Comments on the Proposal to Establish a Specific Regulatory Level Posing No Significant Risk (NSRL) for Acrylamide and Amend Title 22, California Code of Regulations, Section 12705(b)

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By
Robert G. Tardiff [*], Christopher R. Kirman [†], F. Jay Murray [§]

On Behalf Of
The Industry Acrylamide Coalition

Agricultural Council of California
American Bakers Association
American Frozen Foods Institute
American Institute of Baking
American Soybean Association
Baking Association of Canada
California Chamber of Commerce
California Farm Bureau Federation
California Grocers Association
California Hotel and Lodging Association
California League of Food Processors
California Manufacturers and Technology Association
California Restaurant Association
California Retailers Association
Food Products Association
Frozen Potato Products Institute
Grocery Manufacturers Association
Independent Bakers Association
Institute of Shortening and Edible Oils
National Confectioners Association
National Oilseed Processors Association
National Potato Council
National Restaurant Association
North American Millers' Association
Snack Food Association
Tortilla Industry Association
Western Growers Association
Wheat Foods Council

[*] The Sapphire Group, Bethesda, MD

[†] The Sapphire Group, Cleveland, OH

[§] Murray & Associates, San Jose, CA

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EXECUTIVE SUMMARY

OEHHA has proposed revising the NSRL for acrylamide (AA) from 0.2 to 1 microgram (mcg) per person-day based on an estimate of the theoretical cancer potency for humans from studies in rats. Several lines of evidence included in OEHHA's supportive evaluation have been examined to determine whether the weight of evidence offers alternate and more robust interpretations.

Our analysis concludes that the proposed NSRL of one (1) microgram of acrylamide per person-day is flawed, scientifically inappropriate, and unsupported. When all of the information reviewed herein are taken into consideration, OEHHA would be prudent to wait till all relevant data were obtained; however, if not, it is apparent that a significantly higher NSRL for acrylamide is scientifically defensible and appropriate. Our analysis indicates that a significantly higher NSRL, perhaps 100 mcg per person-day or more, is scientifically more appropriate.

The NSRL as currently proposed is seriously flawed for several reasons. First, the two carcinogenicity studies in rats relied upon to derive the proposed NSRL have serious problems in their conduct and have come under serious question. A cancer risk assessment is only as good as the underlying carcinogenicity studies. In the case of the first study, the roof collapsed — literally — which creates substantial questions as to the validity of the findings. The second study was severely criticized by the U.S. Food and Drug Administration (FDA) as inappropriate "for performing a quantitative risk assessment." FDA was so concerned with the quality of these studies that it has sponsored new carcinogenicity studies of acrylamide and its metabolite glycidamide (GA) in rats and mice. These studies are in progress at the National Center for Toxicological Research, and OEHHA should wait for the results of these studies to determine the appropriate NSRL for acrylamide. Should OEHHA choose to proceed, however, the following points become not only germane but essential for OEHHA to consider.

Second, tumors were combined in a manner that is contrary to well accepted scientific principles. A novel method was used to combine risks across multiple tumor sites, a practice that is biologically and statistically inappropriate. For example, both uterine and testicular tumors were combined to estimate individual risks — a biological unlikelihood. Furthermore, tumors of unrelated histological origin were combined, which is a biologically indefensible practice. For example, mammary fibromas (benign connective tissue tumors that never progress to cancer) were combined with mammary adenomas and carcinomas (benign and malignant tumors of epithelial tissue). This practice is particularly troublesome because the proposed NSRL is heavily driven by the inappropriate combining of unrelated mammary tumors, including tumors that never progress to cancer (in either rats or humans). When these tumors are combined properly and the results are extrapolated more appropriately (non-linear dose-response, adopting a margin of safety of

1,000), the NSRL for acrylamide should be no less than 50 mcg/person-day. The potency based solely on malignancies using a non-linear model would provide an estimate of 226 mcg/person-day.

Third, the proposed NSRL uses allometric scaling to convert doses in rats to those in humans. The proposed NSRL assumes that an additional adjustment factor of 3 is necessary to account for interspecies differences. However, the scientific evidence indicates that this factor should be one or less. This change alone would result in an NSRL that is at least 3-fold higher than the proposed NSRL.

Fourth, a statistical trend test was used to analyze the tumor data that assumes a linear dose-response relationship. The linear trend test forces the data to fit a straight line and determines whether the resulting line has a slope different from zero. OEHHA concluded that increases in several tumor types, which were not statistically significant using appropriate statistical tests applied by the study authors, were statistically significant using a less appropriate linear trend test. These "newly-significant" tumors were also combined with the other tumors to estimate risk, thus compounding the errors. Equally importantly, the supporting documentation for the NSRL is misleading because it suggests that the linear trend is equivalent to a linear dose-response at all doses. Actually the trend test is unable to discern whether any effects are occurring at the lowest test doses or beyond the observation range. The application of the trend test provides no justification for the use of a linear model to extrapolate cancer incidence data from experimental levels to those in cooked foods.

Fifth, the proposed NSRL assumes that acrylamide's mode of cancer action is attributable to the genotoxic properties of a reactive metabolite, glycidamide. While the possibility of alternative modes of action is acknowledged, no estimate of risk is provided for any of the alternatives. The acceptance of a genotoxic mode of action is not necessarily indicative of a linear mode of action, as suggested by OEHHA. Rather, it is wholly consistent with a non-linear mode of carcinogenesis, which OEHHA fails to recognize. Even if one were to assume a genotoxic mode of action, the dose-response relationship would not likely be linear, as assumed in deriving the proposed NSRL.

Sixth, the shape of the dose-response curve for acrylamide is influenced by many factors, some of which are apt to be more important at low doses typical of human dietary intake than high experimental doses in rats. Notably, binding of acrylamide and glycidamide to blood proteins (hemoglobin and albumin) is an important detoxification mechanism. Such binding would be saturated at doses much higher than are obtained by humans via the diet, resulting in non-linear dose-response, whereas at doses experienced by humans via the diet saturation would not be possible. The proposed NSRL does not consider the critical role of binding to blood proteins as a detoxification mechanism, particularly at low doses experienced by humans. Saturation of binding to blood proteins contributes an important source of non-linearity in the acrylamide dose-response relationship in rats at high doses, and it justifies the use of a non-linear model to extrapolate to humans exposed to much lower doses via the diet.

And finally, it is highly inappropriate to use the re-analysis of the pancreatic cancer data from the Marsh *et al.* (1999) epidemiological study to "validate" the proposed NSRL. The trend for pancreatic mortality in the re-analysis of the Marsh *et al.* study is not convincing. In fact, earlier in the NSRL document, OEHHA described the potential link to pancreatic cancer as "merely suggestive of an association at best." The NSRL Document is on shaky scientific ground in using the pancreatic cancer data for quantitative risk estimation and toxic potency assessment. In comparison, all of the epidemiogical studies of dietary exposure to acrylamide consistently show no increase in cancer. The overall weight of the epidemiological evidence does not suggest that exposure to low levels of acrylamide in the diet increases human cancer risk.

1.0 INTRODUCTION

In 2002, acrylamide (AA) was discovered to be present in a number of potato and cereal products cooked or heat-processed at high temperatures. AA has subsequently been found in an even wider range of foods after being prepared with traditional cooking and processing methods, whether in a food plant, in a restaurant or at home. AA is formed during reactions involving heating of mixtures of amino acids, particularly asparagine, and glucose. Although only recently detected, human exposures to AA from foods has likely occurred for thousands of years. Recently, California's Office of Environmental Health Hazard Assessment (OEHHA) proposed to revise the No Significant Risk Level (NSRL) for AA and amend Title 22, California Code of Regulations, Section 12705(b). Upon adoption, this proposed level would supersede the current NSRL for AA established in 1990 in Section 12705(c).

Details on the basis for the proposed number are provided in a risk assessment document prepared by OEHHA describing and summarizing the derivation of the regulatory level ("the NSRL Document")[1]. The proposed level described below for inclusion in Section 12705 represents the level of exposure to the chemical that is calculated to result in no more than one excess case of cancer in an exposed population of 100,000, assuming exposure over a 70-year lifetime (10^{-5} lifetime risk of cancer). This proposed regulation would adopt a NSRL (a risk-specific dose) into Section 12705(b) for AA, of 1.0 microgram (mcg)/person-day. The proposed NSRL is based upon a cancer potency estimate for humans of 0.7 $(mg/kg\text{-}day)^{-1}$ based upon several tumor types observed in rats.[2]

The authors have been asked to critically examine and comment upon the appropriateness and rigor of the foundation for, and development of, the NSRL for AA. This report to OEHHA provides our detailed analysis. Should OEHHA have an interest in discussing any of our scientific conclusions, we would welcome the opportunity to do so in the interest of assuring that regulatory policies are based on the soundest scientific information and interpretations.

2.0 COMMENTS ON PROPOSED NSRL FOR AA

We offer comments on the following topics:

1. The questionable quality of the underlying carcinogenicity studies,
2. Combining risks from multiple tumor sites, particularly combining mammary fibromas, adenomas, and adenocarcinomas,
3. Dose adjustments for extrapolating from rodents to humans,
4. Role of statistical trend tests in addressing biologically-based dose-response,

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[1] Office of Environmental Health Hazard Assessment (2005) No Significant Risk Level (NSRL) for the Proposition 65 Carcinogen Acrylamide. March, 2005.

[2] Id.

5. Genotoxic vs. non-genotoxic mode of cancer action,
6. Shape of cancer dose-response curve and role of blood protein binding, and
7. Value of epidemiology data on the weight-of-evidence.

Within the context of these topics, their impact on the NSRL for AA is addressed comprehensively.

2.1. The questionable quality of the underlying carcinogenicity studies

The proposed NSRL is based on two rat carcinogenicity studies. The estimates of cancer potency are only as good as the underlying carcinogenicity studies. In the case of AA, both studies are distinguished with problems. In effect, the NSRL is underpinned by two studies which have come under serious question.

In the first study, that of Johnson *et al.* (1986) study, the roof caved in – literally from the weight of the snow. The roof above the acrylamide study animals collapsed during the middle of the study. As a result, the study animals had to be moved to another room. There was also an outbreak of sialodacryoadenitis, a viral disease, in this study. It is a generally accepted scientific principle in toxicology studies that unnecessary sources of stress (e.g., roof collapse, moving animals) should be avoided and that animals should be disease-free. The impact of these events on the results of the cancer study is unclear. However, they certainly raise questions about the suitability of this study for conducting sophisticated quantitative risk assessment.

The second study, *i.e.*, the Friedman *et al.* (1995) study, was severely criticized by the U.S. Food and Drug Administration (FDA). FDA stated:

> "The Committee does not consider the [Friedman study] to be appropriate for use in determining the carcinogenicity of acrylamide or for performing a quantitative risk assessment, because of serious deficiencies in the conduct of the study."

It is our understanding that FDA auditors reported numerous serious problems with this study, which the study authors have responded to and refuted. We have seen neither the audit report nor the authors response, but given the importance of this matter, OEHHA should evaluate the underlying basis for FDA's concern before revising the NSRL for AA.

In short, the proposed NSRL is based on two studies which have come under serious question. Both studies have clouds over them. The NSRL Document applies sophisticated methodology, and in some cases, incorrectly, to studies of questionable quality.

Because FDA did not consider these studies to be of adequate quality for risk assessment, FDA sponsored new carcinogenicity studies of both AA and GA in rats and mice. These studies are currently underway at the National Center For Toxicological Research (NCTR). It would seem prudent for OEHHA to wait for the results of these studies to revise its NSRL for AA.

2.2 Combining risks from multiple tumor sites, particularly combining mammary fibromas, adenomas, and adenocarcinomas

The NSRL Document used a relatively novel method to combine risk across multiple tumor sites (*e.g.*, mammary gland, central nervous system, thyroid, oral, uterus, clitoral, and testis) using Monte Carlo methods and then taking the geometric mean from four data sets (male and female rats from two studies), yielding a value of 700 (µg/kg-d). This practice has deficiencies from biological and statistical perspectives.

In addition, the combination of risk from different tumor sites, as was done by OEHHA, is a breach of the internationally recognized procedures and principles indicating the limited circumstances in which tumors can be combined (McConnell *et al.*, 1986). To that end, USEPA has codified this view when it recently recommended formally not summing tumors across tumor sites (USEPA, 2005). The National Toxicology Program and the International Agency for Research on Cancer have followed the criteria of McConnell *et al.* (1986) for nearly two decades.

McConnell *et al.* (1986) offer the following criteria for combining tumors:

1. Substantial evidence exists for progression of benign to malignant neoplasms of the same histomorphogenic type. Progression is considered more important if demonstrated within the study in question than if comparisons must be made with past experience.
2. The occurrence of hyperplasia may be used as supporting evidence alone, but more so when the criteria for differentiating hyperplasia from benign neoplasia are not clear (*i.e.*, borderline lesions) or when they are arbitrary and do not reflect the biologic potential of a given lesion.
3. Most neoplasms of the same histomorphogenic type are combined even if they occur in different anatomic sites.
4. Neoplasms of different morphologic classification may be combined when their histomorphogenesis is comparable.

The NSRL Document obscures the fact that it is actually combining tumor types by first estimating risk and only then combining the risk estimate from each. Among the flaws in this approach, risks are combined that made no logical or biological sense: specifically combining the risks for both uterine and testicular tumors for individual risks.

Furthermore, these tumors may differ with respect to mode of action, latency, and relevance to human health. Combining risks from these data sets using Monte Carlo methods appears to invoke an assumption of independence between tumor sites that is invalid. Specifically, if all tumor types were attributed to the formation of GA, then animals with a higher rate for oxidative metabolism would be expected to be at greater risk than animals with a lower rate of metabolism for cancer at all of the tumor sites. It is unclear how correlations between the risks for different tumor sites would be introduced in a defensible manner. Since it is biologically inappropriate, OEHHA should not attempt to combine tumor risks using Monte Carlo methods.

Risk estimates based upon tumors of the oral cavity, uterus, and clitoral gland from the Johnson *et al.* (1986) study should not be included in the assessment since an increased incidence was not observed for these tumor types in a confirmatory study at higher doses (Friedman *et al.* 1995) and because of potential contributions from an oral viral infection to one or more of these tumor types. Based on a preliminary evaluation of the dose-response data, the dose-response data for thyroid and mammary gland tumors are considered to be the most appropriate bases for cancer risk assessment, and to a less extent the CNS and testes tumors.

The NSRL Document combined different data sets (defined by study and sex) using a geometric mean. Two limitations exist in using the geometric mean to combine data sets. First, use of the geometric mean may not be appropriate if a non-linear dose-response relationship is present and the data sets describe different portions of the dose-response curve. This appears to be the case for the dose-response data in female rats (Johnson *et al.* = low [0.01-2 mg/kg-day]; Friedman *et al.* = high [1-3 mg/kg-day]. Second, the use of the geometric mean does not capture important differences in the study design (dose selection, number of animals/group), and, therefore, treats all data sets as if they are of equal quality. To avoid these limitations, OEHHA should consider pooling only the relevant data sets prior to dose-response modeling. Pooling the appropriate dose-response data should improve the statistical power of the regression, resulting in a better characterization of the dose-response curve across a wider range of doses. Preliminary results for pooling the data sets for the critical target tissues are summarized in **Table 1** below.

Table 1. Estimates of cancer potency of AA based upon dose-response data pooled for critical tumor sites from the key cancer bioassays (Johnson *et al.*, 1986; Friedman *et al.*, 1995)*

		Point of Departure (POD) (mg/kg-day)		**Linear Extrapolation** (0.1/POD)		**Non-linear Extrapolation** (POD/UF of 1,000)	
Tumor Site	**Sex**	**ED10**	**LED10**	**ED10**	**LED10**	**ED10**	**LED10**
Mammary gland	F	1.0	0.71	0.10	0.14	0.001	**0.00071**
CNS	MF	5.4	3.1	0.018	0.032	0.0054	0.0031
Thyroid	MF	1.5	1.1	0.065	0.088	0.0015	0.0011
Testes	M	1.2	0.80	0.085	0.12	0.0012	0.00080

*Dose-response modeling was performed using USEPA's BMDS package (version 1.3.2) in accordance with USEPA's final guidelines for carcinogen risk assessment (USEPA, 2005). Human equivalent doses were calculated using an allometric scaling factor of 1.0 (unscaled), based upon a proposed mode of action involving a reactive metabolite. Linear and non-linear extrapolations below the point of departure are presented for comparison.

The largest upper-bound estimate of linear cancer potency (140 per mcg/kg-day) based upon mammary gland tumors (adenocarcinomas and fibroadenomas from Friedman *et al.*; adenomas, fibromas, and adenocarcinomas from Johnson *et al.*) is lower than the value used in the NSRL (700 per mg/kg-day) by a factor of five. This value suggests that under

the condition of low-dose linearity, the NSRL for AA should be 5 mcg/day instead of 1 mcg/day. However, for low-dose non-linearity (adopting a margin of safety of 1,000), the NSRL for AA should be no less than 50 mcg/person-day (0.00071 mg/kg-day * 70 kg * 1,000 µg/mg), based on this consideration alone.

The NSRL Document combined mammary tumors in a scientifically incorrect manner. In deriving the NSRL, mammary fibromas and fibroadenomas were combined with adenomas and adenocarcinomas. However, these tumors have different histological origins, meaning they are distinct and unrelated tumor types. Fibromas and fibroadenomas are benign tumors that originate from connective tissue. In comparison, adenomas and adenocarcinomas are benign and malignant tumors, respectively, that originate from epithelial tissue. Importantly, fibromas and fibroadenomas never progress to become malignant tumors either in rats or humans. While adenomas may progress to become malignant adenocarcinomas, fibromas and fibroadenomas do not become cancer (malignant tumors). Because they are unrelated biologically, it is wrong to combine fibromas and fibroadenomas with adenomas and adenocarcinomas, as indicated by the principles articulated by McConnell *et al.* (1986).

The combining of mammary tumors is important because these tumors play the largest role of all of the tumors in defining the proposed NSRL. Incorrect combination of mammary tumors directly affects the estimate of the NSRL, producing an erroneous NSRL.

It is also important to recognize that the incidences of mammary carcinoma (malignant tumors) were not statistically significantly increased in either of the two rat carcinogenicity studies of AA. Rather, these studies demonstrated an increase in fibromas and fibroadenomas (non-cancerous tumors), as summarized in **Tables 2** and **3**. The incidence of mammary tumors was not significantly increased until fibromas and fibroadenomas were inappropriately combined with adenomas and adenocarcinomas.

Therefore, the ***cancer*** risk assessment and the proposed NSRL for ***cancer*** are based heavily on the results of benign tumors that never progress to ***cancer***. <u>Applying the non-linear dose-response characterization based solely on malignancies would provide an estimated NSRL of 226 mcg AA/person-day.</u>

Table 2. Female Mammary Tumors in the Johnson *et al.* (1986) Study

Tumor Type	**Dose (mg/kg-day)**				
	0	**0.01**	**0.1**	**0.5**	**2.0**
Adenocarcinoma	2/60	1/60	1/60	2/58	6/61
Adenoma	0/60	1/60	0/60	3/58	2/61
Fibroma or fibroadenoma	10/60	11/60	9/60	17/58	21/61

Table 3. Female Mammary Tumors in the Friedman *et al.* (1995) Study

Tumor Type	Dose (mg/kg-day)			
	0	**0.1**	**1.0**	**3.0**
Adenocarcinoma	2/46	0/50	2/94	4/95
Fibroadenoma	5/46	4/50	20/94 *	26/95 *

* $p<0.05$

2.3 Dose adjustments for extrapolating from rodents to humans

Another critical factor in risk assessment is extrapolation of dose from rats to humans. In the NSRL Document, allometric scaling was used to estimate human equivalent dose from rats with a scaling factor of 0.67, *i.e.*, (human body weight/animal body weight)$^{1/3}$. The resulting interspecies factors of 7.05 (male rat) and 5.85 (female rat) were divided into equal kinetic and dynamic components (square root of 7.05 = 2.66 for male rats; square root of 5.85 = 2.42 for female rats). The default allometric scaling component for kinetic factors was replaced by a data-derived value of 1.2 based upon an analysis of hemoglobin adducts; hemoglobin adducts were used to estimate differences between humans and rats in the rate of metabolism of AA and half-lives of AA and GA. Based upon these considerations, the total interspecies adjustments proposed in the NSRL Document were as follows:

Interspecies Adjustment = (Kinetic Factor) * (Dynamic Factor)

Male rats = 1.2 * 2.66 = 3.2

Female rats = 1.2 * 2.42 = 2.9

However, for modes of action involving reactive metabolites (*e.g.*, AA for AA), this practice is inappropriate (and needlessly conservative). A scaling factor of one (1) (unscaled dose) has been shown to be most applicable for modes of action involving one or more reactive metabolites, whereas a scaling factor of 0.75 has been supported for stable metabolites (Clewell *et al.*, 2002; Kirman *et al.*, 2003, etc.). By adopting a scaling factor of 1.0, the toxicokinetic and toxicodynamic factors are each set equal to a value of 1.0 (square root of 1.0).

Rather than normalizing the hemoglobin adduct data to administered dose, to normalize them in terms of internal dose is more defensible. For a given internal dose of AA [based upon AA valine adducts (radiolabeled or unlabeled) in each species], a higher internal dose of GA is observed in rats (0.75-0.83) compared to humans (0.10-0.44). These data suggest that the internal doses of GA experienced in humans are approximately half of those experienced in rats. Therefore, use of a kinetic factor of 1.0 is considered to be protective of human health.

Based upon these considerations, the interspecies adjustment factors should be one (1)

rather than factors of 3.2 and 2.9 for male and female rats, respectively. This change alone would result in an NSRL that is approximately 3-fold higher than calculated by OEHHA in the NSRL Document.

2.4. Role of statistical trend tests in addressing biologically-based dose-response

In the NSRL Document, the incidence data for each tumor site are subjected to a linear trend test which forces the data to fit a straight line and to determine whether the resulting slope is different from zero. Since the line is forced to remain straight, its slope is pulled up by the fact that tumor effects were a high-dose phenomenon with no corresponding tumors at low doses.

Based on the linear trend test, several types of tumors were considered to be "statistically significant" in the NSRL Document, even though the study authors concluded that there was no statistically significant increase in these tumors. The use of the linear trend test to identify which tumor types to use in risk assessment is of limited utility. The accepted practice is to compare various groups against the control group using standard statistical tests, not the linear trend test. Because of OEHHA's controversial use of the trend test, tumor types that would not normally have been included in a conventional cancer risk assessment, were combined and used to propose the NSRL. The trend test should not be used to identify additional tumor types, that otherwise were not significantly increased using appropriate statistical methods, to include in the estimate of cancer potency.

As a case in point, OEHHA's presentation of these trend test results is misleading in a fundamental way. The presentation appears to suggest or imply that AA causes cancer at low test doses and even at doses well below the observation range to where humans are presently exposed via the cooked foods. That would be a misuse of such results. Actually the trend test is unable to discern whether any effects are occurring at the lowest test doses or beyond the observation range. The results certainly do not incorporate any biological information such as the role of toxicokinetics in lowering the toxic potency of AA at doses experienced by humans in their diet. Furthermore, the application of the trend test provides no justification for the use of a linear model to extrapolate cancer incidence data from experimental levels to those in cooked foods.

2.5. Genotoxic vs. non-genotoxic mode of cancer action

The prevailing view expressed in the NSRL Document is that AA's mode of cancer action is likely attributable to the genotoxic properties of a reactive epoxide intermediate of AA, namely GA. OEHHA's proposed NSRL, which is based on an assessment of the toxic potency of AA, relies upon two central assumptions:

1) That the tumors observed in rats are relevant to human health and can be used to estimate risk in humans in a systematically predictable manner; and
2) That the nature of the dose-response relationship is linear for AA dose and tumor response from the high doses associated with tumors in rats to the low doses associated with human exposures.

Because of important species differences and of information that supports non-linearity, each assumption is questionable. In our view, the estimation of toxic potency (also referred to as dose-response assessment) is a scientific endeavor that should be policy neutral, that is, treating the data so as to neither overstate nor understate the toxic potency. Should policy considerations be desired for other ends, they should be proposed in a different context and treated expressly as extra-scientific. Even genotoxic mode of cancer action may have a nonlinear dose-response.

Although the precise mechanism by which AA produces tumors in animals is unknown, several modes of action (*e.g.*, genotoxicity, sulfhydryl reactivity, dopamine agonist, and endocrine activity) have been proposed. Once again, the proposed NSRL is based on an assumption that genotoxicity is the carcinogenic mode of action. This may or may not be true.

Although AA and its reactive metabolite GA are capable of forming DNA adducts, the binding does not occur in the basepairing region, and does not result in mutation in bacterial cells. DNA binding was observed in nearly all tissues examined from mice exposed either orally or dermally to AA (Carlson and Weaver *et al.*, 1985; Carlson *et al.* 1986). AA produces a number of cytogenetic effects (chromosomal aberrations, micro nuclei, and/or heritable translocations) in somatic and germ cells. Given AA's ability to interfere with nerve motor proteins (kinesin, dynein), it may also interfere with the kinesin-like proteins that are responsible for chromosome packaging and segregation. In germ cell lines, these effects may be due to an interaction between AA and protamine:DNA complexes, thereby not allowing DNA to decompress after fertilization (Sega, 1989).

Although it cannot be ruled out as a possible mode of action for some tumor types, the genotoxic potential of GA does not adequately explain the target organ specificity of AA carcinogenesis. Studies of genetic damage do not appear to be consistent with the results of tumor induction (Allen *et al.*, 2005). Although AA may be genotoxic at higher doses, carcinogenicity, particularly at lower doses, in rats may be due to a non-genotoxic mechanism. A non-linear relationship between low dose exposure and carcinogenicity seems likely, as discussed in greater detail in the next section. If the shape of the dose-response curve is non-linear, the correct NSRL could be two or more orders of magnitude higher than the proposed NSRL.

2.6 Consideration of Detoxification

The dose-dependency and species differences associated with key detoxification processes for AA and GA are essential in understanding the relative safety of AA in cooked foods. The following describes information that supports the proposition that cancer is a high dose phenomenon for AA in cooked foods. Our analysis includes first the examination of observations from rodent studies followed by an indication of their applicability to humans.

2.6.1 Dose-Dependency of Detoxification

For all carcinogens including AA, including those with a genotoxic mode of action, several processes can contribute to a nonlinear dose-response. Kinetic factors such as the presence of a saturable metabolic pathway, saturation of binding sites, the depletion of metabolism cofactors, or the induction of metabolizing enzyme at high doses can all contribute to non-linearity across a range of doses. Similarly, dynamic factors such as receptor binding, capacity-limited repair processes, and the introduction of threshold responses (*e.g.*, cytotoxicity), contribute to non-linear dose-response relationships. A recent risk assessment for ethylene oxide, which is similar to GA in that both are reactive, genotoxic epoxides, demonstrates a nonlinear dose-response relationship for exposures to this chemical and cancer (Kirman *et al.*, 2004).

With respect to AA, several toxicokinetic factors contribute to a non-linear dose-response for AA exposure and tumor formation in rats, including:

1) Saturation of binding to blood proteins (hemoglobin and albumin).

2) Saturation of oxidative metabolism, that affects the formation of GA; and

3) Depletion of tissue glutathione levels, that affect the detoxification of both AA and GA.

We submit that the dose-dependency (*i.e.*, saturation) of binding to blood proteins contributes to an important source of non-linearity in the dose-response relationship for tumor production in rats.

By irreversibly binding (*e.g.*, adduct formation) to two specific blood proteins (*i.e.*, hemoglobin and albumin), much less AA and GA are available for distribution to tissues, including those identified as targets of AA chronic toxicity and carcinogenicity. The data from Barber *et al.* (2001) indicate that prior oral exposures to high doses of AA (20 mg/kg) resulted in significantly higher internal doses of AA and GA with subsequent oral exposures (based upon plasma AUC values reported), suggesting that saturation of hemoglobin as a "protective sink" in blood can increase the risk for adverse effects in other tissues. The human equivalent doses used by OEHHA in their dose-response modeling efforts to estimate the cancer potency of AA do not reflect this important source of non-linearity.

Data from Ramsey *et al.* (1984) suggest that saturation of hemoglobin binding in red blood cells was reached following 3 doses of 30,000 mcg/kg — a cumulative dose of 90,000 mcg/kg (*i.e.*, subsequent daily doses of 30,000 mcg/kg on days 4 through 13 did not increase the binding of radiolabel to red blood cells). Based upon a consideration of the doses tested in the two cancer bioassays for AA (Johnson *et al.*, 1986; Friedman *et al.*, 1995), the number of months estimated to reach this saturation dose for each dose level presented is presented in **Table 4**.

Table 4. Assessment of Non-Linearity in the Dose-Response Relationship for Tumor from AA Exposure Resulting from the Saturation of Hemoglobin Binding in Rats

	Dose (mcg/kg-day)					
	10	100	500	1,000	2,000	3,000
Estimated Months of Exposure Needed to Reach a Cumulative Saturation Dose of 90,000 mcg/kg*	300	30	6	3	1.5	1
Is the Saturation of Hemoglobin Anticipated During a 24-Month Lifetime Exposure?	No	No	Yes	Yes	Yes	Yes
Is this aTumorigenic Dose in Rats (Bioassay)?	No[1]	No[1,2]	No[2] Yes[1]	Yes[2]	Yes[1,2]	Yes[2]

[1] Johnson *et al.*, 1986

[2] Friedman *et al.*, 1995

Based upon this analysis, the dose level of 100 mcg/kg-day can be considered as a threshold for tumorigenesis in rats, and is supported by both cancer bioassays (Johnson *et al.*, 1986; Friedman *et al.*, 1995). Accordingly, OEHHA should adopt a "non-linear" method (*e.g.*, consistent with biological threshold) for extrapolating the potential risks from high-dose rat studies to the low doses (*e.g.*, 100 mcg/kg-day; equivalent dose for a 70-kg human of 7,000 mcg/person-day) associated with human dietary exposures.

2.6.2 Species Differences in Detoxification

Differences in detoxification of AA and GA have major impacts on defining safe levels of AA and in estimating a NSRL for AA.

Epoxide Hydrolase Activity

Epoxide hydrolase (EH) is an important detoxification enzyme responsible for the hydrolysis of reactive epoxides such as GA. Although the role of EH in the detoxification of AA and GA has not yet been thoroughly investigated, important differences are likely between rats and humans for this enzyme system based upon data available for structurally similar chemicals. Specifically, EH is active in human liver and is normally absent in rat liver (but is inducible) in catalyzing the hydrolysis of the reactive epoxide metabolite of acrylonitrile, 2-cyanoethylene oxide (Kedderis and Batra, 1993). The presence of an active epoxide hydrolase hydrolysis activity in humans (and its absence in rats) needs to be considered in assessments of cancer risk from AA exposure, as has been done for acrylonitrile (Kirman *et al.*, 2005 - in press).

Blood Protein Binding

There are important species differences in blood protein binding that may have a large impact on the relative susceptibility of rats and humans. Information regarding species differences in hemoglobin and albumin are summarized below.

Hemoglobin — Hemoglobin is the predominant protein present in red blood cells, and readily form adducts with AA and GA. Although binding rates for AA and GA to the terminal valine of hemoglobin are comparable between rats and humans, binding rates for cysteine are dramatically different, the rates in rats exceeding those in humans by approximately 2 orders of magnitude (Bergmark *et al.*, 1993). This explains why such a large fraction of administered dose (>10%) in rats can be measured in red blood cells (Miller *et al.*, 1982). Hemoglobin adducts represent a larger fraction of an administered in rats rather than humans, due largely to the presence of a highly reactive cysteine (Miranda, 2000).

Albumin — Albumin is the predominant thiol-containing protein in serum (extracellular), and forms adducts with AA (and likely GA as well). The binding rate for AA to human albumin was reported to approach that for the predominant intracellular thiol-containing protein, glutathione (Tong *et al.*, 2004). Similar data are unavailable for GA or for binding of either chemical to rat serum albumin. However, data for other chemicals suggest that binding to serum albumin would be greater in humans than in rats (Freeman *et al.*, 1998; Dale and Nilsen, 1984; Lindstrom *et al.*, 1998).

Taken together, these data suggest that a fundamental difference exists between rats and humans with respect binding to blood proteins, with rats relying primarily upon hemoglobin, and humans relying primarily upon albumin as "protective sinks" for adduct formation.

2.6.3 Impact of Species Differences in Detoxification on the NSRL

Because of fundamental differences between rats and humans with respect to the detoxification of AA and GA, the rat tumor data are of questionable relevance to human health, and their use serves to overestimate to potential risks to human populations exposed to low doses of AA in the diet. Prior to developing an NSRL for AA, OEHHA should seek a more thorough understanding of these fundamental species differences, and to the extent possible encode them into a physiologically-based pharmacokinetic (PBPK) model prior. A PBPK model has been developed for the AA and GA in the rat (Kirman *et al.*, 2003), and with the collection of a little more data, could be refined for the purposes of quantifying these important differences in a health risk assessment.

2.7. Value of epidemiology data on the weight-of-evidence

OEHHA used the data for pancreatic cancer mortality of Marsh *et al.* (1999) as reanalyzed by Schulz *et al.* (2001) to derive an alternative potency estimate to provide semi-quantitative support to the NSRL value. The trend for pancreatic cancer mortality from Marsh *et al.* (1999) is not convincing since it is non-monotonic (*i.e.*, does not increase consistently with increasing dose), but becomes monotonic when the two lowest groups were combined by Shulz *et al.* (2001). Inspection of these indicates that a total of 44 pancreatic deaths were observed as compared to a total of 45.3 deaths expected, and, therefore, a small deficit of pancreatic cancer deaths in exposed workers was observed. This deficit serves to indicate that no dose-response relationship exists from which to derive an NSRL.

When stratified into three groups by cumulative exposure, a marginally significant excess of pancreatic cancer deaths in the high exposure group (SMR = 2.26 with a 95% CI of 1.03 to 4.29) is accompanied by a substantial deficit of cancer deaths in the low exposure group (SMR = 0.8 with a 95% CI of 0.54 – 1.14). Based upon these considerations and upon the epidemiology results as recently reviewed (Rice, 2005; Erdreich and Friedman, 2004), use of these data either qualitatively or quantitatively in support of an NSRL value is inappropriate, and as such should be removed from the NSRL documentation.

OEHHA has included this ARL as a means of suggesting that their proposed NSRL of 1 µg/person-day could have been reduced further, had OEHHA relied predominantly upon epidemiologic findings. However, the epidemiologic data say the contrary and support the prospect of a higher NSRL than that currently proposed.

3.0 SUMMARY AND CONCLUSIONS

OEHHA has proposed revising the NSRL for acrylamide (AA) from 0.2 to 1 microgram (mcg) per person-day based on an estimate of the theoretical cancer potency for humans from studies in rats. Our analysis concludes that a significantly higher NSRL, perhaps 100 mcg per person-day or more, is scientifically more appropriate, based on several lines of evidence.

The NSRL as currently proposed is seriously flawed for several reasons. First, the two carcinogenicity studies in rats relied upon to derive the proposed NSRL have serious problems in their conduct, which creates substantial questions as to the validity of the findings, particularly for purposes of quantitative risk assessment. Based on this observation, we recommend that OEHHA wait for the results of ongoing studies to determine the appropriate NSRL for acrylamide. Should OEHHA choose to proceed, however, the following points become not only germane but essential for OEHHA to consider.

OEHHA combined tumors in a manner that is contrary to well accepted scientific principles. When these malignant tumors are combined properly and a more appropriate method of extrapolation is applied (non-linear dose response, adopting a margin of safety of 1,000), the NSRL for AA is estimated to be 226 mcg/person-day.

The proposed NSRL improperly uses allometric scaling to convert doses in rats to those in humans. The proposed NSRL assumes that an additional adjustment factor of 3 is necessary to account for interspecies differences. However, the scientific evidence indicates that this factor should be one or less. This change alone would result in an NSRL that is at least 3-fold higher than the proposed NSRL.

A statistical trend test was used to analyze the tumor data that assumes a linear dose-response relationship. OEHHA concluded that increases in several tumor types, which were not statistically significant using appropriate statistical tests applied by the study authors, were statistically significant using a less appropriate linear trend test. These "newly-significant" tumors were also combined with the other tumors to estimate risk, thus compounding the errors. Equally importantly, the supporting documentation for the NSRL is misleading because it suggests that the linear trend is equivalent to a linear dose-response at all doses.

The proposed NSRL assumes that AA's mode of cancer action is attributable to the genotoxic properties of a reactive metabolite, GA. The acceptance of a genotoxic mode of action is is wholly consistent with non-linear mode of carcinogenesis, which OEHHA fails to recognize. Even if one were to assume a genotoxic mode of action, the dose-response relationship would not likely be linear, as assumed in deriving the proposed NSRL.

Next, the shape of the dose-response curve for AA is influenced by many factors, some of which are apt to be more important at low doses typical of human dietary intake than high experimental doses in rats. Notably, binding of AA and GA to blood proteins (hemoglobin

and albumin) is an important detoxification mechanism. The proposed NSRL does not consider the critical role of binding to blood proteins as a detoxification mechanism, particularly at low doses experienced by humans. Saturation of binding to blood proteins contributes an important source of non-linearity in the AA dose-response relationship in rats at high doses, and it justifies the use of a non-linear model to extrapolate to humans exposed to much lower doses via the diet.

And finally, it is highly inappropriate to use the re-analysis of the pancreatic cancer data from the Marsh *et al.* (1999) epidemiological study to "validate" the proposed NSRL. The NSRL Document is on shaky scientific ground in using the pancreatic cancer data for quantitative risk estimation and toxic potency assessment. In comparison, all of the epidemiogical studies of dietary exposure to AA consistently show no increase in cancer. The overall weight of the epidemiological evidence does not suggest that exposure to low levels of AA in the diet increases human cancer risk.

In conclusion, the proposed NSRL of one (1) microgram of AA per person-day is flawed, scientifically inappropriate, and unsupported. When all of the above factors are taken into consideration, OEHHA would be prudent to wait till all relevant data were obtained; however, if not, it is apparent that a significantly higher NSRL for AA is scientifically more appropriate.

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