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Acrylamide: Consideration of species differences and nonlinear processes in estimating risk and safety for human ingestion

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ABSTRACT

Acrylamide in cooked foods results in wide-spread, low-level human exposure. Potential risks from dietary intake remain unclear due to apparent conflicting results from cancer bioassays conducted in rats that reported tumors and epidemiology studies that are suggestive but provide little or no evidence of increased cancer. Risk estimation often includes two common assumptions: (1) tumor response rates in test species can be extrapolated systematically to estimate human response rates and (2) tumor rates observed following high-dose exposures can be linearly extrapolated to predict response rates following low-dose exposures. The validity of these assumptions was evaluated for acrylamide based upon the examination of relevant toxicokinetic and toxicodynamic differences between humans and rats, including sources of nonlinearity that modify high to low dose extrapolation of cancer incidence. Important species differences and sources of nonlinearity are identified, and recommendations for addressing them within the quantitative framework of a PBTK/TD model are discussed. These differences are likely to estimate risk levels up to several orders of magnitude lower in humans than in rats. Quantitative inclusion of these TK/TD factors will more closely estimate actual human cancer risk derived from high-dose rodent studies, since detoxification processes for acrylamide and glycidamide appear adequately protective against toxicity from human dietary doses.

1. Introduction

Acrylamide (AA) was found several years ago to be formed in assorted carbohydrate-rich foods when cooked at high temperatures (Tareke et al., 2002). In addition, small amounts of AA-based polymers are used in some water systems to treat water prior to distribution to the general population; some very small amounts of monomer may be present in water consumed from the tap. As a result, most humans are exposed repeatedly to low levels of AA from their diet and other sources such as inhalation of tobacco smoke. With respect to its toxicity, the weight-of-evidence for AA from epidemiology studies and from laboratory rodents provides apparently conflicting results. AA has been shown to cause neurotoxicity in laboratory animals and humans, as well as to cause tumors at multiple sites in laboratory rodents exposed to chronic, high doses (Johnson et al., 1986; Friedman et al., 1995). However, results from epidemiologic studies indicate that repeated and prolonged ingestion of AA at current levels in foods and tap water fails to produce any measurable neurotoxicity or increase in cancer morbidity or mortality (Collins et al., 1989; Swaen et al., 2007; Marsh et al., 1999, 2007; Mucci et al., 2003, 2004, 2005, 2006; Mucci and Wilson, 2008; Pelucchi et al., 2006, 2007; Erdreich and Friedman, 2004; Hagmar and Tornqvist, 2003; Hogervorst et al., 2008a,b). Olesen et al. (2008) reported no statistical association between AA-or glycidamide (GA)-hemoglobin levels and estrogen-receptor-positive breast cancer. Yet, after attempting to adjust for smoking without addressing secondary smoke, a weak statistical association was found; however, such adjustment for smoking could not include consideration of indirect exposures to tobacco smoke, strongly suggesting that the association was of questionable significance. Hogervorst et al. (2007) found a minor statistical association between dietary AA intake and ovarian cancer; however, it was only at the highest level of intake (0.53 μg/kg bw-day). An additional slight statistical association was found for endometrial cancer, but only after numerous adjustments for age at menarche, age at menopause, age at first childbirth, parity, use and duration of oral contraceptives, smoking status, alcohol consumption, and

Abbreviations: AA, acrylamide; AAMA, N-acetyl-S-(2-carbamoylethyl)-L-cysteine; AUC, area under the curve; TD, toxicodynamics; EH, epoxide hydrolase; GA, glycidamide; GAMA, N-(R/S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; GSH, glutathione; GST, gluathione-S-transferase; NTP, National Toxicology Program; PBTK, physiologically based toxicokinetics; PD, pharmacodynamic.

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other factors. Hogervorst et al. (2008a) found, among several non-associations, a slight association between the highest level of acrylamide intake (~57 μg/kg bw-day) and renal cell carcinoma; however, this very slight association was noted only after the authors attempted to adjust for smoking, hypertension, body mass index, and fruit and vegetable consumption.

One possible explanation for the apparent disparity between animal and epidemiology data is that the adverse effects of AA are present in the cohorts studied, but cannot be observed in the study populations due to limitations in statistical power to detect pathologic events. Alternatively and equally plausibly, the disparity may be due to invalid assumptions made in risk assessments. Two common assumptions in human health risk assessment include (1) tumor and other pathology response rates in a test species can be extrapolated in a systematic manner to predict response rates in humans, which inherently assumes that no important qualitative or quantitative differences exist between species and (2) tumor and other pathology response rates observed following high-dose exposures can be extrapolated to predict response rates following low-dose exposures, which inherently assumes that no important sources of nonlinear toxicokinetics (e.g., metabolic saturation, cofactor depletion, saturation of binding sites) or toxicodynamics (e.g., threshold, repair) are present. Detoxification processes for AA and GA (e.g., hydrolysis, glutathione (GSH) conjugation, binding to certain macromolecules such as hemoglobin) that are quite adequate and protective against toxicity at lower doses, such as those encountered by humans in their diets, can be overwhelmed at the much higher doses of AA typically used in animal bioassays.

The following analysis focuses on the validity of these two assumptions for AA cancer risk assessment by identifying important toxicokinetic/toxicodynamic differences between rats and humans, and toxicokinetic/toxicodynamic sources of nonlinearity in the dose–response curve for AA exposure and tumor formation. We propose that future risk assessments and analyses of causation for AA (Guzelian et al., 2005) are best accomplished by assessing the data and processes within a quantitative framework, such as PBTK/TD modeling, so more meaningful comparisons of risk can be made between the species of interest.

2. Species differences

Information regarding toxicokinetic and toxicodynamics differences between rats (test species for cancer bioassays conducted to date) and humans (species of interest for human health risk assessment) is discussed below. Potential differences between mice and humans are not included, since no chronic toxicity studies of AA have yet been reported in mice (although as discussed below, one is ongoing).

2.1. Toxicokinetic differences

Except for the differences related to the metabolism of AA and its metabolites, few qualitative differences are expected between humans and rats with respect to absorption, distribution, and excretion, in part because AA is highly water soluble. However, in humans uptake of AA by children was found to be higher (1.3–1.5-fold) than that by adults (Hartmann et al., 2008). More importantly, differences between rats and humans are expected with respect to AA metabolism that generates the toxicologically significant GA. Metabolism of AA proceeds via two pathways: (1) saturable epoxidation by cytochrome P450 to produce GA and (2) conjugation with GSH either nonenzymatically or catalyzed by glutathione-S-transferase (GST) to ultimately yield N-acetyl-S-(3-amino-3-oxopropyl)cysteine (Fig. 1). GA in turn reacts with GSH to yield N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine and N-acetyl-S-(carbamoyl-2-hydroxyethyl)cysteine, or undergoes hydrolysis via epoxide hydrolase (EH) forming 2,3-dihydroxypropionamide (Fig. 1). Differences between rats and humans have been reported with respect to three metabolic pathways: (1) oxidation of AA by cytochrome P450; (2) conjugation of AA and GA with GSH; and (3) hydrolysis of GA by epoxide hydrolase, as discussed below.

2.1.1. Cytochrome P450 epoxidation

Human daily dietary exposures to AA in the US and EU range from an average of 1 μg/kg to a 4 μg/kg for highly exposed individuals (WHO/FAO JECFA, 2005). Differences in the whole-body oxidation of AA to GA in humans and rats have been identified and quantitated using urinary metabolite profiles from ^{13}C NMR (Fennell et al., 2005; Fennell and Friedman, 2005). Rats dosed via gavage with 3 mg/kg 1,2,3-^{13}C3 AA had 41% of the metabolites in urine derived from GA-derived mercapturic acids (Fennell and Friedman, 2005), while human volunteers, also dosed once with 3 mg/kg 1,2,3-^{13}C3 AA, had 14% of their metabolites in the urine at 24 h derived from GA (Fennell et al., 2005). Rats exposed to a single gavage dose of 50 mg/kg 1,2,3-^{13}C3 AA had 28% of their urinary metabolites derived from GA, indicating a dose-dependent decrease in oxidation as AA dose was increased (Sumner et al., 1992). Human exposures were not conducted at doses higher than 3 mg/kg, so no direct comparisons with rats can be made; thus while no direct evidence of dose-dependent changes was observed in fractional AA oxidation in humans, the finding of dose-dependence in rats indicates also the likelihood of its occurrence in humans. However, clearly rats oxidize approximately three times more AA to GA than humans at comparable doses within the range of human dietary exposures. Settels et al. (2008) confirmed *in vitro* that AA was metabolized to GA by CYP2E1 in human liver microsomes.

2.1.2. Glutathione-S-transferase

Species differences are also present in the conjugation of GSH with AA. Fennell and Friedman (2005) found that 59% of the urinary metabolites originated from AA and GSH conjugation in rats dosed with 3 mg/kg 1,2,3-^{13}C3 AA, while humans were found to excrete approximately 30% more (i.e., 86%) of their total urinary metabolites as conjugates AA with GSH at this same dose (Fennell et al., 2005). Humans appear to conjugate approximately 30% more of an equivalent dose of AA than do rats, thus producing lower amounts of GA. GSH also conjugates GA in both rats and humans, with rats excreting almost 20% of their urinary metabolites following a dose of 3 mg/kg as GSH conjugates of GA (Fennell et al., 2005). At this same dose, humans excreted GSH-GA conjugates at levels below the limit of quantitation (i.e., at least in an order or magnitude less than rats) (Fennell and Friedman, 2005). Also, lifestage differences in GSH conjugation exist between human neonates and adults, and are described later herein (Walker et al., 2007).

2.1.3. Epoxide hydrolase

A significant species difference has been found in the hydrolysis of GA in rats and humans. While very little GA produced by humans dosed with 3 mg/kg is conjugated with GSH, the majority is hydrolyzed by EH (11% of the urinary metabolites; see Fennell and Friedman, 2005). Rats that were administered a gavage dose of 50 mg/kg, on the other hand, were found to excrete only 1.2% (or approximately 10% that for humans) of their urinary metabolites as the hydrolysis product of GA (Sumner et al., 1992).

The species differences described above for the GSH and EH reactions with GA are consistent with human and rat metabolism found with other similar epoxides. For instance, rat liver cytosols metabolize cyanoethylene oxide, the epoxide formed from acrylonitrile oxidation, via GSH conjugation at a rate about nine times faster than found in human liver cytosols (Kedderis et al., 1995),