REVIEW

Mode-of-Action Framework for Evaluating the Relevance of Rodent Forestomach Tumors in Cancer Risk Assessment

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Studies have shown that a majority of known human carcinogens also cause cancer in laboratory animals. The converse, however, is not as well established—known animal carcinogens are not equally predictive of human carcinogenicity. A particularly controversial aspect of interspecies extrapolation is application of rodent forestomach tumor data for predicting cancer risk in humans, given that a human counterpart for the rodent forestomach does not exist. Proliferative lesions in the rodent forestomach may result from a combination of factors related to route-specific tissue irritation and/or unnatural dosing regimens and are less likely to be relevant in evaluating human carcinogenic potential, particularly when tumors are exclusive to the forestomach. We review the comparative functional anatomy, physiology, tumor biology, tissue concordance, and historical regulatory practices in the use of forestomach tumors for cancer risk assessment, examining specific chemical examples. We also propose a standardized mode-of-action approach that combines multiple risk characterization criteria, including relevance to human exposure conditions, physiologically based toxicokinetics, genotoxicity, and comparative/mechanistic toxicology. Forestomach tumors associated with chronic irritation of the forestomach epithelium, particularly those induced by repeated oral gavage dosing, should not form the basis for carcinogenic classification or quantitative cancer potency estimates for humans. Genotoxic chemicals and those that cause tumors at multiple sites, at doses at or below the maximum tolerated dose, and in the absence of forestomach irritation, are more likely to be relevant human carcinogens. Cancer risk assessment that utilizes forestomach tumor data should consider relevant human exposures, systemic bioavailability, tissue dosimetry and concordance.

Key Words: forestomach tumors; cancer risk assessment; mode-of-action.

Most recognized human carcinogens cause cancer at the same anatomical site in laboratory animals. 2-Naphthylamine, for example, is known to cause bladder cancer in humans based on epidemiological studies of workers with occupational exposure to 2-naphthylamine, such as through work with dyestuffs (IARC, 1987). In animals, oral administration of 2-naphthylamine causes malignant bladder tumors. Thiotepa, another known human carcinogen, is associated with leukemia in humans and is also carcinogenic at multiple sites in both sexes of rats and mice (IARC, 1975, 1990a; NCI, 1978). Ip injection of thiotepa in rats and mice causes lymphoma or lymphocytic leukemia and benign lung tumors. In addition, in both humans and rodents, vinyl chloride causes angiosarcoma, and benzene causes leukemia.

The converse, however, is not as well established—chemicals known to cause cancer in laboratory animals, and under standardized testing conditions, are not always relevant for carcinogenicity in humans because of species-specific differences in the mode-of-action. For example, alpha 2-l-globulin–induced tumors in the male rat kidney (d-limonene, isophorones) do not predict human kidney cancer (USEPA, 2005). Additionally, reactive hyperplasia from cytotoxicity in the male rat bladder from exposure to chemicals such as saccharin or melamine has not been replicated in the human bladder. Also, sustained excessive hormonal stimulation of the thyroid by goiterogens causes thyroid cancer in animals but not in humans as with ammonium perchlorate.

This review discusses several issues pertinent to the relevance of rodent forestomach tumors in cancer risk assessment. As part of the discussion, we provide an overview of the anatomy, physiology, and tumor biology of the rodent forestomach with comparisons to human tissues and organs. In addition, we review current and historical practices of researchers and regulatory agencies pertaining to the use of rodent forestomach tumors for human cancer risk assessment focusing on specific chemicals as examples. Based on this review, we also propose a mode-of-action decision framework for considering rodent forestomach tumor data in the...
classification of potential human carcinogenicity and for quantitative risk assessment.

ISSUES RELEVANT TO THE USE OF RODENT FORESTOMACH TUMORS IN CANCER RISK ASSESSMENT

While squamous cell carcinomas of the forestomach have been observed in several rodent studies, their relevance for human risk estimation has been considered questionable for many years (Wester and Kroes, 1988). Humans do not have a forestomach, but possess histologically similar organs, including the oral cavity, pharynx, esophagus, and glandular stomach, yet tissue dose in these human organs are not equivalent to that in the forestomach of experimental animals.

The underlying mechanisms of forestomach tumorigenicity have not been completely elucidated. Evidence exists for both genotoxic and nongenotoxic mechanisms of action with different core genetic alterations. For example, genotoxic agents such as 1,3-butadiene and N-methyl-nitrosourea induce tumors by interacting with DNA and causing irreversible genetic alterations, while agents such as butylated hydroxyanisole and ethyl acrylate cause reversible hyperplasia and papillomas. Upon chronic gavage dosing at maximum tolerated doses (MTDs), these compounds cause irreversible forestomach carcinomas (Kaneko et al., 2002). Consistent with tumors at other sites, forestomach carcinogens that appear to act through genotoxic mechanisms involve cell proliferation as well as genetic changes in oncogenes and tumor suppressor genes. For chemicals that tend to act through nonmutagenic mechanisms, chronic inflammation or local irritation of forestomach mucosa due to physical trauma, or chemical-induced irritation and/or ulceration associated with high-dose regimens, may lead to continuous induction of cell proliferation, hyperplasia, and ultimately carcinomas.

In assessing the relevance of rodent forestomach tumor studies to human cancer risk assessment, four issues should be considered: (1) the method of administration and dose level (dose and route extrapolation) and systemic bioavailability, (2) the specificity of the carcinogen to the forestomach (tissue specificity), (3) the applicability of the forestomach to human organs (tissue concordance), and (4) the mode-of-action for tumor formation (genotoxic vs. nongenotoxic mechanisms).

1. Dose and Route Extrapolation

Oral gavage is a method by which a liquid test substance is dosed using a stomach tube or cannula. Generally, the maximum volume of liquid that can be administered at one time depends on the body weight of the test animal. A “good practice” dose volume through oral gavage does not exceed 1–2% of body weight (Diehl et al., 2001). However, larger dose volumes (and high concentrations), which are typical of poorly soluble chemicals, result in doses that far exceed normal environmental exposure conditions (i.e., from drinking water) and may exceed the MTD. Moreover, high dose volumes increase the risk of the chemical reflux into the esophagus or result in unusually long retention times. Finally, the physical nature of repeated administration of excessive dose volumes through the use of an oral gavage needle can result in irritation to the forestomach mucosa and/or abnormal compound absorption (Frederick et al., 1990; Hull, 1995; Poet et al., 2003).

For these reasons, a critical issue in assessing the relevance of rodent forestomach tumors to human cancer risk assessment is whether repeated oral gavage dosing during 2-year bioassays appropriately replicates conditions that are comparable to human exposures. While gavage dosing may represent the manner in which humans ingest certain pharmaceuticals, it is questionable whether rodent forestomach tumor studies with repeated oral gavage dosing are applicable to human cancer assessment of chemical contaminants in environmental media because neither the method of administration nor the administered dose approaches true-life human conditions of exposure.

An important component of our proposed mode-of-action analysis is whether the administered doses exceed the MTD, and whether the dose and delivery conditions are relevant to human exposure conditions. Irritant and mutagenic effects that occur only at doses that are irritating or otherwise unrealistic should be recognized as such in any assessment of an environmental hazard. Studies of ethyl acrylate provide an example of a chemical that was initially considered to pose a potential cancer risk to humans based on forestomach tumors from repeated gavage dosing, but was later demonstrated an effect specific to the gavage administration, such that the carcinogenicity classification was modified (NTP, 2000).

2. Tissue Specificity

A total of 120 substances have been identified as carcinogetic to the forestomach in rodents including rats, mice, and/or hamsters and are listed in the Carcinogenic Potency Database (CPDB) or with the U.S. National Cancer Institute National Toxicology Program (NTP). The CPDB includes results from studies of almost 1500 substances, while the NTP has tested over 400 chemicals for carcinogenicity. Of 144 chemicals tested by NTP for carcinogenicity as administered by oral gavage, 17 chemicals (12%) produced benign or malignant forestomach tumors in rats and mice (IARC, 2003). Although it should be considered that the physicochemical properties of chemicals administered via gavage may differ from those administered in diet (e.g., chemicals unstable in feed are more likely to be administered via gavage and are also more likely to be irritating to forestomach mucosa), of the chemicals administered by other routes, only 2% produced forestomach tumors.

Among the 120 forestomach carcinogens, an overwhelming majority of 101 chemicals (84%) also induced tumors at other sites, while only 19 chemicals (16%) induced tumors exclusively in the forestomach. Also, of 48 chemicals found to
cause forestomach tumors, 31 (64%) were administered y gavage (NTP, 2007). The forestomach is a target tissue for chemicals administered orally but also by other routes of administration. Sixteen of the 120 chemicals (13%) produced forestomach tumors when administered by inhalation, skin painting, buccal painting, sc injection, or ip injection. Chemicals that induce tumors at multiple sites through a single exposure pathway and induce forestomach tumors that are not route specific are more likely to be human carcinogens (Jackson et al., 1997). One such example is vinyl chloride, a known human carcinogen, which induces tumors following ingestion in animals at multiple sites including the forestomach.

Tissue specificity is an important consideration for carcinogenic classification, in that chemicals known to cause cancer only in the forestomach via ingestion should have a recognized mode-of-action relevant for human tissues. Furthermore, tumor data from sites other than the forestomach should be considered with greater weight in dose-response modeling because forestomach tissue dose is not representative of tissue dose for any potentially relevant human tissue. For example, the dose to forestomach tissues is likely much greater than tissue dose to the esophagus or glandular stomach of humans due to significant differences in exposure (gastroesophageal transit) time and tissue mass.

3. Tissue Concordance

In toxicology, the potency of a chemical is defined in terms of the dose of a chemical that will produce a specific response in a specific biological system. Dose-response profiles for a chemical are tissue specific (Klaassen, 1996). When evaluating the applicability of rodent forestomach tumors to human cancer risk assessment, it is important to consider that humans do not have a functional analogue of a rodent forestomach. While humans possess histologically related organs such as the esophagus and stomach, the tissue dose in these organs is not equivalent to that in the rodent forestomach. For instance, tissue exposure in the human esophagus is likely to be minimal compared to tissue exposure in the rodent forestomach. Chemicals pass through the esophagus quickly, thus limiting exposure time. In contrast, the forestomach is a holding compartment, which allows the tissue considerably longer exposure to the chemical. Mode-of-action analyses that consider rodent forestomach tumors should identify the applicable human organ, consider the differences in dosimetry between tissues, and describe the biological basis for relevance. The following sections detail the anatomical and physiological differences between the rodent and human digestive system, as well as differences in tumorigenicity.

COMPARATIVE ANATOMY AND PHYSIOLOGY OF HUMAN AND RODENT DIGESTIVE SYSTEMS

The human digestive system is a complex series of organs, including the esophagus, stomach, small and large intestines, and cecum (Fig. 1). The human stomach is anatomically a single (monogastric) organ lined by a mucosal layer of over 35,000 gastric glands that are folded into ridges.

The digestive systems of rats, mice, and hamsters, which are commonly used in cancer research, share similarities with that of humans but also have distinct differences. Notably, the rodent stomach is multigastric, divided into a proximal, nonglandular forestomach and a distal, glandular stomach (Fukushima et al., 1997). A limiting ridge formed by thickened lamina propria of the forestomach separates these two regions. The mucosa of the rat forestomach is lined with a stratified squamous epithelium. It is heavily cornified, 2–3 layers thick, and devoid of glands and muscularis mucosa. The forestomach constitutes approximately three-fifths of the rodent stomach area and functions as a food reservoir (Nagayu, 1973). In small rodents, the esophagus leads to the forestomach, which then leads to the glandular stomach (Fig. 1).

The absence of a forestomach in humans should be an important consideration in assessing the applicability of rodent forestomach tumor data in human cancer risk assessment. While it is true that the human esophagus and glandular stomach are histologically similar organs, there are functional anatomical differences between these organs and the rodent forestomach, summarized in Table 1.

These structural differences may be particularly relevant in carcinogenicity associated with certain chemicals. In combination with the lack of a protective lining, higher pH levels in the rodent forestomach may contribute to a greater vulnerability to toxicity. The importance of understanding forestomach kinetics is exemplified through the kinetics of 2-butoxyethanol (BE), which is metabolized to 2-butoxyacetic acid (BAA) in the forestomach. Evidence suggests that mouse forestomach epithelial tissues have an increased capacity to metabolize BE to the highly irritating metabolite (BAA) and that the resulting irritation of forestomach tissues causes a nongenotoxic tumor formation (Boatman et al., 2004). Another example of the importance of understanding forestomach kinetics is reduction of hexavalent chromium to the less toxic trivalent form of chromium, which occurs most efficiently in the presence of stomach acid. Because it is devoid of gastric acid–secreting cells, the forestomach is likely more susceptible to tumor formation than the human stomach. These examples demonstrate that the pH conditions and metabolic processes of the rodent forestomach can represent an increase in the toxicity of certain compounds that does not occur in humans. The observation of forestomach tumors must be consistent with human metabolic processes for the mode-of-action to be relevant to cancer risk in humans.

Comparative Tumor Biology

Rat and mouse forestomach. In the forestomach and gastro-intestinal (GI) tract of small rodents, rates of spontaneous tumors vary by species but are relatively rare (Fukushima et al.,

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However, of the spontaneous GI tumors reported, forestomach tumors are the most common in both rats and mice. Proliferative responses in the forestomach following chemical administration are often associated with hyperplasia of the forestomach epithelium, a preneoplastic lesion leading to papillomas and carcinomas. Forestomach papillomas often appear as multiple benign tumors located near the limiting ridge (Fukushima et al., 1997). These tumors are either sessile or pediculated and are formed by hyperplastic squamous epithelial cells arranged in branched finger-like processes supported by stromal connective tissue and usually covered in a hyperkeratotic superficial cell layer. Cellular and structural atypia are absent in the papillomas.

Forestomach squamous cell carcinomas are sessile tumors projecting into the lumen, often with an ulcerated necrotic surface (Fukushima et al., 1997). Metastases to regional lymph nodes and other organs are often observed. These tumors may frequently invade all cell layers of the forestomach and extend into the glandular stomach (Nagayo, 1973). They are composed of squamous epithelium of various degrees of atypia, ranging from poorly differentiated (nonkeratinizing) to well-differentiated, keratinizing squamous cell carcinomas.

**Human esophagus and stomach.** Human organs histologically related to the rodent forestomach are the esophagus and the stomach. The two main histological types of esophageal cancer in humans are squamous cell carcinoma and adenocarcinoma (Adami et al., 2002). The esophagus is composed of smooth and striated muscle tissue, and the lumen of the esophagus is lined with squamous cell epithelium (Table 1). Glandular, columnar cell epithelia are absent in the esophagus. Thus, while the dominant histological type of esophageal cancer is squamous cell carcinoma, adenocarcinoma can still occur. The stepwise accumulation of genetic (somatic) mutations in the esophagus results in the development of both squamous cell carcinomas and adenocarcinomas. The non-genotoxic (epigenetic) mechanisms that result in hyperplasia of the rodent forestomach may not be involved in the development of human esophageal tumors.

Stomach cancer occurs almost entirely as a single histological type, with more than 95% of all stomach neoplasms being adenocarcinomas. While gastric adenocarcinomas can be classified in several ways, a common distinction is made between two main groups: the intestinal type, which is characterized by glandular epithelium composed of absorptive cells and goblet cells; and the diffuse type, which is characterized by poorly differentiated small cells with a noncohesive growth pattern (Adami et al., 2002). In addition to these two main groupings, tumors belonging to mixed types and unclassifiable types also occur. Irrespective of the histotype and
mechanisms of tumorigenesis, human stomach cancers are distinct from those associated with rodent forestomach tumors. Thus, there are significant differences between the functional anatomy and tumor histotypes of the rodent and human digestive systems. These differences should be considered in extrapolating the relevance of rodent forestomach tumorigens to the human digestive system.

Dosing Regimen and Epithelial Irritation

Direct doses to the forestomach as delivered by gavage far exceed those following a dosed feeding regimen or via drinking water. Thus, chemical administration by oral gavage is also likely to irritate the epithelial lining of the forestomach following the deposition of high doses directly in contact with the forestomach epithelium for long periods of time, which is neither representative of natural pathways of exposure nor relevant to human exposure conditions.

Local irritation to forestomach mucosa due to physical trauma by gavage needle, or chemical-induced irritation and/or ulceration associated with high-concentration dosing regimens, may cause local injury that stimulates cell division (compensatory hyperplasia). Irritation to the mucosa, as with oral gavage dosing, appears critical for carcinogenesis for some chemicals (e.g., ethyl acrylate), upon high-dose gavage but not at doses below the forestomach irritation threshold for drinking water exposures (Harrison, 1992; NTP, 2000). Dosing regimens resulting in chronic irritation should be considered above the MTD and contradictory to good laboratory practices (GLP) for cancer bioassays. The NTP's current study design is aimed at administering exposures that do not result in nonneoplastic lesions at the site of application (Bucher, 2000).

4. Genotoxicity

Understanding the mode-of-action and cause of forestomach tumors as either a genotoxic (or mutagenic) or nongenotoxic (not a promutagenic) mechanism is an important consideration for assessing the relevance of forestomach tumors to human cancer risk assessment. It should be recognized that genotoxicity is not always associated with mutagenic changes that are an essential property of tumorigenicity. Thus, labeling a chemical as genotoxic or nongenotoxic can be problematic in this framework and should be accompanied with an understanding of whether the DNA reactivity is relevant to carcinogenicity. International Agency for Research on Cancer (IARC) emphasized genotoxicity observed in in vivo systems in their review of the relevance of forestomach tumors (IARC, 2003). Chemicals that cause forestomach tumors through nongenotoxic mechanisms have typically not been considered relevant for human carcinogenicity (Clayson et al., 1990; IARC, 2003) because the mode-of-action identified is specific to the forestomach. Chemicals that are DNA reactive and cause tumors at multiple sites, in addition to the forestomach, are likely relevant human carcinogens. Thus, the most difficult challenge here is to understand the relevance of chemicals demonstrated to be genotoxic, yet only causing forestomach tumors. One such chemical is the highly reactive diethyl sulfate, an alkylating agent that causes local damage at the site of administration with only low doses distributed to other tissues.
In this case and likely others, systemic bioavailability is limited by the biological instability of the chemical. Thus, chemicals that have low systemic bioavailability due to metabolism in the stomach or liver, or are otherwise biologically unstable, are less likely to be systemic carcinogens; however, these chemicals should be considered potential site-of-contact carcinogens for exposures that might involve prolonged direct contact (e.g., dermal exposures). Toxicokinetics is an important component of human risk assessment for chemicals with low systemic bioavailability because the mode-of-action is likely to be relevant only if the tissue dose is sufficient in a relevant human tissue. Although not specific to the forestomach or to oral exposures, the toxicokinetics work of Conolly et al. (2004) and of Kimbell et al. (2003) to predict target tissue uptake of formaldehyde for human risk assessment provides a comparable example of physiologically based toxicokinetic modeling that may be used to assess both alternative dosimetrics and differences in tissue dose between species.

**RESEARCH AND REGULATORY PRACTICES FOR USING RODENT FORESTOMACH TUMORS IN HUMAN CANCER RISK ASSESSMENT**

**IARC Review**

IARC summarized the views and expert opinions of an IARC working group in its 2003 publication, “Predictive Value of Rodent Forestomach and Gastric Neuroendocrine Tumors in Evaluating Carcinogenic Risks to Humans” (IARC, 2003). The working group assessed the strengths of published scientific evidence from human epidemiology studies and animal bioassays to reach a consensus on the potential of chemicals and other agents to pose hazards to humans. With respect to the relevance of forestomach tumor induction in rodents to human cancer, the group concluded that the exposure conditions of the experiments must be considered. If the exposure conditions are unusual (e.g., as with oral gavage dosing, which may result in high local concentrations of test substances in the forestomach for prolonged periods of time), the responses observed may be unique to the forestomach. However, despite the specificity of conditions to the forestomach, the group concluded that DNA reactive chemicals causing forestomach tumors in rodents should be evaluated for their potential to pose a carcinogenic hazard to humans, even if the forestomach is the only site where the chemical caused cancer. Agents that produce tumors in rodent forest stomachs only after prolonged exposure through non-DNA reactive mechanisms, the group concluded, may be less relevant to humans because human exposure would have to overcome time-integrated dose thresholds to result in a carcinogenetic response. The group recommends that the evaluation of an agent should include the entire toxicological and metabolic evidence specific to the agent, and thus, consideration of whether genotoxicity is an essential property for tumor induction.

**U.S. Environmental Protection Agency Use of Forestomach Tumor Data for Qualitative and Quantitative Risk Assessment**

In early 2005, the U.S. Environmental Protection Agency (EPA) published revised “Guidelines for Carcinogen Risk Assessment,” (Guidelines) which provide a framework for EPA scientists to assess possible cancer risks from exposures to pollutants or other agents in the environment (USEPA, 2005). These new cancer risk assessment guidelines do not specifically address the use of rodent forestomach tumors but do discuss relevant issues, such as dosing, site concordance, and assessment of evidence of carcinogenicity. These issues have indirect implications for the use of rodent forestomach tumor studies and arguably for oral gavage dosing commonly used in such studies. For example, the Guidelines recommend that (1) studies in which excessively high doses were used should be interpreted with caution when extrapolating the results to humans and (2) studies that show tumors only at excessive doses may be compromised, and the results of such studies should generally not be considered if it is determined that the modes of action underlying the tumorigenic response at high doses are not operative at lower doses. The Guidelines also present criteria for assessing evidence of carcinogenicity that may be applied to instances when forestomach tumors are being studied. For example, the observation of tumors by more than one route of administration is more significant than by a single route, as is the observation of tumors in multiple species rather than in a single species. Finally, the Guidelines specify that site concordance of tumor effects between humans and animals should be considered in each case (USEPA, 2005).

While EPA has historically utilized rodent forestomach tumor data to characterize the carcinogenic potential of chemicals, use of the data varies greatly with marked differences depending on the chemical. In the case of certain carcinogens such as benzo(a)pyrene, acrylonitrile, propylene oxide, 1,2-dibromoethane, dichlorvos, and epichlorohydrin (Table 2), EPA has used the dose response observed for forestomach tumors alone, or in combination with that for tumors in other tissues, as the basis of quantitative risk estimates (i.e., to derive cancer slope factors). For other forestomach carcinogens, such as dichloropropene and vinyl chloride, EPA used cancers at other sites as the basis for a cancer slope factor respectively. However, for other carcinogens, such as ethyl acrylate, allyl chloride, and mercuric chloride, EPA either concluded that forestomach tumor data are irrelevant or did not use the available forestomach tumor data for other reasons to qualitatively or quantitatively describe the potential for carcinogenicity. Table 2 presents findings for the five chemicals for which EPA developed quantitative cancer risk assessments based on forestomach tumor data.
EPA approaches for assessing several example chemicals are described below. All data were collected from EPA’s Integrated Risk Information System (IRIS) database, which was accessed most recently for this paper in January 2007 (http://www.epa.gov/iris/).

**Benzo(a)pyrene.** Benzo(a)pyrene is currently classified by EPA as a Group B2—probable human carcinogen, based on sufficient evidence of carcinogenicity in animals (Table 2) and the absence of human data specifically linking human exposure to cancer. The slope factor for benzo(a)pyrene is based on a 1967 study by Neal and Rigdon (1967) that found squamous cell papillomas and carcinomas in the forestomach of male and female mice. Although only forestomach tumors were reported. However, epichlorohydrin also causes nasal cavity tumors at other sites following ingestion in rats—each of which showed a statistically significant increase. This was done separately for three individual studies, and from each study a slope factor was calculated. EPA then took the geometric mean of the slope factors as the slope factor for oral exposures to acrylonitrile.

**Acrylonitrile.** Acrylonitrile is rated as a B1—probable human carcinogen based on a statistically significant increase of lung cancer in exposed workers and observations of tumors in several tissues—most notably astrocytomas in the brain—in studies in two rat strains (Fisher-344 and Sprague-Dawley) exposed by various routes (drinking water, gavage, and inhalation). In the highest dose groups (100 ppm acrylonitrile in drinking water), Biodynamics Inc. (1980) observed an increase in squamous cell carcinoma of the forestomach. Forestomach tumors were also observed following administration of acrylonitrile in olive oil gavage (Maltoni et al., 1977). For the purpose of calculating the oral cancer slope factor, EPA combined the rates of tumors for brain and spinal cord astrocytomas, Zymbal gland carcinomas, and stomach papillomas/carcinomas from three individual studies. The geometric mean of the slope factors developed from the three studies was used as the oral slope factor.

**Epichlorohydrin.** Epichlorohydrin is a strong alkylating agent and a direct-acting mutagen known to produce forestomach tumors in rats following drinking water exposures. Wester et al. (1985) administered 0, 2, or 10 mg/kg/day epichlorohydrin by gavage to groups of 50 male and 50 female Wistar rats. The incidence of forestomach carcinomas was significantly increased in the high-dose rats. Konishi et al. (1980) reported similar results in male Wistar rats administered epichlorohydrin in drinking water. Tumors at other sites following ingestion have not been reported. However, epichlorohydrin also causes nasal cavity tumors following inhalation exposure and as such is described as a “site-of-exposure” carcinogen. EPA concludes that the effect on the forestomach is considered to be a local reaction but does not discuss the relevance, if any, to cancer in humans occurring from oral exposures.

### Table 2

Findings for Chemicals for Which EPA Has Developed Cancer Slope Factors Based on Forestomach Tumor Data

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Route of exposure</th>
<th>Slope factor (mg/kg day)</th>
<th>Cancer classification</th>
<th>Shown to cause tumors in other tissues by oral route</th>
<th>Slope factor based on forestomach tumors alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylonitrile</td>
<td>Drinking water</td>
<td>0.54</td>
<td>B1*</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>Diet</td>
<td>7.3</td>
<td>B2*</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Propylene oxide</td>
<td>Gavage</td>
<td>0.24</td>
<td>B2*</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1,2-Dibromoethane</td>
<td>Gavage</td>
<td>2</td>
<td>“Likely” human carcinogen</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>Gavage</td>
<td>0.29</td>
<td>B2*</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Epichlorohydrin</td>
<td>Drinking water</td>
<td>0.99</td>
<td>B2*</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Based on 1986 Cancer Classification Guideline (USEPA, 1986).
*Based on 1996 Cancer Classification Guideline; not rated under the 1986 Guidelines.
*Slope Factor is the sum of that for forestomach tumors, hemangiosarcomas, and thyroid follicular cell adenomas.
*Slope Factor is calculated by summing the rates of brain and spinal cord astrocytomas, Zymbal gland carcinomas, and stomach papillomas/carcinomas from three individual studies. The geometric mean of the slope factors developed from the three studies was used as the oral slope factor.
*Slope Factor is the sum of that developed for forestomach tumors, leukemia, and pancreatic cancer.
Propylene oxide. Propylene oxide is also classified by EPA as a Group B–probable human carcinogen (Table 2). The oral slope factor is based on a 1982 study in which squamous cell carcinomas of the forestomach were reported in female Sprague-Dawley rats dosed by gavage in a salad oil carrier (Dunkelberg, 1982). Propylene oxide demonstrates mutagenicity in a variety of test systems. Inhalation exposure results in nasal tumors in mice, and subcutaneous injection has been shown to cause tumors at or near the site of administration (Dunkelberg, 1982; NTP, 1985). However, following oral exposures, tumors have been reported only in the forestomach. Similar to epichlorohydrin, propylene oxide is considered to be a site-of-exposure carcinogen but has not been shown to cause tumors at distant sites.

Dichlorvos. The EPA cancer risk assessment for dichlorvos is unlike those conducted for other carcinogens. It is considered to be a B2 probable human carcinogen based on the observation of forestomach tumors in female and male mice, leukemias, and pancreatic acinar adenomas in rats. In the mouse, forestomach tumors are only reported following gavage dosing; but gavage dosing has not resulted in tumors in rats (IARC, 2003). Dichlorvos is also mutagenic in in vitro assays and structurally similar to another forestomach carcinogen, 1,3-dichloropropene. The cancer slope factor of 0.29 (mg/kg day) is the geometric mean of the slope factors developed from forestomach tumor data in female mice, pancreatic adenomas in male rats, and leukemias in male rats. This approach was used because the Agency considered all the tumor findings to be of equal relevance, rather than selecting a slope factor considered the most relevant or selecting the highest slope factor calculated to ensure protection of public health.

1,3-Dichloropropene. Human exposure to dichloropropene is usually occupational, through inhalation or dermal contact, or for the general public, from the inhalation or consumption of contaminated drinking water. There are no chronic human studies suitable for dose-response assessment. EPA has classified dichloropropene as a Group B2–probable human carcinogen under the 1986 guidelines, and a “likely” human carcinogen under the 1996 guidelines, with an oral slope factor of 0.1 (mg/kg day). While 1,3-dichloropropene has been shown to cause tumors of the forestomach and at other sites, the slope factor is based on urinary bladder carcinomas in female mice (NTP, 1985; USEPA, 2007). For this chemical, EPA elected to base the slope factor on bladder tumors, rather than on forestomach tumors; thus, the cancer risk assessment has greater relevance to humans. The inconsistency of approaches for cancer risk assessment between dichlorvos and dichloropropene is notable, because the chemicals are structurally similar, and both cause tumors at multiple sites following oral administration.

1,2-Dibromoethane. The risk assessment for 1,2-dibromoethane demonstrates yet another variation of EPA’s approach to using forestomach tumor data. 1,2-Dibromoethane was not assessed under the 1986 guidelines, but under the 1999 guidelines, and was considered “likely to be a carcinogen to humans” based on strong evidence in animals and inconclusive evidence in humans. The oral cancer slope factor is based on a study in rats administered 1,2-dibromoethane via gavage. Forestomach tumors, hemangiosarcomas, and thyroid follicular cell adenomas/carcinomas were observed. Also, the exposures in both test groups were above the MTD, and the study had to be terminated early due to high rates of mortality. Unlike the approach used for dichlorvos, wherein the cancer slope factors from multiple sites were averaged; unlike the approach with acrylonitrile, wherein the incidence of forestomach tumors was summed with tumors in other tissues by species; and unlike the approach for 1,3-dichloropropene, wherein the cancer slope factor was determined for a tumor type most relevant to humans, for 1,2-dibromoethane EPA summed the cancer slope factors that were calculated separately from the three observed tumor types (forestomach tumors, hemangiosarcomas, and thyroid follicular cell tumors). Perhaps the approach used for 1,2-dibromoethane should be reconsidered, given the approaches used for other chemicals that cause tumors at multiple sites.

Vinyl chloride. Vinyl chloride has been classified as a Group A–human carcinogen (USEPA, 1985). The carcinogenicity of vinyl chloride in humans has been demonstrated in a number of epidemiologic studies and case reports, many of which associate occupational exposure to vinyl chloride to the development of angiosarcomas of the liver. In addition to liver cancer, exposure to vinyl chloride has been linked to an increased risk of lung, brain, hematopoietic, and digestive tract cancers in humans (USEPA, 1985). Numerous animal studies have demonstrated the carcinogenicity of vinyl chloride in many organ systems, including the forestomach. For vinyl chloride, EPA chose to develop the slope factor based on liver tumors in Wister Rats (Feron et al., 1981), which is of greater relevance for human exposures and did not use the observations of forestomach tumors for quantification of cancer risk.

Ethyl acrylate. Human exposure to ethyl acrylate occurs primarily from occupational exposure via inhalation or dermal contact. While epidemiologic studies focusing on occupational exposure to ethyl acrylate/methyl methacrylate have suggested a relationship between the chemical and colorectal cancer, evidence is conflicting or inconclusive. In rodents exposed to ethyl acrylate in corn oil through oral gavage, increased incidence of squamous cell papillomas and carcinomas of the forestomach were observed (NTP, 1983). In these studies, ethyl acrylate caused irritation of the forestomach mucosa in male and female rats and mice. Furthermore, in the oral gavage studies of rodents, increased incidence of forestomach lesions, inflammation, and ulcerations were observed, suggesting an involvement in the carcinogenic mechanism. However, in
studies where rodents were exposed to ethyl acrylate in drinking water, by inhalation or by dermal administration, no statistically significant dose-related increases in tumor incidence were observed. EPA initially set the oral cancer slope factor of 0.048 (mg/kg/day) (USEPA, 1997), but later withdrew it from IRIS, and has not developed a new value.

**Mercuric chloride.** Mercuric chloride has been classified by EPA as a Group C–possible human carcinogen based on limited evidence of carcinogenicity in rats and mice. Focal papillary hyperplasia and squamous cell papillomas in the forestomach, as well as thyroid follicular cell adenomas and carcinomas, were observed in male rats exposed to mercuric chloride through chronic oral gavage over a 2-year period (NTP, 1993). The relevance of the forestomach papillomas to assessment of cancer in humans, however, is questionable because there was no evidence that the papillomas in rats progressed to malignancy (NTP, 1993). In the same study, evidence for increases in squamous cell papillomas in the forestomach of female rats was equivocal. It should be noted that the doses given in the study were considered to exceed the MTD for male rats. Two other negative lifetime rodent studies were considered inadequate, and genotoxicity assays have shown equivocal results. No slope factor was derived using forestomach tumors because the observed forestomach tumors were considered the result of dosing above the MTD, resulting in irritation of the forestomach, subsequent cell death, and epithelial proliferation. While the approach for mercuric chloride seems more scientifically justifiable, the inconsistency between the approach used for this chemical and that used for 1,2-dibromomethane is notable, where EPA used data for forestomach tumors that developed above the MTD. Also, at least one state regulatory agency—New Jersey Department of Environmental Protection—does not treat mercuric chloride as a Group C carcinogen because of the questionable relevance of these observations to humans (NJDEP, 2004).

**Allyl chloride.** Similar to mercuric chloride, EPA classified allyl chloride as a Group C–possible human carcinogen. This classification was based on a low incidence of forestomach tumors in female mice and positive results in a variety of genetic toxicity tests. However, the forestomach tumor data were not used for quantitative cancer risk assessment. Allyl chloride is a strong alkylating agent and is structurally similar to other forestomach carcinogens, such as propylene oxide and epichlorohydrin, which cause tumors at the site-of-exposure. However, in the cases of propylene oxide and epichlorohydrin, EPA elected to use forestomach data to set an oral cancer slope factor in the absence of evidence that the chemicals cause cancers at other sites following oral administration. This comparison demonstrates still other inconsistencies in EPA's approaches for using forestomach tumor data in cancer risk assessment and the need for a standardized, mechanistically-based approach.
orally administered chemicals cause tumors exclusively in the forestomach, others cause tumors in multiple organs and tissues. The forestomach tumors resulting from highly irritating exposures to “site-of-contact” carcinogens (e.g., propylene oxide and epichlorohydrin) only following oral administration are less likely to be relevant to human cancer risk via ingestion than those that cause tumors in multiple tissues. These findings share the common possibility that irritation or ulceration of the forestomach were involved in the carcinogenic mechanism and it is likely that cytotoxicity contributed to the rate and occurrence of tumors. Further, low systemic bioavailability due to the reactive nature of these chemicals is an important consideration.

The EPA approaches for cancer risk assessment for these chemicals have been inconsistent and would benefit from a reevaluation that would consider the mode-of-action and whether the tumors are relevant to human exposure conditions. Oral cancer slope factors developed using these data are also questionable because they do not consider the tissue dose in the forestomach as compared to that in any relevant human tissue, e.g., the risk assessment is based on administered dose rather than tissue dose. Further, for site-of-contact carcinogens, alternative dosimetrics, such as flux into relevant human tissues, should be considered as the conventional body weight–adjusted dose may be of lesser relevance for describing the mode-of-action. Because the tissue dose in the forestomach that resulted in tumorigenesis is unlikely to be representative of that in any other relevant human tissue at the same rate of administration, physiologically-based modeling would advance the use of forestomach tumor data for cancer risk assessment.

**MODE-OF-ACTION FRAMEWORK**

During the nearly 20 years between EPA's issuance of the 1986 Cancer Risk Assessment Guidance and the 2005 current guidelines, an analytical framework for considering the carcinogenic mode-of-action has evolved for assessing the potential for carcinogenicity and relevance of observations in animals at meaningful environmental exposure levels (Dellarco and Baetche, 2005; USEPA, 2005). The current EPA Cancer Risk Assessment Guidance now recommends risk assessment approaches that include linear and nonlinear models as a practical means of considering the processes that precede development of cancer. For example, a mode-of-action framework has been used to conclude that certain tumors, such as the alpha 2\(\mu\)-globulin—induced rat renal tumors, are not relevant to humans. Further, a mode-of-action framework was used to quantify the cancer risk posed by chloroform, in that exposures below levels that cause cytotoxicity, and subsequent regenerative hyperplasia in susceptible tissues, are considered to be below the threshold for cancer risk in humans. In this instance, the cancer risk exists only at doses above those that cause cytotoxicity, and for chloroform, above the reference dose (USEPA, 2007).

To date, no mode-of-action approach has been developed to evaluate human carcinogenic potential based on rodent forestomach tumor data. However, because forestomach tumors occur via more than one mechanism and sometimes in combination with tumors in other organs, designing a universally applicable framework is not practical, and chemical-specific information must be considered for each chemical-specific assessment. Similarly, EPA's 2005 Final Guidelines for Carcinogen Risk Assessment specify that conclusions about the carcinogenicity of a chemical must be derived using a WOE approach, which should include an examination of mode-of-action, species differences in metabolism and toxicokinetics, and exposure conditions that affect cancer expression. These factors, along with genotoxicity and the observation of tumors at other sites, are important considerations in a mode-of-action framework for evaluating the relevance of forestomach tumors in cancer risk assessment.

As discussed previously, many chemicals that cause forestomach tumors are not specific to the forestomach and cause tumors at other sites. Thus, the determination as to whether these chemicals pose carcinogenic hazards to humans is not based solely on findings of tumors in the forestomach but also on tumors observed at other sites. In such cases, the chemical is more likely to pose a cancer hazard. Examples include 1,3-butadiene and vinyl chloride, known human carcinogens, for which neither the classification nor quantitative assessment rely on forestomach tumor data.

Thus, this mode-of-action framework is specific to cancer classification for chemicals that are forestomach specific. Table 3 lists proposed WOE decision criteria for use in considering forestomach tumor data for classification of potential human carcinogenicity and quantitative risk assessment. While the factors described in the WOE framework do not include all possible combinations of data, they do address typical combinations reflected in the IRIS database for forestomach carcinogens and offer a general framework for considering chemical-specific information. As an illustration, examining the WOE for ethyl acrylate in this mode-of-action framework indicates that forestomach tumors, observed only from a gavage dosing regimen, are not relevant to human cancer because drinking water cancer bioassays have revealed that, although it is genotoxic, exposure to ethyl acrylate produces forestomach tumors in rodents only with irritation to the forestomach mucosa occurring via gavage administration (NTP, 2000). This illustration is consistent with current GLP for cancer bioassays for which doses are to be set below those which cause lesions at the site of administration (Bucher, 2000).

When considering the mode-of-action based on forestomach tumors, it is critical to consider realistic human exposure patterns. Evidence from forestomach tumor induction in chronic bioassays suggests that chronic irritation from high concentrations of substances in drinking water and gavage...
dosing can result in irritation, hyperplasia, and tumor formation. However, chronic irritation of epithelial tissue is generally not consistent with human exposure conditions, which are likely to be below a threshold for irritation. Further, cytotoxicity associated with high-dose exposure likely accelerates the rate of tumorigenesis and may be an important consideration in quantifying the dose-response. For example, it is possible that a threshold for human cancers may exist at doses below those that cause nonneoplastic lesions in the forestomach.

Tumor promulgation with cessation of exposure is another key consideration. Kagawa et al. (1993) demonstrated that forestomach lesions induced by genotoxic carcinogens did not regress with removal of exposure, while simple or papillary hyperplasia induced by nongenotoxic carcinogens did regress after cessation of exposure. Ghanayem et al. (1994) showed that forestomach epithelial hyperplasia continued as long as exposure to ethyl acrylate continued via gavage dosing, but cessation of exposure resulted in the regression of hyperplasia and lack of tumor development. Therefore, the effect of temporal dosing regimens on forestomach tumor development should be considered in assessing the mode-of-action and relevance to human exposures.

With respect to genotoxic chemicals that cause site-of-exposure tumors (e.g., propylene oxide, epichlorohydrin, allyl chloride), it is critical to consider whether observations of tumors in the forestomach are relevant to human exposure conditions, accounting for differences in the tissue dosimetry (i.e., tissue dose in the rodent forestomach vs. tissue dose in a tissue determined to be relevant for human exposures for a particular chemical) and whether the observation of tumors is likely a threshold effect (i.e., occurring only at doses or concentrations that induce irritation). In either case, use of physiologically based toxicokinetic modeling might be helpful to describe tissue dose for quantitative risk assessment (i.e., calculation of cancer slope factors). Further, the oral dose rate (mg/kg body weight administered) is not a meaningful

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### TABLE 3

**WOE Decision Criteria for Assessing the Relevance of Forestomach Tumors in Human Cancer Risk Assessment**

<table>
<thead>
<tr>
<th>Oral Cancer Classification Criteria</th>
<th>Genotoxicity deemed potentially relevant to human tissues</th>
<th>Forestomach-specific cancers observed upon oral challenge via gavage, drinking water, or diet&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Not observed in drinking water studies; only observed with gavage dosing</th>
<th>Only observed at doses that irritate the forestomach/doses exceed MTD&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Data provide evidence of potential carcinogenicity in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>NO—even though genotoxic, the conditions of the exposure limit use of the data</td>
</tr>
<tr>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td>NO—even though genotoxic, the conditions of the exposure limit use of the data</td>
</tr>
<tr>
<td>√</td>
<td></td>
<td>√</td>
<td></td>
<td></td>
<td>YES—mode-of-action (MOA) may be relevant, tissue kinetics and dosimetry should be considered in determination</td>
</tr>
<tr>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>YES—tumors at multiple sites from oral exposure suggests increased likelihood of relevant human MOA</td>
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<tr>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NO—even though genotoxic, the conditions of the exposure limit use of the data</td>
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<tr>
<td>√</td>
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<td></td>
<td>NO—conditions of the exposure likely responsible for the tumors and not relevant to human exposures</td>
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<td></td>
<td></td>
<td>YES—MOA may be relevant, tissue kinetics and dosimetry should be considered in determination</td>
</tr>
</tbody>
</table>

<sup>a</sup>Chemicals that cause only forestomach tumors upon oral exposures, not tumors in other tissues, does not include chemicals that cause lung cancer upon inhalation or skin cancer following dermal administration which may be classified as carcinogens for other routes of exposure.

<sup>b</sup>Exposures that result in lesions at the site of administration exceed the MTD and should not be considered relevant for human risk assessment.
measure of the dose for forestomach tumors that act through direct contact, and tissue dose or flux into tissue should be considered as possible alternative dosimetrics.

There are several instances in which metabolic processes should be considered in determining the mode-of-action. For example, evidence suggests that mouse forestomach epithelial tissues have an increased capacity to metabolize certain chemicals to more irritating metabolites, such as is the case with BE being metabolized to BAA, resulting in forestomach chemicals to more irritating metabolites, such as is the case with BE being metabolized to BAA, resulting in forestomach tumors (Boatman et al., 2004). In this case, the metabolic processes occurring in the forestomach are not consistent across species and a relevant mode-of-action in humans is questionable (Poet et al., 2003).

In addition, while hexavalent chromium, a known human respiratory carcinogen via inhalation and a mutagen (IARC, 1990b), is detoxified by the acidic and reductive capacity of the human stomach, the rodent forestomach does not provide equivalent detoxification conditions. For chemicals such as BE and hexavalent chromium, interspecies differences in metabolism and toxicokinetics should be considered in determining the mode-of-action for assessing human carcinogenic potential via ingestion and for determining whether the observation of tumors in rodents is relevant at realistic human exposure conditions.

This review has not addressed the importance, or lack thereof, for forestomach tumors being observed in multiple species and both sexes within species. Haseman and Lockhart (1993) reported a strong correlation between the occurrence of forestomach tumors in mice and rats and between sexes. A more current review of forestomach tumor data, reported in IARC (2003), was reviewed; however, no meaningful trends by species or sex were identified. Several chemicals cause forestomach tumors in only mice or only rats, but most forestomach carcinogens are not species specific (NTP, 2007). Also, there appears to be no species-specific mode-of-action because chemicals that are genotoxic and nongenotoxic in vivo are capable of causing forestomach tumors in both species (IARC, 2003).

CONCLUSIONS

Rodent cancer bioassays are a valuable tool for predicting cancer risk to humans but have inherent limitations. The full WOE should be evaluated for each chemical rather than relying on one type of data to propose a human cancer risk assessment. Approaches based on mode-of-action should encompass not only animal toxicity data but also dose-response information, toxicokinetics, tissue concordance, species-specific dosimetry, assessment of relevant human exposures, and, if available, epidemiological observations. Physiologically based pharmacokinetics may be useful for quantitative risk estimates (e.g., cancer slope factors) in cases where forestomach tumors are deemed relevant for human risk assessment to evaluate the relevant species-specific tissue dosimetry. Exposures that cause chronic irritation of the forestomach exceed the MTD for a cancer bioassay and should not be considered relevant for human risk assessment. Chemicals that induce tumors in multiple tissues upon ingestion are more likely to be relevant human carcinogens because the evaluation is not dependent on the observations of tumors only in the forestomach, and it is important to consider limitations on systemic bioavailability. Chemicals that are direct-acting mutagens and cause forestomach-specific tumors pose the most challenging set of observations for human risk assessment. In these cases, the relevance of human exposures, variation in species-specific and tissue-specific kinetics, and dosimetry are all important considerations in cancer risk assessment.

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