the U.S. ingest an average of 30 μg/d (DiNovi and Howard, 2004). Intake at this rate for 50 years would result in a cumulative dietary exposure of 548 mg. Table 6A compares the ratio of the mean cumulative acrylamide dose in air to the mean cumulative acrylamide dose from food. The ratios indicate that cumulative exposure to acrylamide from the diet is higher than the cumulative exposures received from occupational inhalation in all but the highest exposure category. Thus, it is unlikely that this occupational study would be able to detect increases in cancer risks in any but the highest exposure category.

Recent case-control studies were published which examined potential associations of bowel, kidney and bladder cancer (Mucci *et al.,* 2003) or renal cell cancer (Mucci *et al.*, 2004) and acrylamide intake, as determined by a food frequency questionnaire and reported levels of acrylamide measured in specific food items. These studies are not informative for several reasons.

First, they have very limited power to detect cancer associations (Hagmar and Tornqvist, 2003; Dybing and Sanner, 2003; Erdreich and Friedman, 2004; Koehler, 2004). Second, there is almost certainly considerable exposure misclassification. Specifically, the range of acrylamide intake among non-smokers from the 5th percentile to the 95th percentile is likely to be only about four-fold, based on the (lognormal) distribution of intake inferred from hemoglobin adduct data for acrylamide reported in biomonitoring studies (Schettgen *et al.*, 2002; 2003). Similarly, Hagmar *et al.* (2005) reported that median Hb adduct levels of acrylamide among non-smoking women who consumed low acrylamide diets were indistinguishable from women consuming high acrylamide diets, and median adduct levels among non-smoking men who consumed low acrylamide diets were only 1.4-fold higher than men who consumed high acrylamide diets. Of the 70 individuals tested, the highest acrylamide adduct level was only five-fold higher than the lowest adduct level (Hagmar *et al.*, 2005). In contrast to the biomarker data, the range of acrylamide intake estimated from food frequency questionnaires in the Mucci *et al.* studies was greater than 40-fold. The wide range of acrylamide intake estimates in the Mucci *et al.* studies suggest significant misclassification of exposure, as the likely range of acrylamide intake of the population (based on the more precise, biomonitoring data) is very narrow. The large variability in acrylamide levels within a given type of food, the high percentage of foods in the diet containing acrylamide, and the variability in food preparation methods and food consumption rates among the population suggest that surveys of dietary recall do not provide an accurate measure of acrylamide intake. Non-differential misclassification of exposure, as is likely occurring in these case-control studies, biases the risk measures toward null values (Kelsey *et al.*, 1986).

Third, daily intake estimates based on biomarker data are two- to three- fold higher than intake estimates based on food consumption surveys (Tareke *et al.*, 2002; DiNovi and Howard, 2004). This observation suggests that estimates of acrylamide intake from food frequency questionnaire data are inadequate, and that there may be significant endogenous or other non-food sources of acrylamide. Finally, the tumor sites studied in the Mucci *et al*. studies, namely the large bowel, kidney and bladder, would not necessarily be expected to be target sites for acrylamide. The widespread distribution of acrylamide and its DNA-reactive metabolite, glycidamide, in the human body and the relatively long half-life of acrylamide in humans (about seven hours, Calleman, 1996; Sorgel *et al.*, 2002) provide little insight as to which tissues might be target tumor sites in humans. The occupational cohort study of Marsh *et al.* (1999) suggests the pancreas as a target tissue, although this finding may be confounded by smoking. Studies in rats suggest the testis, thyroid, mammary and central nervous system as possible target sites, and studies in mice suggest the lung and skin as potential targets. Based on current understanding of the pharmacokinetics and the mechanism of action of acrylamide, there is no basis to predict site concordance between rodents and humans. Thus, the negative findings in the dietary exposure studies by Mucci *et al.* (2003, 2004) do not diminish the level of concern over the potential of acrylamide to cause cancer and the studies cannot be used to calculate upper bound estimates of cancer potency, especially given the degree of misclassification bias expected to be present in the studies.

Other recent case-control studies examining cancer rates and consumption of various fried foods, such as fried potatoes, have reported mixed results (Bosetti *et al.*, 2002; Pelucchi *et al.*, 2003). These studies are likewise not informative with respect to the carcinogenicity of acrylamide since any single food, such as fried potatoes, accounts for only a small fraction of an individual's overall dietary exposure (DiNovi and Howard, 2004). These studies also suffer from similar

limitations in statistical power to detect cancer associations as do the Mucci *et al.* (2003, 2004) studies.

APPROACH TO DOSE-RESPONSE ANALYSIS

The approach used to derive the dose-response relationship for acrylamide is described in detail in the Appendix. This section describes the rationale for the method adopted. It begins with a discussion of the mechanistic data and the implications of these data for the shape of the dose-response curve. It then considers differences in pharmacokinetics in rats and humans to take into account in extrapolating cancer potency derived from animal data to humans. The metabolism and excretion of acrylamide and its epoxide metabolite glycidamide involve pharmacokinetic pathways known to be genetically variable in the human population. This section briefly considers the potential for inter-individual variability in response to acrylamide exposure. In addition, exposures to acrylamide occur *in utero* and during infancy and childhood, and the potential for age-dependent sensitivity are also discussed. After accounting for differences in pharmacokinetics, this assessment considers species differences in pharmacodynamics. The fact that the animal studies did not include exposures *in utero* and early after birth is considered in evaluating the pharmacodynamic differences. Finally, since acrylamide induced tumors at multiple sites in male and female rats, a combined cancer potency estimate was derived for the acrylamide treatment-related cancer sites using Monte Carlo analysis.

Shape of the Dose-response Curve

This section reviews the available data related to the proposed mechanisms of carcinogenesis. The carcinogenic mode of action of acrylamide is not well understood, although there are data to indicate that a genotoxic mechanism is likely and that suggest multiple mechanisms may be operative including non-genotoxic mechanisms. There is considerable, ongoing research that may further add to our knowledge of acrylamide's carcinogenic mode of action.

While the carcinogenic mechanism of action of acrylamide remains unknown, and several mechanisms that could account for the tumor findings have been hypothesized, mechanisms involving genotoxicity are the most likely. As summarized by the WHO (2002): "Acrylamide is genotoxic *in vivo* in somatic cells and germ cells, and is known to be metabolized to glycidamide, a chemically reactive epoxide that forms DNA adducts. The finding that acrylamide induces tumours at a number of different sites in both rats and mice is consistent with a genotoxic mode of action of the chemical. The existence of adducts in experimental systems is supportive of a genotoxic mechanism of carcinogenesis of acrylamide. While suggestions have been made that additional modes of action might contribute to the observed spectrum of tumours seen in acrylamide-treated rats, especially tumours of hormone responsive tissues, these suggestions are speculative only."

OEHHA concurs with the WHO characterization and outlines the evidence on the mechanisms of carcinogenesis briefly below. In doing so OEHHA notes that several unpublished industry reports have reviewed the mechanisms of tumor formation in acrylamide-treated rats. Separate documents addressing acrylamide-induced tumors of the mammary gland, thyroid, central nervous system, and testis in the rat have concluded that each of these tumors is either not relevant to humans or occurs through a threshold mechanism of action (KS Crump Group, Inc., 1999a, 1999b, 2000a, 2000b; Shipp *et al.*, 2001; 2002). OEHHA has carefully reviewed these

unpublished reviews and finds, as did the WHO (2002) report, that the evidence for non-genotoxic mechanisms is unconvincing. In all cases, a genotoxic mode of action could explain the tumor formation as well as the hypothesized mechanisms described in the unpublished reports.

Moreover, OEHHA does not expect or presume site concordance among rats and humans, since acrylamide and glycidamide are widely distributed to all tissues and dose accumulation does not appear to correspond to sites of tumor formation. Indeed, site concordance is not seen between mice and rats – of the few tissues examined in mouse experiments, tumors arose in the mouse lung and skin, and such tumors were not found in the rat.

The mechanisms hypothesized for acrylamide carcinogenesis include (1) a genotoxic mode of action through direct binding to DNA, (2) indirect or protein-mediated DNA damage, and (3) alteration of hormones or other growth factors. The evidence for these is briefly discussed here.

1) Genotoxicity through direct reaction with DNA

The genotoxicity of acrylamide has been extensively reviewed elsewhere (Dearfield *et al.*, 1988; 1995; IARC, 1994; WHO, 2002; European Union, 2002), and additional recent studies have been published (Segerback *et al.*, 1995; Generoso *et al.*, 1996; Park *et al.*, 2002; Paulsson *et al.*, 2002, 2003; Abramsson-Zetterberg, 2003; Besaratinia and Pfeifer, 2003, 2004; Gamboa de Costa *et al.*, 2003; Baum *et al.*, 2005; Doerge *et al.*, 2005a; 2005b; Ghanenayem *et al.*, 2005; Glatt *et al.*, 2005; Husøy *et al.*, 2005; Johansson *et al.*, 2005; Puppel *et al.*, 2005; Robinson *et al.*, 2005; Silvari *et al.*, 2005). Acrylamide administered to rodents induces primarily clastogenic effects, including chromosomal aberrations, micronuclei formation, translocations, dominant-lethal effects, spindle disturbances and cell transformation. Acrylamide was a clear tumor initiator in several classic tumor "initiation-promotion" studies in mice (Bull *et al.*, 1984b; Bull *et al.*, 1984a; Robinson *et al.*, 1986).

Acrylamide does not induce gene mutations in standard bacterial assays; however, glycidamide, the primary DNA-reactive metabolite of acrylamide, does induce mutations in *Salmonella* strains TA100 and TA1535 (Dearfield *et al.*, 1995), which detect base-pair substitution mutations. Weakly positive responses for mutation have been observed among acrylamide-treated transgenic mice in a *lacZ* reporter gene (Myhr, 1991; Hoorn *et al.*, 1993) and in mammalian cells *in vitro* (Besaratinia and Pfeifer, 2003; Granath and Tornqvist, 2003). In studies examining the specific locations of DNA adducts formed in the *p53* gene of cultured human bronchial epithelial cells and the *cII* transgene of cultured Big Blue mouse embryonic fibroblasts following *in vitro* exposure to acrylamide or glycidamide, both compounds produced similar patterns of DNA adduct formation (Beseratinia and Pfeifer, 2004). These authors found that at any given dose, the mutagenicity of glycidamide was greater than that of acrylamide in both the human and mouse cells. These investigators concluded that the mutagenicity of acrylamide was largely due to the mutagenic activity of its epoxide metabolite, glycidamide. Beseratinia and Pfeifer (2004) also demonstrated that the mutational spectra formed by glycidamide were statistically different than the spontaneous mutational spectra observed in control cells.

Several very recent genotoxicity studies provide additional evidence that acrylamide and glycidamide induce clastogenic effects and mutations in mammalian cells *in vitro* (Baum *et al.*, 2005; Glatt *et al.*, 2005; Johansson *et al.*, 2005; Manière *et al.*, 2005; Puppel *et al.*, 2005) and *in vivo* in rats (Manière *et al.*, 2005) and mice (Husøy *et al.*, 2005). Additionally, Silvari *et al.* (2005) studied reaction-kinetic studies of glycidamide and ethylene oxide, and concluded that

glycidamide has about a seven-fold higher mutagenic potential in mammalian cells than the well-studied genotoxic carcinogen ethylene oxide.

Available data suggest that a genotoxic mode of action involving direct binding to DNA could be operative for each of the tumor types observed in rodents treated with acrylamide. Glycidamide DNA adducts have been measured in all rodent tissues so examined (Carlson and Weaver, 1985; Segerback *et al.*, 1995; Gamboa da Costa *et al.*, 2003; Doerge *et al.*, 2005b; Manière *et al.*, 2005), including the target tissues thyroid gland, mammary gland, brain and testis in rats. At least five DNA adducts of glycidamide have been characterized (Solomon, 1999; Gamboa de Costa *et al.*, 2003). In terms of dose-response, DNA damage (micronuclei) in polychromatic erythrocytes among mice was linearly correlated to the administered dose of acrylamide (Paulsson *et al.*, 2002) and the internal dose of glycidamide (Paulsson *et al.*, 2003). Similarly, Abramsson-Zetterberg (2003) observed linear formation of micronuclei in polychromatic erythrocytes over a wide range of i.p. doses (1 to 100 mg/kg) in mice. Manière *et al.* (2005) reported dose-related increases in DNA damage (Comet assay) in the brain, testis and other tissues of rats following treatment with single high doses of acrylamide. To date, this is the only study that has examined the dose-response relationships for DNA damage in any of the target tissues for acrylamide carcinogenesis. Additional research to identify and characterize acrylamide's genotoxic effects in tissues that are targets for acrylamide carcinogenicity (e.g., testis, mammary gland, thyroid, and central nervous system for rats and lung for mice) following repeated low doses of acrylamide and glycidamide in rodents could improve risk estimation. Researchers have reported initial findings in mice treated with acrylamide showing DNA-adduct formation in lung (a target tissue for carcinogenesis) and liver at low doses (0, 1, 10 and 50 mg/kg) (Doerge, 2004). Over the dose range tested, the shape of the dose-response curve was supralinear; that is, more DNA adducts were produced per unit dose at lower doses than were produced at higher doses (Doerge, 2004). In the lower dose range the shape of the dose-response curve would be expected to be linear. Additionally, increases in liver DNA adducts were observed among mice fed diets (NIH-31IR autoclaved) representing very low dietary doses of acrylamide (approximately 0.039 mg/kg per day) (Twaddle *et al.* 2004). Concentrations of acrylamide in the NIH-31IR autoclaved diet (240 ppb) are lower than typically found in some human foods such as potato chips and French fries.

Ethylene oxide, a genotoxic carcinogen structurally similar to glycidamide, produced peritoneal mesothelioma and brain glioma in F344 rats treated by inhalation (Snellings *et al.*, 1984; Lynch *et al.*, 1984). These tumor observations from studies of ethylene oxide provide additional support for the hypothesis that the tunic mesotheliomas of the testis and the gliomas of the brain and spinal cord induced in F344 rats by acrylamide arise through a genotoxic mechanism.

2) Genotoxicity through indirect or protein-mediated DNA damage

There is some evidence to suggest that acrylamide or glycidamide also induces DNA damage indirectly through protein binding or receptor-mediated processes (Dearfield *et al.*, 1995; Park *et al.*, 2002). For example, acrylamide binds with high affinity to microtubules in cells from the brain and spinal cord *in vitro*; however, binding to microtubules *in vivo* could not be confirmed (Carrington *et al.*, 1991). Binding to histones or mitotic-spindle proteins would be consistent with observations that acrylamide disrupts mitosis and induces aneuploidy *in vitro* (Dearfield *et al.*, 1995). Also, glycidamide binding to protamines correlates well with the sensitivity of germ cells to the formation of dominant lethal mutations or heritable translocations, suggesting a protein-mediated effect (Generoso *et al.*, 1996; Favor and Shelby, 2005). Recent data in

CYP2E1-knockout and wild-type mice clearly show that epoxidation of acrylamide to glycidamide is necessary for the formation of germ-cell mutations (Ghanayem *et al.*, 2005). Sickles *et al.* (1995, 1996) demonstrated that acrylamide could arrest mitosis of cultured cells by inhibiting kinesin, a protein important for proper migration of the microtubules during cell division. Inhibition of kinesin may represent a mechanism of aneuploidy or other clastogenic effects. However, others observed that acrylamide does not significantly inhibit kinesin when kinesin is bound to other macromolecules as is typically found under cellular conditions, questioning whether this mechanism is operative *in vivo* and at low doses (Martenson *et al.*, 1995).

Separately, Park *et al.* (2002) suggested a potential carcinogenic mechanism involving glutathione depletion. They suggested that acrylamide binding to glutathione might alter the redox status of the cell, which can affect apoptosis, cell proliferation and transformation. However, repeated acrylamide treatment at high doses (50 mg/kg) reduced glutathione stores in the brain by only 20 % (Srivastava *et al.*, 1986). It is unlikely that this mechanism would be operative at lower doses.

3) Alteration of hormones or other growth factors

Some of the acrylamide-induced tumors in rats arose in hormonally responsive tissues, namely the thyroid gland, mammary gland, uterus, clitoral gland, testis, and central nervous system. Alterations in hormone or growth factor levels might be an underlying mechanism of acrylamide-induced cancer. There is evidence that moderately high doses of acrylamide (≥ 20 mg/kg-d) significantly reduced blood concentrations of prolactin and testosterone in male rats (Ali *et al.*, 1983). Increased concentrations of prolactin, if sustained, result in increased proliferation of fibroblast cells in the mammary gland and may represent a mechanism of induction of mammary fibroadenomas in female rats. However, since acrylamide did not alter prolactin levels in the blood or pituitary gland of female rats administered acrylamide up to 15 mg/kg per day (Khan *et al.*, 1999), this does not appear to be a viable mechanism for the observed acrylamide-induced mammary tumors.

Acrylamide administered to rats at doses of 5 mg/kg or higher significantly increased dopamine receptor binding levels in some regions of the brain (especially in the striatum) but not others (namely the frontal cortex, cerebellum and medulla) (Agrawal *et al.*, 1981a; Agrawal *et al.*, 1981b; Bondy *et al.*, 1981). However, acrylamide administered to rats at doses of 10 mg/kg or higher only slightly decreased dopamine levels in the frontal cortex, but not the striatum or other regions of the brain (Ali *et al.*, 1983; Agrawal *et al.*, 1981b). Dopamine receptors in certain tissues are linked with various hormonal control systems, and theoretically represent a pathway through which acrylamide-induced alterations in dopamine receptor levels could affect cell cycle control and differentiation in the brain, mammary gland and other tissues. An unpublished industry study reported a correlation between brain regions high in dopamine D2 receptors and sites within the central nervous system where acrylamide-induced astrocytomas were observed in the rat bioassays of Johnson *et al.* (1986) (as reported by Shipp *et al.*, 2002). Acrylamide appeared to up-regulate dopamine receptors in the striatum following doses of ≥5.0 mg/kg while not affecting dopamine concentrations in the brain at higher doses (Ali *et al.*, 1983; Agrawal *et al.*, 1981b; Bondy *et al.*, 1981). Reserpine, a carcinogen that lowers dopamine levels as well as other catecholamines, induces adrenal tumors in the F344 rat, but not brain or mammary gland tumors (NCI, 1982). More research is needed to determine if dopamine-mediated effects occur as a result of exposure to acrylamide at doses associated with tumor formation, and if these

effects represent a viable mechanism for acrylamide-induced tumor formation in the brain, or any other hormonally responsive tumor site.

With respect to thyroid cancer, rats are sensitive to the increased cell proliferation accompanying prolonged disruption of thyroid hormone balance, which can lead to thyroid hyperplasia and can progress to neoplasia (U.S. EPA, 1998). However, in published and unpublished rodent studies, acrylamide did not disrupt thyroid hormone balance to any significant extent (Khan *et al.*, 1999; Shipp *et al.*, 2002). Moreover, acrylamide-treated male and female rats administered up to 20 mg/kg per day in 90-day toxicity studies (Dow, 1979) or up to 3.0 mg/kg per day in the two-year drinking water studies (Friedman *et al.*, 1995) exhibited no evidence of dose-related thyroid follicular cell hyperplasia, a hallmark of the thyroid-hormone-disruption mechanism. Thus, the available data do not support a non-genotoxic, proliferative mechanism of thyroid tumor induction for acrylamide. Thyroid cancer has been observed at high incidences among male and female rats treated with other genotoxic agents that do not alter thyroid hormone balance, such as the chlorination byproduct MX (Komulainen *et al.*, 1997; OEHHA, 2001a).

Considering the mechanistic information that is currently available, a genotoxic mode of action for acrylamide that is mediated by the metabolite glycidamide is most likely. There are very limited data suggesting that hormonal- or other receptor-mediated processes may also be operative at higher exposure levels. However, even if other non-genotoxic mechanisms with upward-turning nonlinearities are also operative at higher doses, the shape of the dose-response relationship would be expected to be linear at low doses (Hattis, 1990). Therefore, the low dose-linear, default approach fitting the linearized multistage model to tumor dose-response data has been applied.

Pharmacokinetic Adjustments

The pharmacokinetics of acrylamide have been reviewed by Calleman (1996) and others (European Union, 2002; Dybing *et al.*, 2005), and a pharmacokinetic model for the rat has been published (Kirman *et al.*, 2003). Acrylamide is readily absorbed via the oral and dermal routes and is widely distributed to all tissues, including the fetus. Rapid absorption and wide distribution by the inhalation route is also expected (European Union, 2002). Both acrylamide and glycidamide, which also is widely distributed, have relatively long half-lives in blood, sufficient to be transported to all tissues: 1.4 hours in rats, and two to seven hours in adult humans (Calleman, 1996; Sorgel *et al.*, 2002). This compares with about ten minutes for ethylene oxide in rats and 40 minutes for ethylene oxide in humans (Hattis, 1987).

Metabolism via cytochrome P4502E1 to the reactive epoxide, glycidamide, is linear at low doses and begins to saturate and become non-linear with dose at doses above ~10 mg/kg in the rat (Calleman, 1996; Kirman *et al.*, 2003). Metabolism to the epoxide is quite efficient at very low doses in rats, where an estimated 58 percent of absorbed acrylamide is converted to glycidamide (Calleman *et al.*, 1993). At acrylamide doses greater than 50 mg/kg, about 20 to 30 percent is converted to glycidamide (Calleman *et al.*, 1993; Calleman, 1996). Thus, based on the analyses of Calleman (1996), efficient conversion (> 50 %) of acrylamide to glycidamide is expected at the doses employed in the long-term drinking water studies in rats (i.e., 0.01 to 3.0 mg/kg-d). Conjugation of glycidamide with glutathione was observed to be the primary route of elimination of acrylamide in rodents, irrespective of the route of exposure (Sumner *et al.*, 2003). A pharmacokinetic model in the rat suggests that liver glutathione levels would not become

appreciably deleted at acrylamide doses lower than 10 mg/kg (Kirman *et al.*, 2003); thus, glutathione depletion at doses used in the animal cancer studies (0.01 to 3.0 mg/kg-d) is expected to be minimal. Interestingly, studies in human blood indicate that glutathione transferases and epoxide hydrolase play no role in determining the extent of acrylamide or glycidamide Hb-adduct formation (Paulsson *et al.*, 2005). The area under the concentration-versus-time curve (AUC) of glycidamide was found to be linearly related to the concentration of the epoxide administered in rats (Calleman, 1996). At low doses, the AUC for glycidamide resulting from exposures to acrylamide will be a fixed fraction of the AUC for acrylamide itself.

The relatively long half-life of glycidamide may relate to limited detoxification activity in the liver. It has been hypothesized that glycidamide is a poor substrate for epoxide hydrolase, as has been observed for the structurally similar compound cyanoethylene oxide (Calleman, 1996). The long half-lives also suggest that acrylamide and glycidamide do not bind rapidly to glutathione or other electrophilic sites in the blood or tissues.

At occupational exposure levels, conversion of acrylamide to glycidamide increases linearly with increasing air concentration, based on hemoglobin adduct formation among acrylamide-exposed workers (Bergmark *et al.*, 1993; Perez *et al.*, 1999). Calleman (1996) compared the formation of acrylamide and glycidamide hemoglobin adducts in 51 human workers and controls to those produced in rats following oral or i.p. administration. Human samples were from adult males working in China. Utilizing knowledge of the half-lives and the second order reaction-rate kinetics of acrylamide and glycidamide binding to hemoglobin, Calleman (1996) estimated AUC for both acrylamide and glycidamide from the hemoglobin adduct data (Bergmark *et al.*, 1993; Calleman, 1996). The ratio of AUCs for glycidamide relative to acrylamide (AUC_{gly}/AUC_{AA}) was estimated to be about 0.3 in humans and 0.58 in rats at low doses (Table 8).

Findings from pharmacokinetic studies in human volunteers and rats administered up to 3.0 mg/kg-day acrylamide have recently been published (Fennell *et al.*, 2005). Groups of five sterile human male volunteers who had been non-smoking for at least six months were orally administered [^{13}C-labeled] acrylamide for three days at daily doses in water of 0, 0.5, 1.0 or 3.0 mg/kg or topically at 3.0 mg/kg daily for three days. In addition each group had a naïve control. Blood and urine were collected for 24 hours post-treatment. Additionally, rats were given oral doses of [^{13}C-labeled] acrylamide in water at doses of 0 or 3.0 mg/kg. Blood and urine were also collected from the rats. The researchers derived kinetic information related to hemoglobin (Hb)-adduct formation of acrylamide (N-(2-carbamoylethyl)valine) and glycidamide (N-(2-carbamoyl-2-hydroxyethyl)valine) and utilized that information to estimate the AUC for acrylamide and glycidamide for both rats and humans. Based on the Hb-adduct data, the ratio of AUC_{gly}/AUC_{AA} was estimated to be about 0.25 in humans and 0.65 in rats (Fennell *et al.*, 2005) (Table 8).

Table 8. Rat Versus Human Conversion of Low Doses of Acrylamide to Glycidamide

	Calleman (1996)[a]	Fennell *et al.* (2005)[b]	
basis	Hb Adducts	Hb Adducts	Total Urinary Metabolites
metric	AUC_{Gly}/AUC_{AA}	AUC_{Gly}/AUC_{AA}	% Gly metabolites/%AA metabolites
Human	0.30	0.25	0.12
Rat	0.58	0.65	0.41
Rat/Human	1.9	2.6	3.4

Abbreviations: Hb, hemoglobin; AUC, area under the concentration-versus-time curve; Gly, glycidamide; AA, acrylamide

[a] Based on occupational exposure of humans to acrylamide characterized as "low-dose," and administration of 0.05, 1.0, 2.0, 5.0, or 10 mg/kg acrylamide to rats.

[b] Based on administration of 0.5, 1.0, or 3.0 mg/kg-day acrylamide to humans and 3.0 mg/kg to rats.

Urinary metabolites were assayed and the proportions of acrylamide- or glycidamide-based urinary metabolites were estimated for both rats and humans (Friedman, 2003). The proportion of absorbed acrylamide that was converted to glycidamide and excreted in the urine was estimated to be about 12% in humans and 41% in rats (Table 8).

Thus, for two sets of Hb-adduct data following acrylamide exposure, the ratio of AUCs of glycidamide to acrylamide for rats relative to humans were similar: 1.9 for the Calleman (1996) data, and 2.6 for the Fennell *et al.* (2005) data (Table 8). The rat-to-human ratio from urinary metabolite data among rats and human volunteers was 3.4 (Fennell *et al.,* 2005). These sets of data suggest that rats are more efficient in metabolizing acrylamide to glycidamide than are adult male humans.

The available mechanistic data implicate glycidamide as the primary DNA-reactive species mediating acrylamide carcinogenesis. Accordingly, interspecies differences in the rate of conversion of acrylamide to glycidamide need to be accounted for in the cancer dose-response assessment, as well as the differing half-lives of acrylamide and glycidamide in humans and rats. The AUC of glycidamide (AUC_{gly}) divided by the acrylamide dose applied (D_{AA}) is a measure of internal glycidamide per unit acrylamide exposure.

Table 9 compares for humans and rats estimates of AUC_{gly}, normalized by D_{AA} (i.e., AUC_{gly}/D_{AA}). AUC_{gly} is based upon Hb adduct levels resulting from relatively low-dose acrylamide exposures in humans and rats as reported by Calleman (1996) and Fennell *et al.* (2005). Due to the longer half-lives of acrylamide and glycidamide in humans as compared to rats, the human internal dose of glycidamide per unit acrylamide dose, is greater (i.e., human AUC_{gly}/D_{AA} > rat AUC_{gly}/D_{AA}), even though there is greater conversion of acrylamide to glycidamide in rats. Specifically, the data of Calleman (1996) indicate that human exposures to acrylamide result in estimated internal blood doses of glycidamide per unit acrylamide exposure (AUC_{gly}/D_{AA}) that are 1.1-fold higher than the estimated internal blood doses observed in rats, and the data of Fennell *et al.* (2005) indicate that the human AUC_{gly}/D_{AA} is 1.2 fold higher than that of the rat. The more recent human to rat ratio AUC_{gly}/D_{AA} of 1.2 estimated from the Fennell *et al.* (2005) study was used as the inter-species adjustment factor for pharmacokinetics in the

cancer potency estimates (Table 10). This pharmacokinetic adjustment factor of 1.2 accounts for interspecies differences in 1) the rate of conversion of acrylamide to glycidamide and 2) the half-lives of acrylamide and glycidamide.

Table 9. **Rat versus Human Estimates of AUC of Glycidamide (AUC_{gly}) Normalized by Acrylamide Dose Applied (D_{AA}) [(mM-hr)/(mg acrylamide/kg-body weight)]**

Species	Calleman (1996)[a]	Fennell *et al.* (2005)[b]
Human AUC_{gly}/D_{AA}	0.0313	0.0614
Rat AUC_{gly}/D_{AA}	0.0280	0.0520
Ratio (human/rat)	**1.1**	**1.2***

* Value used for interspecies pharmacokinetic adjustments to cancer potency.

[a] Based on occupational exposure of humans to acrylamide characterized as "low-dose," and administration of 0.05, 1.0, 2.0, 5.0, or 10 mg/kg acrylamide to rats.

[b] Based on administration of 0.5, 1.0, or 3.0 mg/kg-day acrylamide to humans and 3.0 mg/kg to rats.

Inter-individual Variation in Sensitivity

Although it is difficult to quantitate at this time, inter-individual variation in sensitivity to acrylamide-induced cancer is expected to be significant. The expected variability relates to many factors including differences in pharmacokinetics, co-exposures, and age at exposure. At least two metabolic pathways where there is genetic variability in the human population – cytochrome P4502E1 (*CYP*2E1)-mediated metabolism of acrylamide to glycidamide, and glutathione S-transferases (GST)-mediated conjugation of glycidamide – are likely to contribute to this variability. Thus, as noted by WHO (2002), the sensitivity of humans to the effects of acrylamide may be variable. Individuals likely to be at higher risk of acrylamide-induced carcinogenesis may be those that are simultaneously high in *CYP*2E1 activity and low in GST activity. The possible contribution of GST polymorphisms to inter-individual variation in sensitivity to acrylamide-induced cancer is unclear, however, in light of recent studies with human blood indicating that GSTs play no role in determining the extent of formation of acrylamide and glycidamide Hb adducts (Paulsson *et al.*, 2005). However, it is not known whether GST facilitates the removal of acrylamide or glycidamide in tissues such as the liver.

Stephens *et al.* (1994) noted a 50-fold variation in *CYP*2E1 activity among humans, and it is unclear whether this variation stems from genetic or environmental factors. Indeed, many common human exposures such as ethanol (e.g., alcoholic beverages) and acetaminophen are known inducers of P4502E1. Stephens *et al.* (1994) investigated the frequency of two polymorphisms in the *CYP*2E1 gene among different ethnic groups. Among the 695 individuals examined, statistically significant differences in allelic frequencies were observed between Taiwanese and African-Americans or European-Americans for each polymorphism. Allelic frequencies for the two polymorphic variants were 24 to 28 percent in Taiwanese, one to eight percent in African-Americans, and four to 11 percent in European-Americans. Similarly, de Vries *et al.* (1994) observed among 17 volunteers of varying age, gender and ethnicity a 28-fold

difference in the ability to metabolize chlorzoxazone to 6-hydroxychlorzoxazone. Chlorzoxazone is a drug that is metabolized almost exclusively by cytochrome P4502E1.

Large inter-individual differences in activity of GST exist within the population (reviewed in Warmhoudt *et al.*, 1999). The subclasses μ (GSTM1) and θ (GSTT1) are effective catalysts for conjugation of glutathione with epoxides. Seidegard and Pero (1985) tested the activity of GST (towards a model epoxide, trans-stilbene oxide) in a population of 248 individuals. They observed 100- to 200-fold inter-individual differences in overall GST activity in peripheral blood leukocytes. The role of polymorphisms of glutathione S-transferases in acrylamide toxicity is unclear but could be low based on *in vitro* findings that Hb-adduct formation is not affected by GSTs (Paulsson *et al.*, 2005).

Iyer and Sinz (1999) investigated the metabolic activities of both cytochrome P4502E1 and GST in human livers from 21 individuals, varying in ethnicity, age, and smoking and alcohol consumption status. Even in this small sample set, a nearly five-fold difference in P4502E1 activity and a three-fold difference in GST activity were observed. We examined the ratios of P4502E1/GST activity for the 21 liver samples, which fit a lognormal distribution (Crystal Ball 2000, Decision Engineering, Inc.). The mean of the distribution differed by only 20 % from the median; however, the 95th percentile value was 2.4-fold higher than the median activity ratio. As noted in other larger datasets above, the variability in P4502E1 or GST activity was greater than those found by Iyer and Sinz (1999). This suggests that the variability in the ratio of P4502E1 to GST activity (e.g., the pharmacokinetic variability most relevant to acrylamide toxicity) is likewise going to be greater in the general human population.

In addition to inter-individual variability due to pharmacokinetics, there is likely to be variability due to the age at which exposure occurs. Acrylamide and presumably glycidamide are widely distributed to the fetus (Ikeda *et al.*, 1983, 1985, 1987; Schettgen *et al.*, 2004), thus *in utero* exposures occur. Exposure via mother's milk is also known to occur (Sorgel *et al.*, 2002), and direct consumption of acrylamide containing foods is also expected early in life. Children's exposures to acrylamide have been estimated to be greater than adult exposures on a body weight basis (Konings *et al.*, 2003). The enzyme that bioactivates acrylamide to glycidamide, P4502E1, is not highly expressed for the first months of life, but is expressed at levels approaching those seen in adults by one year of age (OEHHA, 2001c). However, maternally formed glycidamide is expected to reach the fetus through placental transfer and to the nursing baby via breastmilk. Some isoforms of GST, such as GST μ and α, have low levels of expression at birth, and by about six months of age are expressed at levels approaching those seen in adults (OEHHA, 2001c). Since conjugation with glutathione is thought to represent a major detoxification pathway for acrylamide, low GST *in utero* and in young infants may translate to greater sensitivity for this group.

Although no carcinogenicity studies have employed early-in-life exposures, there are reasons to suspect that early-life exposures to acrylamide may result in greater tumor induction than exposures in adulthood. For example, compounds structurally similar to acrylamide, such as vinyl chloride and urethane (ethyl carbamate), induced a higher incidence of tumors following early-life exposure compared to adult exposure (Maltoni *et al.*, 1981; Kaye and Trainin, 1966; Rogers, 1951). Like acrylamide, both vinyl chloride and urethane are metabolized to epoxide intermediates that bind to DNA. Vinyl chloride DNA adducts levels were higher in the livers of

newborn mice compared to levels in the livers of mice treated as adults (Laib *et al.*, 1989, Swenberg *et al.*, 1992). Since cancer is a multi-step process, DNA damage early in life has more time to express itself and increases the likelihood that subsequent DNA damage will result in neoplasia. Acrylamide also is well recognized to cause germ cell mutations, which may indicate a multigenerational risk from exposure (Dearfield *et al.*, 1995). Carcinogenicity testing of acrylamide employing early-in-life exposures is critically needed.[3]

Variability in acrylamide-induced carcinogenesis may also stem from co-exposures to other carcinogens or promoters. For example, orally administered acrylamide distributes readily to the skin of mice (Carlson and Weaver, 1985), initiating tumors when expressed by phorbol ester promotion (Bull *et al.*, 1984a; 1984b).

Thus, there are many potential sources of variability in sensitivity within the population to the carcinogenic effects of acrylamide, including (1) wide variability among humans in the enzymes involved in activation (P450 2E1) and possibly detoxification (GST), (2) variability due to age sensitivities, and (3) co-exposures.

Such variability is difficult to quantitate at this time. Given that the animals used in the rat cancer bioassays (Johnson *et al.*, 1986; Friedman *et al.*, 1995) were adults at the initiation of dosing, potency estimates based on these studies do not account for variability due to early-life susceptibility. No specific adjustments to the acrylamide cancer potency estimates were made to address inter-individual variability. The use of a default inter-species pharmacodynamics factor (2.66 for male rats and 2.42 for female rats, see Dose-Response Assessment and Appendix) may or may not be adequate to account for both interspecies differences in pharmacodynamics and inter-individual human variability in response. Thus, the cancer potency derived may not be adequately protective of children and other sensitive groups. Research in this area is critically needed.

DOSE-RESPONSE ASSESSMENT

Animal Data

Cancer potency estimates were derived for tumor responses in acrylamide-treated rats (Tables 2-5), using methods described in the Appendix.

The interspecies conversion factor is based on surface area scaling, i.e., (human body weight / animal body weight)$^{1/3}$ [Title 22, California Code of Regulations, section 12703(a)(6)]. Based on the average body weights of the rats in the Johnson *et al.* (1985) and Friedman *et al*. (1995) studies, the interspecies conversion factor is calculated to be 7.05 for male rats and 5.85 for female rats (see Appendix). Human potency is estimated from rat data as follows:

$$\textit{Cancer potency (human)} = \textit{cancer potency (animal)} \times \textit{interspecies factor}$$

[3] The U.S. Food and Drug Administration, in conjunction with the National Toxicology Program, is planning to conduct a series of cancer bioassays in the newborn mouse (U.S. FDA, 2004b).

The default interspecies factor assumes that dose in amount per surface area produces the same cancer incidence in different species. It is the ratio of human to animal body weights to the 1/3 power:

Interspecies factor *male rat to human:* $(70/0.35)^{1/3} = 7.05$

female rat to human: $(70/0.20)^{1/3} = 5.85$

This interspecies adjustment factor accounts for differences in pharmacokinetics (e.g., differences in the internal dose) and pharmacodynamics (e.g., differences in response to the internal dose). This assessment chose to proportion equally the interspecies scaling factor into a pharmacokinetics (PK) factor and a pharmacodynamic (PD) factor. Thus, the equation for estimating the human cancer potency can be expressed as:

Cancer potency (human) = cancer potency (animal) × PK factor × PD factor.

The default PK and PD factors are the square root of the interspecies factor. Sufficient data exist to depart from the default PK factor but not the default PD factor.

- The PK factor is taken as the ratio of the human to rat internal dose of glycidamide, normalized by the applied acrylamide dose, or **1.2** (Table 9). This adjustment factor takes into account the differences between humans and rats in the rate of metabolism of acrylamide to glycidamide, and the differences between humans and rats in the half lives of acrylamide and glycidamide.

- The PD factor is taken as the square root of the default interspecies scaling factor [$(7.05)^{1/2} = 2.66$ for male rats, and $(5.85)^{1/2} = 2.42$ for female rats]. It is unclear whether this PD factor is adequate to account for both interspecies differences in PD and inter-individual human variability in response.

Cancer potency (human) = cancer potency (male rat) × 1.2 × 2.66

Cancer potency (human) = cancer potency (female rat) × 1.2 × 2.42

Human-equivalent cancer potency estimates for each tumor site are presented in Table 10.

Table 10. Upper 95 % confidence bound, human-equivalent cancer potency estimates for acrylamide, $(mg/kg\text{-}d)^{-1}$

	Johnson *et al.* (1986)		**Friedman *et al.* (1995)**	
Tumor site in rats	females	males	females	males
Mammary	1.0	--	0.40	--
Central nervous system	0.14	--	0.071	0.13
Thyroid	0.24	0.32	0.33	0.44
Testis	--	0.58	--	0.40
Oral cavity	0.31	--	--	--
Uterus	0.15	--	--	--
Clitoral gland	0.26	--	--	--
All sites combined[a]	1.5	0.75	0.69	0.77
Geometric mean[a]	**0.70**			

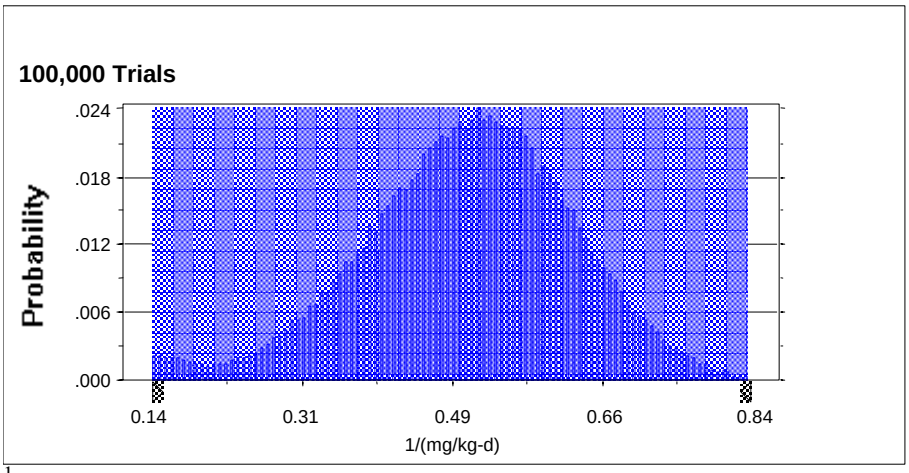
[a] Derived using Monte Carlo techniques.

No adjustment factors were applied to the cancer potency estimate to account for early-in-life susceptibility or other sources of inter-individual variability in response such as genetic differences in metabolism. The animals used in the rat cancer bioassays (Johnson *et al.*, 1986; Friedman *et al.*, 1995) were adults at the initiation of dosing; thus, early-life susceptibility is not captured by the tumor responses. The use of the PD factor above may or may not be adequate to account for both interspecies differences in pharmacodynamics and inter-individual variability in response. Research is needed to address this data gap.

Since acrylamide induced tumors at multiple sites in male and female rats, combined potency estimates were derived for each experiment using Monte Carlo analysis for those tumor sites judged to be associated with exposure to acrylamide. For each tumor site, a distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model was generated with the MSTAGE computer program (Crouch, 1998), which had been modified to tabulate percentile values. For each rat cancer study, a combined distribution was created by adding q_1 for each tumor site, according to its distribution, through 100,000 Monte Carlo trials (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The four (multisite) potency distributions derived from the two female and two male rat studies were further combined using Monte Carlo analysis to estimate a distribution of geometric mean potencies (Figure 1). The geometric mean was taken as the basis of the cancer potency estimate for the combined tumor sites across the four studies, yielding a mean potency of 0.51 $(mg/kg\text{-}d)^{-1}$ and the upper 95 % confidence bound potency estimate of 0.70 $(mg/kg\text{-}d)^{-1}$.

The cancer potency estimates for all treatment-related tumors (combined), based on the upper 95 % confidence bound, from the studies in male and female rats are quite consistent (Table 10). The four multisite potency estimates from the rat studies differed only by about a factor of two.

Figure 1. Distribution of human-equivalent cancer potency estimates[1] based on all acrylamide-responding tumor sites observed in four cancer studies in rats.



[1] This distribution represents the geometric mean estimates from four rat studies (Tables 2-5) and accounts for interspecies differences in the conversion of acrylamide to glycidamide and pharmacokinetic differences in response. The 95 % upper confidence bound from this distribution, 0.70 $(mg/kg\text{-}d)^{-1}$, is taken as the basis of the NSRL for acrylamide.

Human Data

For comparative purposes only, a cancer potency estimate was derived from the pancreatic tumor data from the Marsh *et al.* (1999) epidemiological study. A linear relative risk model was applied to the available data using Poisson regression. In the relative risk model (shown below), the rate of observed deaths λ(dose) is a linear function of the cumulative exposure (dose), where λ_0* is the application of the background rate of expected deaths based on age-dependent U.S. cancer mortality rate tables. The slope parameter of the exposure-response curve, β_1, was estimated by Poisson regression, a maximum likelihood procedure.

$\lambda(dose) = \lambda_0^*[1 + \beta_1(dose)]$ (Excess relative risk model)

Analysis was performed using the program AMFIT, which is part of the EPICURE computer software package (Preston *et al.*, 1993). AMFIT computes maximum likelihood estimates of parameters in a general class of hazard function models, including excess and relative risk models. This program has been used by other risk assessment programs to estimate human cancer risk, including both the BEIR IV and BEIR V Committees to estimate the cancer potency of ionizing radiation (NRC, 1990).

Once the slope estimate, β_1, has been calculated from the cohort data, the final stage of the dose-response assessment involves the calculation of excess lifetime (70-year) risk for a pattern of exposure in the population of interest (e.g., continuous exposure of the general population). This is done with life table techniques (NRC, 1990). The life table was developed based on the

methods described by Chiang (1984). Previous cancer risk assessments developed using these life table techniques include assessments for cadmium (DHS, 1986), diesel exhaust (OEHHA, 1998) and benzene (OEHHA, 2001b). Data for age-specific background incidence rates in the California population for all races combined were used in the life table calculations (Kwong *et al.*, 2000). Also, a lag time of ten years was used for pancreatic cancer since it is generally a late-forming tumor (Kwong *et al.*, 2000). The population-based human cancer potency estimate reflects dose adjustments from an occupational exposure scenario to that expected for exposure of the general population. Specifically, estimates were divided by a factor of 0.33 to account for the difference in days per year exposed and for volume of exposed air consumed in a standard work-day relative to 24-hour estimates; e.g., (240 days/365 days)*(10 m^3/20 m^3) = 0.33. The excess 70-year risk in units of ppm^{-1} was converted to a population-based human cancer potency estimate for acrylamide in units of $(mg/kg\text{-}d)^{-1}$ using standard default values for daily intake of air. This yielded an upper-bound cancer potency estimate for pancreatic tumors of 2.8 $(mg/kg\text{-}d)^{-1}$.

The upper-bound cancer potency estimate of 2.8 $(mg/kg\text{-}d)^{-1}$ derived from the Marsh *et al.* (1999) occupational cohort study for exposure of the general population to acrylamide over a lifetime is higher than the upper-bound cancer potencies estimated from the long-term rat drinking water studies, which ranged from 0.69 to 1.5 $(mg/kg\text{-}d)^{-1}$ (Table 10). This comparison illustrates that, if humans are equally or less sensitive to acrylamide-induced cancer as rats, then the expected relative risk estimates in the worker study would have been small. If the animal cancer studies are predictive of human risk, one would not expect to observe a statistically significant increase in cancer rates from the exposures experienced by the workers, a conclusion also reached by others (Calleman, 1996; Granath *et al.*, 2001; Dybing and Sanner, 2003; Erdreich and Friedman, 2004). Thus, the available human cancer data on acrylamide do not reduce OEHHA's concern regarding the potential induction of cancers by acrylamide.

A cancer potency estimate of 0.70 $(mg/kg\text{-}day)^{-1}$ was derived from the geometric means of combined distributions of cancer potency estimates for all acrylamide-related tumor sites across four studies in rats (Johnson *et al.*, 1986; Friedman *et al.*, 1995) (Figure 1). This is associated with a dose of 0.014 μg/kg-day at a lifetime cancer risk of 10^{-5}.

COMPARISON OF CANCER RISK-SPECIFIC DOSE WITH PUBLIC HEALTH LEVELS FOR OTHER TOXIC ENDPOINTS

Neurotoxicity is a sensitive non-cancer endpoint of acrylamide toxicity (WHO, 2002; NIOSH, 1991), which has been manifest primarily as neuropathy in occupational studies. This toxicity defines the critical endpoint for non-cancer effects evaluated by public health institutions such as the U.S. EPA (1991) and WHO (2002). The U.S. EPA (1991) has established a reference dose (RfD) for acrylamide of 2×10^{-4} mg/kg-day based on neurotoxic effects. The U.S. EPA definition of an RfD is "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." This RfD was based on nerve damage observed in a drinking water study in rats (Burek *et al.*, 1980). An uncertainty/adjustment factor of 1000 was applied to the NOEL (0.2 mg/kg-day) observed in that study. The WHO (2002) evaluated non-cancer endpoints and found a NOEL of 0.5 mg/kg-day, noting that "Rodent studies (sub-chronic and chronic oral dosing), primate studies (oral and

subcutaneous) and a human occupational study, support a NOEL for acrylamide neuropathy of 0.5 mg/kg bw per day."

In addition, acrylamide has been shown to cause adverse effects on reproduction and fetal development (CERHR, 2004a, 2004b; NIOSH, 1991, U.S. EPA, 1990), and it is also recognized that acrylamide can induce heritable DNA damage (WHO, 2002). In a draft report by the Expert Panel on Acrylamide of the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction, the lowest observed effect level for both developmental and male reproductive toxicity was about 5 mg/kg-day (CERHR 2004b). Dividing this level by a standard uncertainty factory of ten (for LOEL to NOEL extrapolation) results in a NOEL estimate of 0.5 mg/kg-day, essentially the same as that derived by WHO (2002) for neurotoxic effects. U.S. EPA (1991) established a NOEL of 0.2 mg/kg-day (= 200 μg/kg-day), and a corresponding reference dose (RfD) of 0.2 μg/kg-day for non-cancer effects based on experimental neurotoxicity data. The daily dose level posing a 10^{-5} lifetime risk of cancer of 0.014 μg/kg-day (=1.0 μg/day ÷ 70 kg) is more than 10,000 times lower than the NOELs of 200 or 500 μg/kg-day for non-cancer (reproductive, developmental or neurotoxic) endpoints, and more than 10 times lower than the U.S. EPA RfD. This suggests that cancer is the most sensitive health endpoint for acrylamide.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The combined cancer potency estimate for all acrylamide-related tumor sites, 0.70 $(mg/kg\text{-}day)^{-1}$, derived above was used to calculate the NSRL for acrylamide (1.0 μg/day).

REFERENCES

Abramsson-Zetterberg L (2003). The dose-response relationship at very low doses of acrylamide is linear in the flow cytometer-based mouse micronucleus assay. *Mutat Res* **535**(2):215-222.

Agrawal AK, Seth PK, Squibb RE, Tilson HA, Uphouse LL, Bondy SC (1981a). Neurotransmitter receptors in brain regions of acrylamide- treated rats. I: Effects of a single exposure to acrylamide. *Pharmacol Biochem Behav* **14**(4):527-531.

Agrawal AK, Squibb RE, Bondy SC (1981b). The effects of acrylamide treatment upon the dopamine receptor. *Toxicol Appl Pharmacol* **58**(1):89-99.

Ali SF, Hong JS, Wilson WE, Uphouse LL, Bondy SC (1983). Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. *Arch Toxicol* **52**(1):35-43.

Bergmark E (1997). Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem Res Toxicol* **10**(1):78-84.

Bergmark E, Calleman CJ, He F, Costa LG (1993). Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. *Toxicol Appl Pharmacol* **120**(1):45-54.

Besaratinia A, Pfeifer GP (2003). Weak yet distinct mutagenicity of acrylamide in mammalian cells. *J Natl Cancer Inst* **95**(12):889-896.

Besaratinia A, Pfeifer GP (2004). Genotoxicity of acrylamide and glycidamide. *J Natl Cancer Inst* **96**(13):1023-1029.

Baum M, Fauth E, Fritzen S, Herrmann A, Mertes P, Merz K *et al*. (2005). Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood. *Mutat Res* **580**(1-2):61-19.

Bondy SC, Tilson HA, Agrawal AK (1981). Neurotransmitter receptors in brain regions of acrylamide- treated rats. II: Effects of extended exposure to acrylamide. *Pharmacol Biochem Behav* **14**(4):533-537.

Bosetti C, Talamini R, Levi F, Negri E, Franceschi S, Airoldi L, La Vecchia C (2002). Fried foods: a risk factor for laryngeal cancer? *Br J Cancer* **87**(11):1230-1233.

Bull RJ, Robinson M, Laurie RD, Stoner GD, Greisiger E, Meier JR, Stober, J (1984a). Carcinogenic effects of acrylamide in Sencar and A/J mice. *Cancer Res* **44**(1):107-111.

Bull RJ, Robinson M, Stober JA (1984b). Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Lett* **24**(2):209-212.

Burek, JD, RR Albee, JE Beyer, *et al.* (1980) Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. *J Environ Pathol Toxicol* 4: 157-182.

Calleman CJ (1996). The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk estimation. *Drug Metab Rev* **28**(4):527-590.

Calleman CJ, Bergmark E, Stern LG, Costa LG (1993). A nonlinear dosimetric model for hemoglobin adduct formation by the neurotoxic agent acrylamide and its genotoxic metabolite glycidamide. *Environ Health Perspect* **99**:221-223.

Carlson GP, Weaver PM (1985). Distribution and binding of 14C-acrylamide to macromolecules in SENCAR and BALB/c mice following oral and topical administration. *Toxicol Appl Pharmacol* **79**(2):307-313.

Carrington CD, Lapadula DM, Dulak L, Friedman M, Abou-Donia MB (1991). In vivo binding of [14C]acrylamide to proteins in the mouse nervous system. *Neurochem Int* **18**(2):191-197.

Center for the Evaluation of Risks to Human Reproduction (CERHR, 2004a). Meeting Summary May 20, 2004. CERHR, National Toxicology Program, Research Triangle Park, NC.

Center for the Evaluation of Risks to Human Reproduction (CERHR, 2004b). DRAFT. NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Acrylamide, CERHR, National Toxicology Program, Research Triangle Park, NC.

Chiang CL (1984). *The Life Table and Its Implications*. Robert E. Krieger Publishing Co., Malabar, Florida.

Collins JJ, Swaen GM, Marsh GM, Utidjian HM, Caporossi JC, Lucas LJ (1989). Mortality patterns among workers exposed to acrylamide. *J Occup Med* **31**(7):614-617.

Crouch AC (1998). *MSTAGE A program to fit end-of-life carcinogenesis bioassay data to the multistage formula Version 2.01.* Cambridge Environmental Inc., Cambridge Massachusetts, Crouch@CambridgeEnvironmental.com, February 11, 1998.

Damjanov I, Friedman MA (1998). Mesotheliomas of tunica vaginalis testis of Fischer 344 (F344) rats treated with acrylamide: a light and electron microscopy study. *In Vivo* **12**(5):495-

502.

Dearfield KL, Abernathy CO, Ottley MS, Brantner JH, Hayes PF (1988). Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. *Mutat Res* **195**(1):45-77.

Dearfield KL, Douglas GR, Ehling UH, Moore MM, Sega GA, Brusick DJ (1995). Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. *Mutat Res* **330**(1-2):71-99.

de Vries JD, Salphati L, Horie S, Becker CE, Hoener BA (1994). Variability in the disposition of chlorzoxazone. *Biopharm Drug Dispos* **15**(7):587-97.

DHS (1986). *Part B. Health Effects of Cadmium. Report to the Air Resources Board on Cadmium.* Department of Health Services, State of California.

DiNovi M, Howard D (2004). The updated exposure assessment for acrylamide. Presentation at the JIFSAN meeting entitled "Acrylamide in Food: Update -- Scientific Issues, Uncertainties, and Research Strategies," April 13, 2004, located at http://www.jifsan.umd.edu/ acrylamide2004.htm, Chicago, Illinois.

Doerge D (2004). Toxicology and Metabolic Consequences. Presentation at the JIFSAN meeting entitled "Acrylamide in Food: Update -- Scientific Issues, Uncertainties, and Research Strategies," April 13, 2004, Chicago, Illinois.

Doerge DR, Young JF, McDaniel LP, Twaddle NC, Churchwell MI (2005a). Toxicokinetics of acrylamide and glycidamide in B6C3F(1) mice. *Toxicol Appl Pharmacol* **202**(3):258-267.

Doerge DR, Gamboa da Costa G, McDaniel LP, Churchwell MI, Twaddle NC, Beland FA (2005b). DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutat Res* **580**(1-2):131-141.

Dow (1979). *Initial submission: results of a 90-day toxicity study in rats with acrylamide administered in the drinking water followed by up to 144 days of recovery w-cover letter dated 043092.* Unpublished report, EPA/OTS, Doc #88-920002384 .

Dybing E, Sanner T (2003). Risk assessment of acrylamide in foods. *Toxicol Sci* **75**(1):7-15.

Dybing E, Farmer PB, Andersen M, Fennell TR, Lalljie SP, Muller DJ *et al*. (2005). Human exposure and internal dose assessments of acrylamide in food. *Food Chem Toxicol* **43**(3):365-410.

Erdreich LS, Friedman MA (2004). Epidemiologic evidence for assessing the carcinogenicity of acrylamide. *Regul Toxicol Pharmacol* **39**(2):150-157.

European Union (2002). European Union Risk Assessment Report: Acrylamide. Institute for Health and Consumer Protection, European Chemicals Bureau, PL-1, Volume 24, European Communities, Luxemborg.

Favor J, Shelby MD (2005). Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure. *Mutat Res* **580**(1-2):21-30.

Fennell TR, Sumner SCJ, Snyder RW, Burgess J, Spicer R, Bridson WE *et al*. (2005). Metabolism and hemoglobin adduct formation of acrylamide in humans. *Toxicol Sci* --advance access online version.

Friedman MA, Dulak LH, Stedham MA (1995). A lifetime oncogenicity study in rats with acrylamide. *Fundam Appl Toxicol* **27**(1):95-105.

Gamboa da Costa G, Churchwell MI, Hamilton LP, Von Tungeln LS, Beland FA, Marques MM *et al*. (2003). DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem Res Toxicol* **16**(10):1328-1337.

Generoso WM, Sega GA, Lockhart AM, Hughes LA, Cain KT, Cacheiro NL *et al*. (1996). Dominant lethal mutations, heritable translocations, and unscheduled DNA synthesis induced in male mouse germ cells by glycidamide, a metabolite of acrylamide. *Mutat Res* **371**(3-4):175-183.

Ghanayem BI, Witt KL, El-Hadri L, Hoffler U, Kissling GE, Shelby MD *et al*. (2005). Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. *Biol Reprod* **72**(1):157-63.

Glatt H, Schneider H, Liu Y (2005). V79-hCYP2E1-hSULT1A1, a cell line for the sensitive detection of genotoxic effects induced by carbohydrate pyrolysis products and other food-borne chemicals. *Mutat Res* **580**(1-2):41-52.

Granath F, Ehrenberg L, Paulsson B, Tornqvist M (2001). Cancer risk from exposure to occupational acrylamide. *Occup Environ Med* **58**(9):608-609.

Granath F, Tornqvist M (2003). Who knows whether acrylamide in food is hazardous to humans? *J Natl Cancer Inst* **95**(12):842-843.

Hagmar L, Tornqvist M (2003). Inconclusive results from an epidemiological study on dietary acrylamide and cancer. *Br J Cancer* **89**(4):774-775.

Hagmar L, Wirfalt E, Paulsson B, Tornqvist M (2005). Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutat Res* **580**(1-2):157-165.

Haseman JK (1995). Data analysis: statistical analysis and use of historical control data. *Regul Toxicol Phamacol* **21**:52-59.

Hattis D (1987). A pharmacokinetic/mechanism-based analysis of the carcinogenic risk of ethylene oxide. NTIS/PB88-188784, M. I. T. Center for Technology, Policy and Industrial Development, CTPID 87-1, August, 1987.

Hattis D (1990). Pharmacokinetic principles for dose-rate extrapolation of carcinogenic risk from genetically active agents. *Risk Anal* **10**(2):303-316.

Hoorn AJ, Custer LL, Myhr BC, Brusick D, Gossen J, Vijg J (1993). Detection of chemical mutagens using Muta Mouse: a transgenic mouse model. *Mutagenesis* **8**(1):7-10.

Husøy T, Abramsson-Zetterberg L, Olstorn HB, Paulsen JE, Alexander J (2005). Adenomatous polyposis coli influences micronuclei induction by PhIP and acrylamide in mouse erythrocytes. *Mutat Res* **580**(1-2):111-118.

Ikeda GJ, Miller E, Sapienza PP, Michel TC, King MT, Turner VA, Blumenthal H (1983). Distribution of 14C-labelled acrylamide and betaine in foetuses of rats, rabbits, beagle dogs and miniature pigs. *Food Chem Toxicol* **21**(1):49-58.

Ikeda GJ, Miller E, Sapienza PP, Michel TC, King MT, Sager AO (1985). Maternal-foetal

distribution studies in late pregnancy. II. Distribution of [1-^{14}C]acrylamide in tissues of beagle dogs and miniture pigs. *Food Chem Toxicol* **23**(8):757-61.

Ikeda GJ, Miller E, Sapienza PP, Michel TC, Inskeep PB (1987). Comparative tissue distribution and excretion of [1-^{14}C]acrylamide in beagle dogs and miniature pigs. *Food Chem Toxicol* **25**(11):871-5.

International Agency for Research on Cancer (IARC, 1994). Acrylamide. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 60.* World Health Organization, IARC, Lyon, France

International Agency for Research on Cancer (IARC, 2002). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 83 Tobacco Smoke and Involuntary Smoking*. World Health Organization, IARC, Lyon, France.

Iyer KR, Sinz MW (1999). Characterization of Phase I and Phase II hepatic drug metabolism activities in a panel of human liver preparations. *Chem Biol Interact* **118**(2):151-169.

Johansson F, Lundell T, Rydberg P, Erixon K, Jenssen D (2005). Mutagenicity and DNA repair of glycidamide-induced adducts in mammalian cells. *Mutat Res* **580**(1-2):81-89.

Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA *et al*. (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* **85**(2):154-168.

Kaye AM, Trainin N (1966). Urethan carcinogenesis and nucleic acid metabolism: factors influencing lung adenoma induction. *Cancer Res* **26**:2206-12.

Kelsey JL, Thompson WD, Evans, AS (1986). *Methods in Observational Epidemiology.* Monographs in Epidemiology and Biostatistics Volume 10, Oxford University Press, New York, pp. 293-300.

Khan MA, Davis CA, Foley GL, Friedman MA, Hansen LG (1999). Changes in thyroid gland morphology after acute acrylamide exposure. *Toxicol Sci* **47**(2):151-157.

Kirman C, Gargas M, Deskin R, Tonner-Navarro L, Andersen M (2003). A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. *J Toxicol Environ Health A* **66**(3):253-274.

Koehler KM (2004). Epidemiology studies of acrylamide and cancer. Presentation at the JIFSAN Meeting Entitled "Acrylamide in Food: Update -- Scientific Issues, Uncertainties, and Research Strategies," April 13, 2004, Chicago, Illinois

Komulainen H, Kosma VM, Vaittinen SL, Vartiainen T, Kaliste-Korhonen E, Lotjonen S *et al*. (1997). Carcinogenicity of the drinking water mutagen 3-chloro-4- (dichloromethyl)-5-hydroxy-2(5H)-furanone in the rat. *J Natl Cancer Inst* **89**(12):848-856.

Konings EJ, Baars AJ, van Klaveren JD, Spanjer MC, Rensen PM, Hiemstra M, van Kooij JA, Peters PWJ (2003). Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem Toxicol* **41**(11):1569-1579.

KS Crump Group, Inc. (1999a). *Consideration of the Potency Classification of Acrylamide Based on the Incidence of Tunica Vaginalis Mesotheliomas (TVMs) in Male Fischer 344 Rats*. Unpublished, Ruston, Louisiana, June 11, 1999.

KS Crump Group, Inc. (1999b). *Mechanism of Acrylamide Induction of Benign Mammary Fibroadenomas in the Aging Female Fischer 344 Rat: Relevance to Human Health Tisk Assessment.* Unpublished, Ruston, Louisiana, December 14, 1999.

KS Crump Group, Inc. (2000a). *The Biological Role of Acrylamide-induced Astrocytomas in the Aging Fischer 344 Rat to Human Health Outcomes*. Unpublished, Ruston, Louisiana, November 2000.

KS Crump Group, Inc. (2000b). *The Biological Role of Acrylamide-induced Thyroid Follicular Cell Tumors in the Aging Rat to Human Health Outcomes*. Unpublished, Ruston, Louisiana, November 2000.

Kwong SL, Perkins CI, Morris CR *et al*. (2000). *Cancer in California 1988-1998.* California Department of Health Service, Cancer Surveillance Section, Sacramento, California:

Laib RJ, Bolt HM, Cartier R, Bartsch H (1989). Increased alkylation of liver DNA and cell turnover in young versus old rats exposed to vinyl chloride correlates with cancer susceptibility. *Toxicol Lett* **45**(2-3):231-9.

Lynch DW, Lewis TR, Moorman WJ, Burg JR, Groth DH, Khan A *et al*. (1984). Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. *Toxicol Appl Pharmacol* **76**(1):69-84.

Maltoni C, Lefemine G, Ciliberti A, Cotti G, Carretti D (1981). Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. *Environ Health Perspect* **41**:3-29.

Manière I, Godard T, Doerge DR, Churchwell MI, Guffroy M, Laurentie M *et al*. (2005). DNA damage and DNA adduct formation in rat tissues following oral administration of acrylamide. *Mutat Res* **580**(1-2):119-129.

Marsh GM, Lucas LJ, Youk AO, Schall LC (1999). Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occup Environ Med* **56**(3):181-190.

Martenson CH, Odom A, Sheetz MP, Graham DG (1995). The effect of acrylamide and other sulfhydryl alkylators on the ability of dynein and kinesin to translocate microtubules *in vitro*. *Toxicol Appl Pharmacol* **133**(1):73-81.

Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K (2003). Dietary acrylamide and cancer of the large bowel, kidney, and bladder: Absence of an association in a population-based study in Sweden. *Br J Cancer* **88**(1):84-89.

Mucci LA, Lindblad P, Steineck G, Adami HO (2004). Dietary acrylamide and risk of renal cell cancer. *Int J Cancer* **109**(5):774-776.

Myhr BC (1991). Validation studies with Muta Mouse: a transgenic mouse model for detecting mutations in vivo. *Environ Mol Mutagen* **18**(4):308-315.

NCI (1982). *Bioassay of Reserpine for Possible Carcinogenicity.* National Toxicology Program and the National Cancer Institute (NTP/NCI), Technical Report 193.

NICNAS (2002). *Acrylamide. Priority Existing Chemical Report No. 23*. National Institute of Chemical Notification and Assessment Scheme, Commonwealth of Australia, www.nicnas.gov.au/publications/CAR/PEC/PEC23/PEC23_whole.pdf.

NIOSH (1991). *NIOH and NIOSH Basis for Occupational Health Standard. Acrylamide: A Review of the Literature.* Department of Human Health Services, National Institute of Occupational Safety and Health (NIOSH) Publication No. 91-115, Cincinnati, Ohio.

NRC (1990). *Health Effects of Exposure to Low Levels of Ionizing Radiation*. National Research Council. BEIR V, Committee on the Biological Effects of Ionizing Radiation. National Academy Press: Washington, D.C.

OEHHA (1998). *Part B: Health Risk Assessment for Diesel Exhaust*. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento.

OEHHA (2001a). *Evidence of the Carcinogenicity of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone).* Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, California, located at www.oehha.ca.gov/Prop65/Pdf/MX-Final.Pdf.

OEHHA (2001b). *Public Health Goal for Benzene in Drinking Water*. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, California, www.oehha.ca.gov/water/phg/pdf/BenzeneFinPHG.pdf.

OEHHA (2001c*). Prioritization of Toxic Air Contaminants Under the Children's Environmental Health Protection Act.* Final, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, California.

OEHHA (2003). Transcript, Meeting of the Science Advisory Board's Carcinogen Identification Committee, held October 17, 2003, Sacramento, California.

Park J, Kamendulis LM, Friedman MA, Klaunig JE (2002). Acrylamide-induced cellular transformation. *Toxicol Sci* **65**(2):177-183.

Paulsson B, Grawe J, Tornqvist M (2002). Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or N-methylolacrylamide treatment. *Mutat Res* **516**(1-2):101-111.

Paulsson B, Kotova N, Grawe J, Henderson A, Granath F, Golding B *et al*. (2003). Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. *Mutat Res* **535**(1):15-24.

Paulsson B, Rannug A, Henderson AP, Golding BT, Tornqvist M, Warholm M (2005). In vitro studies of the influence of glutathione transferases and epoxide hydrolase on the detoxification of acrylamide and glycidamide in blood. *Mutat Res* **580**(1-2):53-59.

Pelucchi C, Franceschi S, Levi F, Trichopoulos D, Bosetti C, Negri E, La Vecchia, C (2003). Fried potatoes and human cancer. *Int J Cancer* **105**(4):558-560.

Perez HL, Cheong HK, Yang JS, Osterman-Golkar S (1999). Simultaneous analysis of hemoglobin adducts of acrylamide and glycidamide by gas chromatography-mass spectrometry. *Anal Biochem* **274**(1):59-68.

Preston DL, Lubin JH, Pierce DA (1993). *Epicure User's Guide*. Seattle, WA: HiroSoft International Corporation.

Public Health Service (PHS, 2004). *The Health Consequences of Smoking : a Report of the Surgeon General*. Department of Health and Human Services, PHS, located at http://www.cdc.gov/tobacco/sgr/sgr_2004/index.htm.

Puppel N, Tjaden Z, Fueller F, Marko D (2005). DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells. *Mutat Res* **580**(1-2):71-80.

Robie D, DiNovi M (2003). The Exposure Assessment for Acrylamide . Presentation at the U.S. Food and Drug Administration Food Advisory (FDA) Committee meeting, February 24, 2003, U.S. FDA Office of Food Additive Safety, located at www.cfsan.fda.gov.

Robinson KO, Petersen AM, Morrison SN, Elso CM, Stubbs L (2005). Two reciprocal translocations provide new clues to the high mutability of the Grid2 locus. *Mamm Genome* **16**(1):32-40.

Robinson M, Bull RJ, Knutsen GL, Shields RP, Stober J (1986). A combined carcinogen bioassay utilizing both the lung adenoma and skin papilloma protocols. *Environ Health Perspect* **68**:141-145.

Rogers S (1951). Age of the host and other factors affecting the production with urethane of pulmonary adenomas in mice. *J Exp Med* **93**:427-449.

Schettgen T, Broding HC, Angerer J, Drexler H (2002). Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. *Toxicol Lett* **134**(1-3):65-70.

Schettgen T, Weiss T, Drexler H, Angerer J (2003). A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. *Int J Hyg Environ Health* **206**(1):9-14.

Schettgen T, Kutting B, Hornig M, Beckmann MW, Weiss T, Drexler H *et al*. (2004). Trans-placental exposure of neonates to acrylamide-a pilot study. *Int Arch Occup Environ Health* **77**(3):213-216.

Schulz MR, Hertz-Picciotto I, van Wijngaarden E, Hernandez JC, Ball LM (2001). Dose-response relation between acrylamide and pancreatic cancer. *Occup Environ Med* **58**(9):609.

Segerback D, Calleman CJ, Schroeder JL, Costa LG, Faustman EM (1995). Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of acrylamide. *Carcinogenesis* **16** (5):1161-1165.

Seidegard J, Pero RW (1985). The hereditary transmission of high glutathione transferase activity towards trans-stilbene oxide in human mononuclear leukocytes. *Hum Genet* **69**(1):66-68.

Shipp AM, Lawrence GA, Van Landingham CB, Gentry PG (2001). *Hazard Identification and Dose-response Analysis for Acrylamide*. Unpublished, K.S. Crump Group of ICF Consulting, Monroe, Louisiana, November 2001.

Shipp A, Lawrence G, Gentry R, Bartow H, McDonald T, Bounds J, Clewell H, Van Landingham C, Macdonald, N (2002). *Toxicological Review of Acrylamide (CAS No. 79-06-1)*. Unpublished, Environ International Corporation, Ruston, Louisiana, November 2002.

Sickles DW, Welter DA, Friedman MA (1995). Acrylamide arrests mitosis and prevents chromosome migration in the absence of changes in spindle microtubules. *J Toxicol Environ Health* **44**(1):73-86.

Sickles DW, Brady ST, Testino A, Friedman MA, Wrenn RW (1996). Direct effect of the neurotoxicant acrylamide on kinesin-based microtubule motility. *J Neurosci Res* **46**(1):7-17.

Silvari V, Haglund J, Jenssen D, Golding BT, Ehrenberg L, Tornqvist M (2005). Reaction-kinetic parameters of glycidamide as determinants of mutagenic potency. *Mutat Res* **580**(1-2):91-101.

Snellings WM, Weil CS, Maronpot RR (1984). A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. *Toxicol Appl Pharmacol* **75**(1):105-117.

Solomon JJ (1999). Cyclic adducts and intermediates induced by simple epoxides. *IARC Sci Publ* (150):123-35.

Sorgel F, Weissenbacher R, Kinzig-Schippers M, Hofmann A, Illauer M, Skott A *et al*. (2002). Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. *Chemotherapy* **48**(6):267-274.

Sumner SC, Williams CC, Snyder RW, Krol WL, Asgharian B, Fennell TR (2003). Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. *Toxicol Sci* **75**(2):260-270.

Srivastava S, Sabri MI, Agrawal AK, Seth PK (1986). Effect of single and repeated doses of acrylamide and bis-acrylamide on glutathione-S-transferase and dopamine receptors in rat brain. *Brain Res* **371**(2):319-323.

Stephens EA, Taylor JA, Kaplan N, Yang C-H, Hsieh LL, Lucier GW, Bell DA (1994). Ethnic variation in the CYP2E1 gene: Polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese. *Pharmacogenetics* **4**(4):185-192.

Swenberg JA, Fedtke N, Fishbein L (1992). Age-related differences in DNA adduct formation and carcinogenesis of vinyl chloride in rats. In: Guzelian PS, Henry CJ, Olin SS, eds. *Similarities and differences between children and adults: Implications for risk assessment*. Washington, DC: ILSI Press, pp. 163-171.

Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* **50**(17):4998-5006.

Twaddle NC, Churchwell MI, McDaniel LP, Doerge DR (2004). Autoclave sterilization produces acrylamide in rodent diets: implications for toxicity testing. *J Agric Food Chem* **52**(13):4344-4349.

U.S. Environmental Protection Agency (U.S. EPA, 2004). *Acrylamide (CASRN 79-06-1).* Integrated Risk Information System (IRIS), carcinogenicity assessment last updated July 1, 1993, located at http://www.epa.gov/iris.

U.S. Environmental Protection Agency (U.S. EPA, 1998). *Assessment of Thyroid Follicular Cell Tumors*. U.S. EPA, National Center for Environmental Assessment, EPA/630/R-97/002 March 1998, located at http://www.epa.gov/ncea/thyroid.htm.

U.S. Environmental Protection Agency (U.S. EPA, 1991). Acrylamide (CASRN 79-06-1). Integrated Risk Information System (IRIS). Assessment of the oral RfD last updated March 1, 1991, located at http://www.epa.gov/iris/subst/0286.htm.

U.S. Environmental Protection Agency (U.S. EPA, 1990). Assessment of Health Risks from Exposure to Acrylamide. U.S. EPA Office of Toxic Substances, Washington DC, June 1990.

U.S. Food and Drug Administration (U.S. FDA, 1996). Memorandum of conference. Cancer Assessment Committee Meeting on acrylamide, May 31, 1996, Washington, D.C., located at http://www.fda.gov/ohrms/dockets/ ac/03/briefing/3925b1_13.pdf.

U.S. Food and Drug Administration (U.S. FDA, 1998). Report of the Quantitative Risk Assessment Committee, Memorandum, April 10, 1998, located at http://www.fda.gov/ohrms/dockets/ac/03/briefing/3925b1_14.pdf .

U.S. Food and Drug Administration (U.S. FDA, 2004a). FDA action plan for acrylamide in food, CFSAN/Office of Plant & Dairy Foods, March 2004, located at http://www.cfsan.fda.gov/~dms/acrypla3.html.

U.S. Food and Drug Administration (U.S. FDA, 2004b). Exploratory data on acrylamide in food. U.S. FDA, CFSAN Office of Plant & Dairy Foods, March 2004, located at http://www.cfsan.fda.gov/~dms/acrydata.html.

Van Gemert (2001). *The Tegeris Laboratory's Acrylamide Study: Response to FDA Concerns. December 2001.* Unpublished document sent to Dr. T. McDonald, OEHHA, California EPA by Dr. Marvin Friedman, SNF Floerger on November 11, 2003.

World Health Organization (WHO, 2002). *Health Implications of Acrylamide in Food.* Report of a joint FAO/WHO consulation, Geneva, June 25-27, 2002.

Wormhoudt LW, Commandeur JN, Vermeulen NP (1999). Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. *Crit Rev Toxicol* **29**(1):59-124.

APPENDIX: METHODOLOGY USED TO DERIVE AN NSRL FOR ACRYLAMIDE

Procedures for the development of Proposition 65 NSRLs are described in regulation (Title 22, California Code of Regulations, sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRL for acrylamide are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

"Multistage" polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (CDHS, 1985; U.S. EPA, 1996; Anderson *et al.*, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_i d^i)] \quad (1)$$

with constraints,

$$q_i \geq 0 \text{ for all i.}$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter q_0 represents the background lifetime incidence of the tumor. The parameter q_1, or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1^*, the upper 95% confidence bound on q_1 (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units of mg/kg-day, the parameters q_1 and q_1^* are given in units of $(\text{mg/kg-day})^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977). To estimate potency in animals (q_{animal}) from experiments of duration T_e, rather than the natural life span of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$q_{animal} = q_1^* \bullet (T/T_e)^3 \quad (2)$$

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural life span of mice and rats is assumed to be two years, so that for experiments lasting T_e weeks in these rodents:

$$q_{animal} = q_1^* \bullet (104/T_e)^3 \quad (3)$$

Since the rat cancer bioassays (Johnson *et al.*, 1986; Friedman *et al.*, 1995) used in this assessment followed the animals for 104 weeks, the less-than-lifetime study correction was not needed. To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson *et al.*, 1983) as "extra risk," and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

Calculation of the lifetime average dose

For the rat drinking water studies, the average daily doses were provided by the study authors (Johnson *et al.*, 1986; Friedman *et al.*, 1995). Dosing was continued for life; no adjustments to the doses are needed.

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California risk assessment guidelines (CDHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-third power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \bullet (bw_h / bw_a)^{1/3} \quad (7)$$

Average body weights for acrylamide-treated female and male F344 rats in the Johnson *et al.* (1986) study, 0.2 and 0.35 kg respectively, were estimated by U.S.EPA (1990) from the individual animal data. These estimates of body weight appeared to be appropriate for the second set of rat drinking water studies (Friedman *et al.*, 1995), based on graphs of body weight over the study period. The average body weights for male and female F344 rats in these studies are lower than average body weights observed in the National Toxicology Program. The reason for the lighter animals is not clear. A default body weight of 70 kg for humans was assumed (Gold and Zeiger, 1997). The default interspecies scaling factors, on the average body weights of the rats in these studies, would be 7.05 for male rats and 5.85 for female rats.

However, in the case of acrylamide, pharmacokinetic data in human volunteers and rats (Fennell, 2004) were available and applied in the risk assessment. The internal dose of glycidamide (AUC_{gly}), the DNA-reactive metabolite thought be primarily responsible for mediating acrylamide carcinogenesis, was observed to be 1.2-fold higher in humans than rats (Table 9). The default interspecies scaling factor accounts for differences in pharmacokinetics (e.g., differences in the internal dose) and pharmacodynamics (e.g., differences in response to the internal dose). This assessment chose to proportion equally the interspecies scaling factor into a pharmacokinetics (PK) factor and a pharmacodynamic (PD) factor. Thus, the equation for the human cancer potency estimate can be expressed as:

Cancer potency (human) = cancer potency (animal) * PK factor * PD factor,

The interspecies PK factor was 1.2, based on pharmacokinetic data in rats and humans, whereas the interspecies PD factor was taken as the square root of the default factor [i.e., $(7.05)^{1/2} = 2.66$, for male rats; $(5.85)^{1/2} = 2.42$, for female rats]. Cancer potency estimates for each tumor site presented in Table 10 were adjusted in this manner.

A.3 Risk-Specific Intake Level Calculation

The intake level (I, in mg/day) associated with a cancer risk R, from exposure is:

$$I = \frac{R \times bw_h}{q_{human}} \quad (8)$$

where bw_h is the body weight, and q_{human} the theoretical cancer potency estimate for humans.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the no significant risk level for cancer under Proposition 65 (Title 22, California Code of Regulations, section 12703).

Thus for a 70 kg person, the NSRL is given by:

$$\mathrm{NSRL} = \frac{10^{-5} \times 70\,\mathrm{kg}}{q_{\mathrm{human}}} \tag{9}$$

APPENDIX REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265-267.

Anderson EL and the U.S. Environmental Protection Agency Carcinogen Assessment Group (1983). Quantitative approaches in use to assess cancer risk. *Risk Analysis* **3**:277-295.

California Department of Health Services (CDHS, 1985). Guidelines for Chemical Carcinogen Risk Assessment and Their Scientific Rationale. California Department of Health Services, Health and Welfare Agency, Sacramento, CA.

Crump KS (1981). An improved procedure for low-dose carcinogenic risk assessment from animal data. *J Environ Path Toxicol* **52**:675-684.

Crump KS, Guess HA, Deal LL (1977). Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. *Biometrics* **33**:437-451.

Gold LS, Zeiger E (1997). *Handbook of Carcinogenic Potency and Genotoxicity Databases.* CRC Press, Inc., Boca Raton.

Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA *et al*. (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* **85**(2):154-168.

Friedman MA, Dulak LH, Stedham MA (1995). A lifetime oncogenicity study in rats with acrylamide. *Fundam Appl Toxicol* **27**(1):95-105.

U.S. Environmental Protection Agency (U.S. EPA, 1988). *Recommendations for and Documentation of Biological Values for Use in Risk Assessment*. Office of Health and Environmental Assessment, Washington D.C. EPA/600/6-87/008.

U.S. EPA (1990). *Assessment of health risks from exposure to acrylamide,* U.S. EPA, Office of Toxic Substances, Washington, D.C.

U.S. Environmental Protection Agency (U.S. EPA, 1996). *Proposed guidelines for carcinogen risk assessment*. Federal Register **61**(79):17960-18011 (April 23, 1996).