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RESEARCH ARTICLE

Iodomethane human health risk characterization

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Abstract

Iodomethane is a new pre-plant soil fumigant approved in the United States. Human exposure may occur via inhalation due to the high vapor pressure of iodomethane. A quantitative human health risk assessment was conducted for inhalation exposure. The critical effects of acute duration iodomethane exposure are: (1) fetal losses in rabbits, (2) lesions in rat nasal epithelium, and (3) transient neurotoxicity in rats. Chronic exposure of rats resulted in increased thyroid follicular cell tumors from sustained perturbation of thyroid hormone homeostasis. A physiologically based pharmacokinetic (PBPK) model for iodomethane was developed to characterize potential human health effects from iodomethane exposure. The model enabled calculation of human equivalent concentrations (HECs) to the animal no-observed-adverse-effect levels (NOAELs) using chemical-specific parameters to determine the internal dose instead of default assumptions. Iodomethane HECs for workers and bystanders were derived using the PBPK model and NOAELs for acute exposure endpoints of concern. The developmental endpoint NOAEL was 10 ppm and corresponding bystander HEC was 7.4 ppm. The nasal endpoint NOAEL was 21 ppm and the HEC was 4.5 ppm. The transient neurotoxicity endpoint NOAEL was 27 ppm and the HEC was10 ppm. Data demonstrated that humans are less sensitive to the effect that causes developmental toxicity in rabbits and the PBPK model incorporated this information, resulting in a higher HEC for the developmental endpoint than for the nasal endpoint. Nasal olfactory degeneration is the primary endpoint for risk assessment of acute exposure to iodomethane.

Introduction

Iodomethane is a pre-plant agricultural soil fumigant used to control insects, parasitic nematodes, soil borne pathogens, and weed seeds. Iodomethane is a viable alternative for methyl bromide, an ozone-depleting fumigant currently being phased out of use around the world. Iodomethane is approved in the United States for pre-plant application into soil used to grow crops including: strawberries, tomatoes, peppers, cut flowers, turf, trees, and vines. Iodomethane is stored as a liquid under pressure, but volatilizes rapidly following injection into soil. Iodomethane is a new agricultural active ingredient, and there are no human exposure incident reports related to agricultural uses. Iodomethane is used as an intermediate in the manufacture of some pharmaceuticals, in methylation processes, and in the field of microscopy; thus, sporadic reports of human exposure to excess iodomethane are available in the open literature (Hermouet et al., 1996).

The general public may be exposed to low levels of iodomethane in air from agricultural uses due to volatilization following application. Specifically, fumigants can off-gas into air and be transported off-site by meteorological processes. Agricultural field workers may be exposed to iodomethane during or after the application process. Bystander exposure to iodomethane following application to agricultural soil is expected to occur over a time period of approximately 24 hours due to the emission patterns of the compound and air dispersion patterns (EPA OPP Health Effects Division (HED), 2007). Occupational exposure to iodomethane may occur 8 hours per day, 5 days per week, during the application season. For risk assessment purposes, potential inhalation exposures could be acute (24 hours

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or less), short-term (1–30 days), intermediate-term (1–6 months), or long-term in duration.

The toxicity from iodomethane exposure via the inhalation route has been characterized by generation of a complete set of laboratory studies performed according to US Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines to support registration of iodomethane in the USA (EPA OPP Health Effects Division (HED), 2007). These studies provide sufficient information to identify critical effects for each relevant exposure scenario and form the bases for a human health risk assessment. A standard risk assessment performed to evaluate the potential risk to bystanders and workers who might be exposed to iodomethane resulting from fumigation activities would apply default methodologies to extrapolate from the no-observed-adverse-effect levels (NOAELs) in the animal studies to estimate human equivalent concentrations to the external animal exposures.

To reduce the uncertainties in the human health risk assessment of iodomethane that result from the use of default assumptions to extrapolate from animal to human exposure, chemical-specific data and physiologically based pharmacokinetic (PBPK) models were developed. A series of studies was conducted to develop and validate hybrid computational fluid dynamics (CFD) models of nasal airflows coupled with PBPK models of the systemic disposition of iodomethane in rats, rabbits, and humans. Studies were performed to identify the appropriate dose metric for each endpoint of potential concern; to characterize the airflow in the nasal passages of rabbits; to describe the kinetic uptake and metabolism of iodomethane in the nasal passages of rats and rabbits; and to determine partition coefficients for iodomethane in a variety of tissues in rats, rabbits, and humans. These studies are summarized briefly in this paper and are published in separate articles in this journal issue.

Methods

Human equivalent concentrations (HECs) corresponding to NOAELs in laboratory animal studies were derived as points of departure for risk assessment of potential iodomethane exposure to humans. HECs for endpoints of concern for acute exposure NOAELs were derived using PBPK models for iodomethane and iodide as described by Sweeney et al. in this issue (2009). PBPK models were not applied to endpoints of concern identified for subchronic or chronic exposure. HECs for intermediate- and long-term exposures to iodomethane were derived using the methodology developed by the EPA Office of Research and Development (ORD) for the derivation of inhalation reference concentrations (RfCs) (EPA, 1994). Bystanders potentially exposed to iodomethane were assumed to be exposed for 24 hours in 1 day. Workers exposed to iodomethane were assumed to be exposed for 8 hours per day, 5 days per week.

In this assessment the most sensitive endpoint and the ultimate basis for a given acute exposure assessment scenario is the endpoint represented by the lowest HEC. The lowest HEC may or may not correspond to the lowest animal NOAEL. In both the PBPK modeling and the EPA RfC approaches, different HECs may be identified for the same experimental NOAEL due to: (1) the different algorithms used to derive HECs for systemic versus portal of entry effects; (2) different dose metrics used in the PBPK model; or (3) time adjustments conducted for non-occupational versus occupational exposure scenarios. Differences between systemic versus portal of entry effects may arise from the use of different calculations to estimate the inhalation risk to humans that are dependent on the regional gas dose ratio (RGDR). For non-occupational versus occupational exposure, differences may arise because, while it is presumed that non-occupational exposure may occur 24 hours/day, 7 days/week, occupational exposure occurs only during the course of an average work week (8 hours/day and 5 days/week). An 8-hour work day is not anticipated to result in an 8-hour exposure duration, so this assumption is considered to be conservative.

Data

Database summary

A complete database of toxicity studies was developed to support government registration of iodomethane as a pesticide. Studies available that were conducted by the inhalation route include an acute neurotoxicity study and developmental studies in rats and rabbits, as well as a multigenerational reproductive toxicity study and a combined chronic/carcinogenicity study in rats. A chronic dietary exposure study was conducted in mice. The full database of studies performed according to the US EPA OPPTS guidelines is summarized in Table 1. Additional studies were performed to characterize the metabolism, toxicokinetics, and mode of action (MOA) of iodomethane.

Acute endpoint studies

The studies listed in Table 1 considered to have endpoints appropriate for risk assessment for acute exposure duration are the acute neurotoxicity study, the developmental toxicity study, and studies that resulted in effects on the rat nasal epithelium. The transient neurotoxicity occurred in a 6-hour exposure, and so the relevance to acute duration assessment is evident. Developmental toxicity is generally considered an effect that could occur due to a single day of exposure; consequently, this effect is subject to a risk assessment for a 24-hour exposure (EPA, 1991). The nasal olfactory degeneration observed in a number of subchronic studies is considered a potential endpoint for acute exposure because the effect did not progress in severity with increasing time of exposure, and because studies in the literature report this effect following short exposure to high concentrations of iodomethane (Chamberlain et al., 1998b). The studies to be the basis for the acute exposure duration assessments are summarized below.

Acute neurotoxicity study in rats (Schaefer, 2001). In the acute neurotoxicity study, rats were exposed to 0, 27, 93, or

Table 1. Iodomethane toxicity profile.

EPA OPPTS guideline no./ study type	Exposure conditions	Results
870.1100 Acute oral: rat		LD_{50} = 79.8 mg/kg (males); 131.9 mg/kg (females) The up/down procedure was used in Sprague Dawley (SD) rats Toxicity category II
870.1100 Acute oral: mouse		LD_{50} = 155 mg/kg (males); 214 mg/kg (females) The up/down procedure was used in CD-1 mice Toxicity category II
870.1200 Acute dermal: rat		LD_{50} > 2000 mg/kg (limit test) There were no deaths Toxicity category III
870.1300 Acute inhalation: rat	581, 710, 797, or 1198 ppm	LC_{50} = 691 ppm = 4 mg/L (combined sexes) The test article was administered as a vapor in a dynamic whole-body chamber for 4 hours Toxicity category IV
870.2400 Primary eye irritation: rabbit		Corrosive Toxicity category I
870.2500 Primary skin irritation: rabbit		Well defined erythema, slight-severe edema Toxicity category II
870.2600 Dermal sensitization (Magnusson–Kligman maximization test): guinea pig		Not a dermal sensitizer
870.3100 Subchronic feeding: mice	0, 133, 400 or 1200 ppm iodomethane microencapsulated in the diet.	Increased thyroid weights, accumulation of follicular colloid, hyperkeratosis of esophagus in 133-ppm group; NOAEL < 133 ppm NOAEL = 133 ppm based on decreased body weight less than 10% with corresponding decreased food consumption, and adaptive response of thyroid
870.31870.3465 13-week inhalation: rat (Kirkpatrick, 2001)	0, 5, 21, or 70 ppm, 6 h/day, 5 days/week for 4 weeks (interim sacrifice) or 13 wks	NOAEL = 21 ppm (0.12 mg/L/day) LOAEL = 70 ppm (0.41 mg/L/day) based on initial decreases in body weights, body weight gains, and food consumption (males); and nasal degeneration
870.3700 Inhalation developmental toxicity: rat (Nemec, 2001a)	0, 5, 20, 60 ppm, 6 h/day on GDs 6–19	Maternal NOAEL = 20 ppm (0.12 mg/L/day) Maternal LOAEL = 60 ppm (0.35 mg/L/day) based on decreased body weight gain (14–19% ~5–6% absolute body weight) Developmental NOAEL = 60 ppm (0.35 mg/L/day) Developmental LOAEL was not observed
870.3700 Inhalation developmental toxicity: rabbit (Nemec, 2002, Nemec, 2009)	0, 2, 10, or 20 ppm, on GDs 6–28	Maternal NOAEL = 20 ppm Maternal LOAEL: not identified Developmental NOAEL = 10 ppm Developmental LOAEL = 20 ppm based on increased fetal losses and decreased fetal weights (19.6%, combined male and female)
Non-guideline Inhalation phased-exposure developmental toxicity: rabbit (Nemec, 2003, Nemec, 2009)	0 or 20 ppm on GDs 6–28; 20 ppm on GDs 6–14, 15–22, 23–24, 25–26, or 27–28, 6 hrs/day	This study was intended to determine the critical period of exposure during gestation that resulted in fetal loss as observed in a previously evaluated guideline developmental toxicity study in rabbits. Increased fetal losses at 20 ppm on GDs 6–28 (~21%), 23–24 (~9%), and 25–26 (~11%)
870.3800 Inhalation two-generation reproductive toxicity: rat (Nemec, 2001b)	00, 5, 20, or 50 ppm Note: offspring directly exposed on PND 28	Parental NOAEL = 20 ppm based on decreased body weight gain, body weight, degeneration of the olfactory epithelium at 50 ppm Offspring NOAEL = 5 ppm based on decreases in body weight gain, and delays in vaginal patency at 20 ppm Reproductive NOAEL = 20 ppm based on decreased live births at 50 ppm
870.4200 Carcinogenicity feeding: mouse (18 months) (Harriman, 2005)	0, 60, 200, or 600 ppm iodomethane microencapsulated in the diet	Decreased body weight and food consumption at 200 and 600 ppm; decreased T4 and increased TSH in males at 600 ppm; macroscopic and microscopic effects in thyroid
870.4300 Chronic inhalation toxicity/ carcinogenicity: rat (Kirkpatrick, 2005)	0, 5, 10, 60 ppm for 6 hrs/day, 5 days/week	Systemic NOAEL = 5 ppm based on increased incidence of salivary gland squamous cell metaplasia at 20 ppm Portal of entry NOAEL = 20 ppm based on degeneration of the olfactory epithelium at 60 ppm At 60 ppm, perturbations of the thyroid–pituitary axis and thyroid histopathology were reported

Table 1. Continued on next page.

Table 1. Continued.

EPA OPPTS guideline no./ study type	Exposure conditions	Results
870.5100 Bacterial reverse mutation test (Ames assay) (Wagner, 2001)		Non-mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537; and in *Escherichia coli.* Negative in all strains
870.5300 In vitro mammalian cell mutation test in Chinese hamster ovary cells (San, 2001)		Negative
870.5375 In vitro chromosomal aberration in Chinese hamster ovary (Gudi, 2001)		Positive for the induction of structural chromosome aberrations (clastogenesis), but negative for induction of numerical aberrations in CHO cells in this assay
870.5395 In vivo micronucleus assay: mice (Gudi, 2001)		Negative
870.6200 Inhalation acute neurotoxicity: rats (Schaefer, 2001)	0, 27, 93, 401 ppm whole-body, 6-hour exposure	Systemic NOAEL = 27 ppm Systemic LOAEL = 93 ppm based on functional observational battery (FOB) findings (clonic convulsions in 1/12 females, decreased body temperature), and decreased motor activity Portal of entry effects not assessed
870.7485 Metabolism: rat (Sved, 2002)		SD rats were orally dosed or exposed via inhalation with [^{14}C]iodomethane. Maximum blood concentrations were achieved within 4 hours (oral) and 0–2 hours (inhalation), and were proportional to dose/concentration. Initial t_2 was 5.1–7.2 hours, and terminal t_2 was 116–136 hours Recovered radioactivity was primarily as CO_2 (39.40–60.81% dose) and in the urine (26.50–33.40% dose) in all treated groups, while feces accounted for <2% dose. Radioactivity remained in the carcasses (11.92–14.39% dose) of all treated animals 168 hours following treatment in the main test The major metabolites were expired CO_2, and N-(methylthioacetyl) glycine and S-methyl glutathione which were excreted in the urine. Minor metabolites were methylthioacetic acid, methyl mercapturic acid, and S-methyl cysteine

401 ppm iodomethane vapor by inhalation for 6 hours in a whole-body exposure chamber. Rats were evaluated for potential effects on the nervous system using a functional observational battery (FOB). The systemic NOAEL identified was 27 ppm based on FOB findings including decreased motor activity (75–78% in males, 81–84% in females), repetitive movements of the mouth and jaws (i.e. clonic convulsions) in 1 of 12 females, and decreased body temperature, at the systemic LOAEL (lowest observed adverse effect level) of 93 ppm. These effects were observed on the day of exposure only and did not persist to the day-7 and day-14 evaluations.

Developmental toxicity studies in rabbits (Nemec, 2002, 2003; Nemec et al., 2009). In a developmental toxicity study, groups of 24 female New Zealand White (NZW) rabbits were exposed to iodomethane vapor (99.6% active ingredient, a.i.) in whole-body inhalation chambers at concentrations of 0, 2, 10, or 20 ppm (0, 0.012, 0.058, or 0.12 mg/L/day) 6 hours per day, gestation days (GDs) 6 through 28. A significant increase in late resorptions per doe was observed following exposure to 20 ppm iodomethane (1.6/doe, compared to 0.1/doe, control), contributing to an increased post-implantation loss of 2.0/doe compared to 0.7/doe in the controls.

The maternal NOAEL identified by the EPA is 20 ppm and no maternal LOAEL was identified (EPA OPP Health Effects Division (HED), 2007). The developmental toxicity LOAEL identified by the EPA is 20 ppm based on increased fetal losses, specifically late resorptions, and decreased fetal weights (~20%). The developmental toxicity NOAEL is 10 ppm.

In a second developmental toxicity study iodomethane (99.7% a.i.) was administered via the inhalation route (whole body) to 24 New Zealand White rabbits/group at concentrations of 0 or 20 ppm during GDs 6–28 (control and group 2), GDs 6–14 (group 3), GDs 15–22 (group 4), GDs 23–24 (group 5), GDs 25–26 (group 6), or GDs 27–28 (group 7) for 6 hours/exposure per day. This study was not intended to fulfill the guideline requirement or establish NOAELs and LOAELs, but rather was conducted to determine if there was a critical period of exposure during gestation that resulted in fetal loss as observed in the standard developmental toxicity study in rabbits.

As in the standard developmental toxicity study, a significant increase in late resorptions/doe was observed in rabbits exposed to 20 ppm iodomethane during GDs 6–28 (1.2/doe compared to 0.1/doe control). No other significant increases in late resorptions were observed, but non-significant increases

were observed in does exposed during GDs 23–24 (0.6/doe), and GDs 25–26 (0.7/doe). Thus, a window of sensitivity to iodomethane exposure during GDs 23–26 was identified.

Subchronic inhalation toxicity study in rats (acute effect) (Kirkpatrick, 2001). In a subchronic inhalation toxicity study, iodomethane (99.7% a.i.) was administered via whole-body inhalation to Sprague Dawley rats (20/sex/concentration) for 6 hours/day, 5 days/week for 13 weeks at concentrations of 0, 5, 21, or 70 ppm (0, 0.029, 0.12, or 0.41 mg/L/day). Ten rats/sex/concentration were sacrificed after 4 weeks, and the remaining 10 rats/sex/concentration were sacrificed after 13 weeks.

There were no effects of treatment on mortality, ophthalmology, urinalysis, hematology, organ weights, or gross pathology. Irritation of the nasal olfactory epithelium was observed in rats exposed to 70 ppm iodomethane at both interim and terminal sacrifices. The nasal irritation at 4 and 13 weeks was characterized by inflammation and cellular degeneration and regeneration. Body weights and body weight gains were reduced during the first 6 weeks of the study in male and female rats exposed to 70 ppm iodomethane.

The systemic LOAEL identified for this study is 70 ppm based on initial decreases in body weights and body weight gains. The systemic NOAEL is 21 ppm. The port-of-entry LOAEL identified is 70 ppm based on degeneration of the nasal olfactory epithelium. The port-of-entry NOAEL is 21 ppm.

Subchronic endpoint studies

Two-generation inhalation toxicity study in rats (Nemec, 2001b) In a two-generation reproduction toxicity study, iodomethane (99.7% a.i.) was administered via whole-body inhalation to Sprague Dawley rats (30/sex/concentration) for 6 hours/day at concentrations of 0, 5, 21, or 50 ppm. These P generation animals were exposed to the test article for at least 70 days prior to mating to produce the F_1 litters. Exposure of the P males continued throughout mating and until the day prior to euthanasia. The P females were exposed throughout mating and through GD 20, at which point exposure was discontinued. Daily exposure of the P females was reinitiated on lactation day (LD) 5 and continued until the day prior to euthanasia. After weaning, F_1 animals (30/sex/concentration) were selected, equalized by sex, to become the parents of the F_2 generation and, beginning on postnatal day (PND) 28, were exposed to the same concentration test atmosphere as their dam.

The systemic parental NOAEL was 20 ppm and the LOAEL of 50 ppm was based on decreases in body weight, body weight gain, changes in organ weights, and gross pathology and histopathology findings. The developmental NOAEL was 5 ppm based on decreases in body weight gain and delays in vaginal patency observed at 20 ppm.

Chronic endpoint studies

Combined chronic toxicity/carcinogenicity study in rats (Kirkpatrick, 2005) In a combined chronic toxicity/carcinogenicity study in rats, iodomethane (99.7% a.i.) was administered to Sprague Dawley rats via whole body inhalation at concentrations of 0, 5, 20, or 60 ppm for 6 hours/day, 5 days/week for 104 weeks. Sixty animals/sex/concentration were exposed to 0, 5, or 20 ppm iodomethane while 70/sex were exposed at the 60-ppm level. Animals were observed for moribundity and mortality twice daily and clinical signs of toxicity once daily. Once a week a detailed physical examination was conducted, including but not limited to evaluations of changes in appearance, autonomic activity (e.g. lacrimation, piloerection, pupil size, breathing patterns), gait, posture, response to handling, and stereotypic and/or bizarre behavior. In addition, evaluations of clinical chemistry, hematology, urinalysis, gross pathology, and histopathology parameters were conducted.

The systemic NOAEL was 5 ppm based on increased incidence of salivary gland squamous cell metaplasia observed at 20 ppm. The NOAEL for portal of entry effects in the nasal olfactory epithelium was 20 ppm. An increased incidence of thyroid adenomas was observed in male rats exposed to 60 ppm.

Studies to determine mode of action

Studies were performed to identify the likely MOA of iodomethane in eliciting developmental toxicity in rabbits. It is important to determine the MOA that results in the adverse effect, because that allows determination of the appropriate internal dose metric to be modeled using the PBPK model. The two studies performed to identify the mode of iodomethane action and to support development of the CFD-PBPK model are: (1) a combined baseline inhalation exposure study of iodomethane-related fetotoxicity in rabbits, and (2) a MOA study to evaluate iodomethane-related fetotoxicity in rabbits. The baseline study was performed in rabbits because the ontogeny of rabbit thyroid development had not been described in the literature in detail, and the information available from other iodomethane exposure studies suggested that effects on the developing rabbit thyroid could be important. The goals of each study and the results are summarized briefly below, and presented in full as stand-alone papers in this issue (Sloter et al., 2009).

A baseline study to describe rabbit fetal development was combined with an inhalation exposure study of iodomethane-related fetotoxicity in rabbits (Sloter et al., 2009). The objective of this study was to identify maternal and/or fetal biomarkers of iodomethane exposure or toxicity in rabbits to be measured in a definitive MOA study. Baseline parameters for each potential biomarker were established using unexposed does and fetuses from GD 21 to GD 27. Potential biomarkers included serum chemistry and hematology parameters, maternal progesterone and estradiol levels, thyroid and pituitary hormones, and glutathione (GSH) concentrations in blood and various tissues. Microscopic examinations of fetal thyroids were conducted to compare the ontogeny of thyroid structure and function in unexposed versus exposed fetuses. Kinetic markers of iodomethane exposure (i.e. hemoglobin adducts and serum iodide levels) were measured in maternal and fetal blood. Iodomethane was administered by whole-body inhalation to two exposure groups (groups 8 and 9)

consisting of 10 time-mated female New Zealand White rabbits for 6 hours per day following either a 2-day (GDs 23 – 24, group 8) or 4-day (GDs 23 – 26, group 9) exposure regimen. The gestational day of the laparohysterectomy for each exposure group was GD 24 (group 8) or 26 (group 9) following the 6-hour exposure on each respective day. Seven baseline groups (groups 1 – 7) consisting of 10 time-mated female New Zealand White rabbits were not exposed to the test article; the GD of the laparohysterectomy for the baseline groups ranged from GD 21 (group 1) to 27 (group 7).

Iodide concentrations in maternal and fetal serum following maternal exposure to iodomethane (groups 8 and 9) were 100- to 500-fold higher than baseline concentrations (groups 4 and 6). Serum iodide levels were approximately two-fold higher in does and fetuses following 4 days of maternal exposure (group 9) compared to 2 days of maternal exposure (group 8). Serum iodide concentrations in the fetuses were approximately two-fold higher than maternal concentrations in both exposure groups. Following maternal exposure to iodomethane, iodide accumulation in the developing fetuses was associated with significant effects on fetal serum thyroid and pituitary hormone concentrations. Diminished fetal thyroxine (T4) and increased thyroid stimulating hormone (TSH) serum concentrations correlated with microscopic changes present in the thyroids of 56% (group 8) and 99% (group 9) of fetuses following the 2-day and 4-day exposure regimens, respectively. The changes in the fetal thyroids were characterized by decreased amounts of colloid in the follicular lumen, a hypertrophic follicular epithelium, and vacuolation of the epithelial cytoplasm.

A follow-on study was performed to characterize the mode of action for iodomethane-related fetotoxicity in rabbits (Sloter et al., 2009). The objective of the study was to characterize the MOA for iodomethane-related fetotoxicity observed in previous prenatal developmental toxicity studies in rabbits. The previous study suggested the MOA was an increase in fetal iodide level from iodomethane exposure. One group of 40 time-mated female New Zealand White rabbits was exposed by whole body inhalation to 20 ppm iodomethane for 3 or 6 hours daily. A concurrent control group of 40 pregnant females was exposed to filtered air. The females in the control and iodomethane groups were exposed on GDs 23, 24, 25, and/or 26. A comparator group of 40 females received 81.2 μ*M* sodium iodide in sterile water via four, 15-minute intravenous infusions (20.3 μ*M* per infusion) 2 hours apart over a 6-hour period or via two, 15-minute infusions 2 hours apart over a 3-hour period on the same gestation days. Serum iodide levels and tissue GSH concentrations were measured as potential metrics of internal dose and metabolism for the PBPK model, while thyroid hormone levels, thyroid stimulating hormone, 5′-deiodinase inhibition, and thyroid gland histopathology were evaluated to provide insight into the MOA of iodomethane in pregnant rabbits. The hemoglobin adduct S-methylcysteine was examined as a marker of maternal and fetal blood exposure to the parent compound, iodomethane. The pattern of sampling in relation to exposures was designed to characterize the time courses of adsorption, metabolism, distribution, and elimination.

Following maternal exposure to iodomethane on GDs 23 – 26, the mean litter proportion of late fetal resorptions on GD 29 was increased approximately 10-fold (50.4% per litter) compared to the control group (5.0% per litter). In the sodium iodide-exposed group, the mean litter proportions of late fetal resorptions and viable fetuses and mean gravid uterine weight were similar to control group values. Maternal and fetal serum iodide concentrations were increased in the iodomethane (inhalation) and sodium iodide (intravenous infusion) groups and increased with the duration of maternal exposure. Fetal serum iodide concentrations were 2 – 3-fold higher than maternal concentrations in both the iodomethane and sodium iodide exposed groups. Maternal and fetal serum iodide concentrations in the iodomethane group were approximately twice the serum iodide concentrations in the sodium iodide group. Thyroid follicular cell hypertrophy and colloid depletion were apparent in the thyroid glands of fetuses exposed to either iodomethane or sodium iodide. On GDs 23, 24, 25, and 26, the incidence and severity of these fetal thyroid findings were similar between exposure groups and increased with the duration of maternal exposure to iodomethane or sodium iodide. On GD 29 (following 3 days of recovery), the incidence of follicular cell hypertrophy and colloid depletion was similar between the iodomethane and sodium iodide groups; however, the severity remained greater in fetuses exposed to iodomethane. These differences between exposure groups may be due to the fact that the internal dose of iodide in the maternal and fetal serum following sodium iodide infusion was about one-half that achieved by 20 ppm iodomethane inhalation exposure. Microscopic findings in the thyroid glands of does euthanized at GD 26 were generally considered to be within normal limits and could not clearly be distinguished from normal biologic variation.

The time course data show an increase in fetal TSH beginning after 3 days of iodomethane exposure on GD 25, further increasing after 4 days of exposure on GD 26, and decreasing slightly 3 days after exposure was discontinued, on GD 29, though remaining well above the control level. In contrast to the iodomethane-exposed group, fetal TSH levels in the NaI-exposed group returned to near control levels 3 days after exposure was discontinued on GD 29. The mechanism responsible for the increases in TSH appears to be mediated by a prolonged increase in fetal serum iodide level reported in the same experimental subjects.

Studies to support PBPK models

A variety of data were generated to support development of the PBPK models of iodomethane for the rabbit, rat, and human. These PBPK models were developed to address the endpoints of developmental toxicity observed in rabbits and the nasal effects and neurotoxicity observed in rats exposed to iodomethane. The mechanistic studies were intended to either provide compound-specific inputs for the PBPK model or define the dose metric for interspecies extrapolation. The models constitute a sophisticated effort to describe

the kinetics of iodomethane following inhalation exposure and the kinetics of iodide as a metabolite. The data developed are critical in the development of the iodomethane models to describe nasal tract dosimetry and GSH depletion in the rat to evaluate nasal toxicity, to describe distribution of iodomethane to tissues including brain, to evaluate transient neurotoxicity, and to capture iodomethane metabolism and iodide kinetics in the pregnant rabbit to address developmental toxicity.

The studies performed to support development of the CFD-PBPK model include: (1) an inhalation mechanistic toxicity study in rats, (2) a pulmonary function study in rabbits, (3) a study to derive iodomethane partition coefficients, (4) computational fluid dynamics simulations to describe rabbit nasal airflows, (5) quantification of nasal absorption of iodomethane in rats and rabbits, (6) quantification of systemic iodomethane absorption in rabbits, (7) characterization of iodide kinetics in pregnant rabbits, and (8) determination of metabolic rate constants. These studies are summarized briefly below, and presented in full as stand-alone papers in this issue.

An in vivo 2-day inhalation mechanistic toxicity study—rats (Himmelstein et al., 2009). The objective of this study was to evaluate the toxicokinetic behavior of iodomethane in rats exposed by inhalation. Key study endpoints included evaluation of GSH status in selected target tissues, inorganic serum iodide and hemoglobin adducts as measures of internal dose, and clinical chemistry, hematology, thyroid hormone status, and pulmonary function as measures of toxicity. Effects on clinical pathology, thyroid hormone status and uridine diphosphate (UDP)-glucuronyltransferase, and concentrations of S-methylcysteine were determined the morning after 2 days of 6 hour/day whole body inhalation exposure to 0, 25, or 100 ppm iodomethane ($n = 10$ rats/group). Additional main inhalation exposure groups ($n = 3$ rats per 0, 25, or 100 ppm) were sampled at 1, 3, 6, 9, 24, 25, 27, 30, 33, and 48 hours for quantification of GSH and inorganic serum iodide.

Treatment-related changes from exposure to 25 and 100 ppm iodomethane were minimal to mild increases in total cholesterol concentrations (due to proportional increases in both high density lipoprotein (HDL) and non-HDL cholesterol), and minimal to mild decreases in triglyceride concentrations. Serum TSH concentrations were significantly increased at exposure concentrations of 25 and 100 ppm iodomethane. Serum triiodothyronine (T3) and T4 concentrations were significantly decreased at exposure concentrations of 100 ppm iodomethane, and serum reverse T3 (rT3) concentrations were not altered under the conditions of this study. S-methylcysteine was detected in globin from control rats at an average concentration of 161.2 nmol/g globin, and in rats exposed to 25 and 100 ppm iodomethane, the mean concentrations were increased to 201.6 and 345.3 nmol/g globin, respectively. Iodomethane exposure caused time- and concentration-dependent reductions in tissue GSH concentrations. Depletion was less pronounced in blood, kidney, and liver than in olfactory and respiratory epithelia. Iodomethane exposure of rats resulted in increased inorganic serum iodide concentrations that were concentration and time dependent. Concurrent pulmonary function measurement during exposure of rats to 0, 25, or 100 ppm iodomethane did not lower the breathing frequency, indicating that iodomethane did not induce a respiratory irritant response in rats.

A pulmonary function study—rabbits (in Sweeney et al., 2009). The objective of this study was to evaluate the toxicokinetic behavior of iodomethane in rabbits exposed by inhalation. To estimate the amount of iodomethane that is actually delivered into the respiratory tract of exposed rabbits, whole-body plethysmography was utilized. Key study endpoints included breathing frequency, tidal volume, serum iodide, and hemoglobin adduct analysis. Rabbits were exposed to average iodomethane concentrations of 0 or 18.46 ppm for 6 hours. None of the rabbits exposed to iodomethane demonstrated clinical signs of respiratory irritation and the overall mean respiratory rate of animals exposed to iodomethane was 4 breaths per minute (bpm) lower than the mean control value 130.6 bpm. Rabbits exposed to iodomethane demonstrated an overall mean minute volume that was 34% higher than the control value of 403.9 mL/min. There was no statistical difference in S-methylcysteine blood values between the control and iodomethane exposed animals. Rabbits exposed to 18.46 ppm iodomethane demonstrated an over 1000-fold increase in inorganic serum iodide levels.

Partition coefficients in rat and rabbit tissues and human blood (in Sweeney et al., 2009). The objective of this study was to determine tissue:blood partition coefficients in selected tissues from rat and rabbit, and from human blood, to support PBPK modeling. Iodomethane tissue-to-air partition coefficients (PCs) were determined in rat tissues (blood, brain, fat, kidney, liver, muscle, thyroid, nasal tissue), rabbit tissues (fetal blood, maternal blood, brain, fat, kidney, liver, muscle, thyroid, placenta, nasal tissue), human blood (male and female), and saline. The respective PCs for brain, fat, kidney, muscle, and nasal tissue were similar across animal species. The rabbit thyroid PC was three times higher than the rat thyroid PC (rabbit: 39, rat: 11). The rat liver PC was twice as high as the rabbit liver PC (rat: 24, rabbit 13). The rat blood PC was 2.5 times higher than the rabbit blood PC (rat: 39, rabbit: 16). The human blood PC (18) was more similar to the rabbit blood PC than the rat blood PC. The rabbit fetal blood PC (12) was similar to rabbit maternal blood PC (16). The iodomethane data collected for rat, rabbit, and human tissues was used as chemical-specific input into the physiologically based models (Sweeney et al., 2009).

Magnetic resonance imaging and computational fluid dynamics simulations (Corley et al., 2009). The purpose of this study was to develop the three-dimensional (3-D) nasal CFD models for the rabbit to determine airflow splits to different regions of the nasal airways, and the corresponding regional surface areas and volumes necessary to construct similar hybrid models in this species. CFD simulations were based upon 3-D computational meshes derived from magnetic resonance images of three adult female NZW rabbits. In the anterior portion of the nose, the maxillary

turbinates of rabbits are considerably more complex than comparable regions in rats, mice, monkeys or humans. This leads to a greater surface area to volume ratio in this region, and thus the potential for increased scrubbing of water-soluble or reactive gases and vapors in the anterior portion of the nose, compared to other species such as monkeys and humans. Although there was considerable inter-animal variability in the fine structures of the nasal turbinates and airflows in the anterior portions of the nose, there was remarkable consistency between rabbits in the percentage of total inspired airflows that reached the olfactory epithelium lining the ethmoid turbinate region (~20%). These latter results (airflows reaching the ethmoid turbinate region) were also consistent with previous published estimates for the male F344 rat.

Uptake of iodomethane by the rat and rabbit nasal cavities (Thrall et al., 2009b). The purpose of this study was to determine the nasal absorption of iodomethane in the intact nasal cavities of the rat and rabbit to provide experimental data to validate the computational fluid dynamics portion of the models. Uptake of iodomethane in the nasal cavities of the intact rat and rabbit was evaluated by measuring iodomethane concentration in the nasopharynx region of anesthetized animals via a small-diameter air-sampling probe, and comparing that concentration with the concentration in the exposure chamber. The exterior portion of the probe was connected directly to a mass spectrometer to provide a continual real-time analysis of concentrations of iodomethane in the nasal cavity. Rats were placed in a sealed glass chamber and exposed to iodomethane at a chamber concentration of approximately 1 ppm. Studies were conducted on six rats at a single exposure concentration. An average of 63% of iodomethane was absorbed in the rat nasal cavity. Rabbits were placed in a sealed glass chamber and exposed to iodomethane at chamber concentrations ranging from approximately 2 to 50 ppm. The results show that the percent of iodomethane absorbed in the nasal cavity of the rabbit ranged from 57 to 92% (average 72 ± 11) regardless of the initial exposure concentration.

In vivo gas uptake in rabbits (in Sweeney et al., 2009) The purpose of this study was to provide kinetic gas uptake data for rabbits. A series of gas uptake curves were generated for rabbits exposed to varying initial chamber concentrations of iodomethane by inhalation using a closed-chamber gas-uptake system. Studies involved three individual rabbits per dose group exposed at initial chamber concentrations of 50 ppm, 10 ppm, or 2 ppm iodomethane. Animals were unanesthetized and unrestrained throughout the exposures, which were approximately 3.5 – 4 hours in duration.

The pharmacokinetics of sodium iodide in pregnant rabbits (Thrall et al., 2009a) The purpose of this study was to determine the kinetics of radiolabeled sodium iodide ($Na^{131}I$) in timed-pregnant rabbits and fetuses during the critical period of gestation in which exposures to iodomethane can produce fetal resorptions. Iodomethane releases iodide (I^-) via metabolism or reactions with blood and tissue macromolecules. Radioiodide accumulated as expected in the thyroid of maternal animals. Radioiodide also accumulated in fetal blood and tissues, levels of which were consistently higher than maternal levels and, unlike maternal tissues, showed no evidence of clearance over the 24-hr sampling period. In contrast to observations in the maternal animals, fetal stomach contents showed the highest accumulation of radioiodide for both dose groups by 1 – 2 hrs after dosing, followed by the trachea and thyroid tissues, with the lowest concentrations of radioiodide in the amniotic fluid and blood. There was no evidence for preferential accumulation of radioiodide in fetal thyroid tissues on GDs 25 – 26 as there were no significant differences in radioactivity detected between samples of tracheas containing thyroid and samples of trachea alone.

In vitro GSH conjugation with iodomethane in rat, rabbit, and human blood and tissues (Poet et al., 2009) The purpose of this study was to determine the rate constants for the conjugation of iodomethane with GSH in whole blood and cytosols from liver and kidney tissues from female human donors, male Sprague Dawley rats, and pregnant NZW rabbits and fetuses as well as in nasal respiratory and olfactory epithelium from pregnant female NZW rabbits. Iodomethane is metabolized via conjugation with GSH or via a minor CYP450 oxidation to formaldehyde. Potential modes of action for adverse effects due to iodomethane exposure may involve iodide release or GSH depletion. Iodomethane was well metabolized in most of the tissue cytosol samples, but not in blood or fetal rabbit kidney. Limiting velocity V_{max} rates were similar in liver and kidney cytosol from rats and human donors (40 and 48 in liver and 15 and 12 nmol/min/mg in kidney for rat and human tissue, respectively), but were lower in rabbit tissues (10 and 0.72 nmol/min/mg in liver and kidney, respectively). The metabolism in olfactory and respiratory epithelial cytosol had K_m (Michaelis–Menton) values that were several times higher than for any other tissue (on the order of 25 m*M* for olfactory and 2.5 m*M* for respiratory), suggesting an essentially first order rate of metabolism in the nasal area. The results from these in vitro metabolism studies in conjunction with results from the in vivo gas uptake inhalation studies (Thrall et al., 2009b) can be used to estimate human in vivo metabolism rate constants using a parallelogram approach.

Discussion

Endpoints and corresponding modes of action: acute exposure

The critical endpoints identified for acute exposure to iodomethane by the inhalation route include developmental toxicity in rabbits, lesions in the nasal olfactory epithelium, and transient neurotoxicity. Effects on the thyroid have also been reported in a number of the studies of longer duration. Modes of action for these endpoints are summarized briefly below and are described in greater detail in a stand-alone paper in this issue (Kirman et al., 2009).

Developmental toxicity

Developmental and/or offspring toxicity is observed in rabbits and possibly rats. Two developmental toxicity

studies in rabbits conducted via the inhalation route have been described. In the guideline study, an increase in fetal losses was noted at the highest exposure concentration. Subsequently, a phased-exposure rabbit developmental toxicity study was conducted in which animals were exposed for different time periods. This second study reproduced the fetal losses seen in the guideline study and defined a narrow dosing window that may elicit this effect. Only exposure on GDs 23 – 24 or GDs 25 – 26 resulted in fetal losses. It is noteworthy that the time of fetal loss coincides with the time of ontogeny of fetal thyroid function in the rabbit (GD 22). Given the essential role of iodine in the proper function of the thyroid gland (both iodine deficiency and excess can have profound effects on thyroid function and thyroid hormone biosynthesis) and the fact that iodomethane exposure may lead to an excess accumulation of iodine in the thyroid, a MOA for the fetal losses involving perturbations of fetal thyroid function as a result of excess iodide has been proposed. In the case of rats, no fetal losses were reported in the developmental toxicity study, yet a decrease in the number of live births was reported in the multigenerational reproduction toxicity study. It is interesting to note that while iodomethane exposure in the developmental study ceased on GD 17 before ontogeny of rat fetal thyroid function, in utero exposure during the multigenerational toxicity continued until GD 20 (i.e. during ontogeny of fetal thyroid function). Thus, the data suggest that fetal losses may have occurred in the rat developmental study had exposure continued beyond GD 17. Similar effects have been reported for another iodine-rich compound, amiodarone (an antiarrhythmic drug), after treatment of pregnant rabbits and rats (FDA, 2003).

Developmental toxicity—mode of action The biochemical measure associated with the developmental effects observed in rabbits following iodomethane exposure is fetal iodide accumulation. Data from early studies suggested that there are important species differences in fetal iodide accumulation, such that guinea pigs, sheep, and rabbits possess an active mechanism for transporting iodide from mother to fetus, while other animals such as rats do not concentrate iodide in the fetus (Logothetopoulos & Scott, 1956; Roti et al., 1983). Recent studies described in this issue confirmed that control fetal rabbits have blood iodide levels that are approximately nine times the blood iodide levels in maternal blood (Sloter et al., 2009). The rabbit has been shown to be more sensitive with regard to fetal viability than the rat, hamster, and swine when dosed with potassium iodide or sodium iodide in feed at certain times during gestation (Arrington et al., 1965). Iodide accumulation in the fetus is the critical step in the MOA that ultimately leads to fetal loss in rabbits exposed to iodomethane.

The fact that the rabbit fetus concentrates iodide has been known for decades, and in studies performed to support iodomethane registration, the fetuses from unexposed control pregnant rabbits in the GD 23 – 26 timeframe had plasma iodide concentrations that were 9–11-fold higher than their respective mothers (Sloter et al., 2009). Thus, the control rabbit fetal/maternal iodide concentration ratios were 9 to 11. In contrast to rabbits, the human fetus was not believed to concentrate iodide from the maternal circulation in such high ratios. Available data from the literature indicate that normal human fetal iodide concentrations are generally lower than or equal to human maternal concentrations, resulting in a fetal/maternal plasma iodide concentration ratio of approximately 1. A study by Rayburn et al. (2008) was designed to characterize the fetal and maternal plasma iodide concentrations in unexposed fetal maternal pairs of human subjects. These data were used to inform the PBPK modeling and increase the certainty in the human health risk assessment.

The fetal and maternal iodide concentrations and fetal/maternal plasma iodide ratios characterized in the Rayburn study (2008) confirmed that the human conceptus does not highly concentrate iodide relative to the maternal circulation as the rabbit fetus does. Maternal plasma iodide concentrations were 1.6 ± 0.4 μg/dL for premature deliveries and 1.5 ± 0.5 μg/dL for term deliveries. Cord plasma iodide concentrations were 1.4 ± 0.5 μg/dL for premature deliveries and 1.7 ± 0.7 μg/dL for term deliveries. The average fetal:maternal plasma iodide ratio for all subject pairs combined was 1.2 ± 0.7. The average fetal:maternal plasma iodide ratio for premature delivery pairs was 0.9 ± 0.4 ($n = 29$), and the average fetal:maternal plasma iodide ratio for term deliveries was 1.3 ± 0.8 ($n = 92$). The human fetal:maternal plasma iodide ratio for premature deliveries is less than one. Thus, the data lead to the conclusion that iodide could not be concentrated in premature infants as it is in the rabbit. This MOA supports the use of the cumulative iodide concentration (area-under-the-curve or AUC) in fetal plasma as an appropriate internal dose measure for PBPK modeling.

Nasal histopathology

Histopathologic changes caused by iodomethane exposure occurred in the respiratory tract, and the salivary and thyroid glands. The respiratory tract histopathology was characterized by lesions of the nasal cavity described as degeneration of the olfactory epithelium (portal of entry effects). These lesions were identified in the 13-week inhalation toxicity study, the multigenerational reproductive toxicity study, and the combined chronic toxicity/carcinogenicity study in rats and were limited to the extrathoracic region with no involvement of the tracheobronchial or pulmonary regions. Furthermore, they did not appear to progress with time (i.e. nasal lesions of comparable severity were seen after 4, 13, and 52 weeks of exposure at the same concentration), thus suggesting that the nasal lesions were the result of reaching a critical concentration (C_{max}) rather than being time-dependent (i.e. $C \times t$; Haber's law). In contrast, a $C \times t$ relationship is assumed for all systemic effects.

Nasal histopathology—mode of action The weight of evidence suggests that the MOA responsible for iodomethane effects in the olfactory epithelium of rats is GSH depletion. A variety of potential modes of action of iodomethane on the nasal olfactory epithelium were investigated in a study by Chamberlain et al. (1998b). This in vitro study ruled out two

potential modes of action: (1) cellular protein methylation and (2) cytochrome P450 metabolism of iodomethane to formaldehyde. The only parameter studied that correlated with the site-selective lesion of the olfactory epithelium is GSH depletion catalyzed by conjugation of GSH and iodomethane by glutathione S-transferase (GST) theta.

GSH depletion as a key factor in the MOA of iodomethane on the nasal epithelium of rats was confirmed in a second study by Chamberlain et al. (1998a). In the second study, rats pretreated with an isopropyl ester of GSH to increase tissue GSH levels were protected against the nasal effects of iodomethane. Alternatively, rats pretreated with phorone and buthionine sulfoximine to deplete GSH tissue levels exhibited a potentiated response of the nasal effects following iodomethane exposure (Chamberlain et al., 1998a). This study concluded that GSH depletion by conjugation with iodomethane is the MOA for the nasal effects and a detoxification pathway for iodomethane.

Neurotoxicity

In regards to the potential role of iodomethane as a neurotoxicant, the inhalation acute neurotoxicity study in rats revealed that iodomethane exposure elicited transient behavioral signs of neurotoxicity. These signs of neurotoxicity included: an 80% decrease in motor activity, a 2–3°C decrease in body temperature, and repetitive mouth and jaw movement (clonic convulsions), in the absence of neuropathology. All effects were transient, and recovery was complete. Clinical signs of neurotoxicity were not observed in any of the subjects in the subchronic or chronic studies performed on iodomethane.

Neurotoxicity—mode of action A wide variety of inhaled solvents are known to produce anesthetic or sedative effects in animals and humans, similar to those reported for iodomethane, by altering nerve cell membrane properties (Snyder & Andrews, 1996). Iodomethane may depress spontaneous and evoked activity of neurons in the brain, possibly through non-specific actions with the lipid matrix of the nerve membrane or, as with anesthetic agents, by a more specific action on the γ-aminobutyric acid ($GABA_A$) receptor chloride channel, or by inhibition of neurotransmission at excitatory NMDA (*N*-methyl-D-aspartate) receptors (Balster, 1998; Trevor & White, 2004). These modes of action have not been linked specifically to iodomethane, but the temporal association, the type of effects, and the transient nature of the effects are similar, and indicate that the effects of iodomethane on the nervous system are due to the concentration of the parent compound in the brain.

Derivation of HECs using PBPK modeling for acute exposures

Iodomethane and iodide PBPK models were used to derive HECs for three potential endpoints for acute iodomethane exposures of 24 hours. The general design and assumptions used in the PBPK models for developmental toxicity, effects on the nasal olfactory epithelium, and acute neurotoxicity, are summarized in the following section and described in detail in the paper presented in this issue by Sweeney et al. (2009).

PBPK modeling of developmental effects

The endpoint of fetal losses identified in the developmental toxicity studies in rabbits is also considered appropriate for this risk assessment since it is presumed that developmental effects may be the outcome of an acute exposure. In the case of iodomethane, this presumption has been substantiated somewhat by the results of the phased developmental toxicity study in rabbits in which a slight increase in fetal losses was observed after two 6-hr exposures to 20 ppm iodomethane. The MOA identified for this effect is an excess in serum iodide in the rabbit fetus following exposure to 20 ppm iodomethane during GDs 23 through 26. In a MOA study, excess iodide has been shown to lead to fetal thyroid hormone disruptions (possible Wolff–Chaikoff effect) resulting in fetal loss. Consequently, the dose metric used for this assessment is the area under the concentration curve (AUC) for fetal serum inorganic iodide during a single day of exposure. An HEC of 7.4 ppm was calculated for the non-occupational risk assessment, assuming a single 6-hour iodomethane exposure in the rabbit and a corresponding 24-hour bystander iodomethane exposure. This HEC was derived based on the assumption that human fetal serum iodide levels are 120% of the maternal levels at equilibrium (Rayburn et al., 2008).

In deriving an HEC that corresponds to the 10-ppm rabbit study NOAEL described in the previous paragraph, the assumption that a single day of iodomethane exposure to the rabbit has the potential to produce an effect was employed. However, given that the window of sensitivity for the effect of fetal loss from iodomethane exposure is GDs 23 through 26, the relevant single day of exposure to produce the critical effect during the study is a single day that was preceded by 17 or more days of iodomethane exposure (Nemec, 2002, 2003; Nemec et al., 2009). In modeling repeated rabbit exposures to 10 ppm iodomethane, rabbit fetal plasma iodide increases with daily iodomethane exposure, approaching steady state around day 5. A repeat exposure modeling scenario was used to derive an AUC corresponding to the fetal plasma iodide level on a single day after four previous days of exposure. This AUC provides an alternate NOAEL-equivalent internal dose for a single-day 10-ppm iodomethane exposure in the rabbit. This 1-day AUC is not the 1-day AUC for the first day of exposure (GD 6), but rather an AUC that reflects the likely iodide profile that occurred during the window of susceptibility.

If 10 ppm is accepted as the rabbit NOAEL and prior exposure of the rabbit to iodomethane is taken into account in the model, the calculated bystander HEC is 26 ppm for this endpoint. This HEC can be compared to the HEC of 7.4 ppm, which results when the impacts of continued daily dosing of the rabbit prior to the window of susceptibility are not taken into account. Similarly, the worker (8 hr/day) HEC is calculated as 67 ppm if prior daily dosing of the rabbit is taken into account, compared to an HEC of 23 ppm when the impact of

prior dosing is ignored. The details of these PBPK modeling simulations are described by Sweeney et al. (2009). This repeat dose modeling of the rabbit exposure demonstrates the conservative nature of the proposed HECs of 7.4 ppm (non-occupational) and 23 ppm (occupational), which do not account for the prior days' dosing.

Nasal histopathology and PBPK model design

The nasal histopathology was reported in rats after 13 weeks of daily exposure to iodomethane; however, data from the published literature indicate that nasal lesions can occur in rats after acute exposures if the time profile of the exposure concentration leads to an overall iodomethane exposure of greater than or equal to 200 ppm-hr (for example, approximately 2 hours at 100 ppm-hr) (Reed et al., 1995). The design of the nasal olfactory epithelium (NOE) compartments of the PBPK model provides for iodomethane exposure to all cell layers in the compartment via diffusion through the cell stacks. Each compartment provides for diffusion of iodomethane from the nasal lumen through a layer of mucus, through four layers of epithelial cells, and the blood exchange layer. This layered or "stacked" structure of the NOE compartment allows the model to approximate the iodomethane concentration gradient across nasal tissue from the mucus layer to the vascularized region under the basal lamina. This corresponds to the degeneration that occurred through the epithelium following exposure to high concentrations of iodomethane.

The proposed MOA for nasal histopathology involves GSH depletion as a key event in the toxicity pathway leading to damage of the nasal olfactory epithelium (Chamberlain et al., 1998a, 1998b). Consequently, GSH depletion is the dose metric used in the PBPK model for interspecies extrapolation for the nasal lesions and to identify the HEC. Selection of the appropriate degree of GSH depletion to predict nasal olfactory toxicity depends on judgments about the relationship of this measure with toxicity and the time-course of exposure concentrations with the prediction of GSH depletion. The PBPK modeling has been performed assuming that in order for GSH-related toxicity to be produced in the nasal olfactory epithelium, GSH concentrations must drop below 50% of control and remain depleted. A transient 50% depletion of GSH achieved only at the end of a 24-hour iodomethane exposure is an appropriate HEC to the rat NOAEL for the acute exposure endpoint of nasal toxicity because the benchmark GSH depletion would barely be achieved, and not sustained. Average GSH depletion in the four epithelial layers of the human PBPK model is the basis for the HEC.

A number of researchers have presented data that indicate that in order for GSH-related toxicity to be produced in tissues other than the liver (including nasal tissue), GSH concentrations in extrahepatic tissues must (1) drop below 50% of control and (2) remain depleted (Frederick et al., 1992; Plopper et al., 2001; Lee et al., 2005). These studies are summarized below.

Two examples of research programs that have characterized GSH depletion in the respiratory tract are summarized for the compounds naphthalene and propylene oxide. These examples illustrate the function of the GSH detoxification pathway in the respiratory tract tissue. Cellular structure and function may be disrupted when GSH levels are reduced to approximately 40% of normal control levels, while normal cell structure and function are maintained during sustained GSH depletion to approximately 50% of control levels.

Naphthalene example. Studies performed on naphthalene provide data to clarify the relationship between GSH depletion and the susceptibility of the respiratory tract tissue to damage from chemical exposure. These studies show that a substantial depletion in GSH in respiratory tissue is necessary for toxicity to occur.

The primary target cells for naphthalene toxicity are the Clara cells in the distal airways of the lung, and injury may extend into the proximal airways as the dose increases. This difference in relative sensitivity of cells in different airway levels of the mouse was exploited in a study by Plopper et al. (2001) to show that Clara cells exposed to naphthalene that maintained at least 50% of the control levels of GSH did not exhibit changes in organelles indicative of toxicity. Clara cells that did not maintain GSH levels of 50% of control levels were susceptible to naphthalene-induced injury.

In a more recent study on naphthalene, mice were exposed to 15 ppm naphthalene and GSH levels were measured and correlated with histological analyses (Phimister et al., 2004). A 2-hour exposure to 15 ppm naphthalene resulted in a 90% loss of GSH in the distal airways, but after 24 hours, no signs of cellular injury were observed. Conversely a 4-hour exposure to 15 ppm naphthalene did cause the Clara cells in the distal airways to be swollen and vacuolated. Thus the Clara cells of the distal airways could survive a 2-hour, 90% depletion of GSH without alterations in cellular organelles, but a longer severe depletion in GSH resulted in toxicity.

Propylene oxide example. A combination of studies was performed using propylene oxide exposure to evaluate the degree of GSH depletion required for minimal damage to occur in nasal tissue of rats (Rios-Blanco, et al., 2003; Lee et al., 2005). Cell proliferation and GSH levels were measured in the same nasal epithelial tissue. An exposure to 50 ppm propylene oxide for either 3 days or 4 weeks (20 days) resulted in GSH levels that were 43% of control levels. No alteration in cell proliferation compared to control occurred in the nasal epithelium of the rats exposed to 50 ppm propylene oxide for 3 days or 4 weeks (Figure 6 of Lee et al., 2005). There was no difference in the amounts of GSH depletion or cell proliferation observed after 3 days or 4 weeks of exposure to 50 ppm propylene oxide. The next highest exposure level (300 ppm) resulted in a significant increase in cell proliferation and GSH levels in the nasal epithelia that were 16% of control. The dose response curves for cell proliferation and GSH levels of exposed rats were used by the authors to determine that a daily GSH depletion in the nasal epithelium of approximately 60% is required for an increase in cellular proliferation over controls to occur.

Iodomethane data. Himmelstein et al. (2009) examined the GSH levels in the nasal epithelium at various times during and after iodomethane exposure in rats. Exposure of rats to 25 ppm for 6 hours per day for 2 days resulted in GSH levels that gradually drop during exposure and then rebound when exposure ends, such that GSH levels return to normal levels or greater, 15 hours after exposure ends. The same pattern occurs in the nasal epithelium of rats exposed to 100 ppm iodomethane.

The data from the naphthalene studies demonstrate that a substantial depletion of GSH is required before the cells are susceptible to toxicity, and that the cells can withstand a transient 90% GSH depletion with no adverse effect, but a longer severe depletion results in toxicity to sensitive cells. The data from the propylene oxide studies demonstrate that the nasal epithelial cells remain normal even with a sustained 57% GSH depletion due to daily exposure to propylene oxide.

The available evidence demonstrates that a substantial depletion of GSH is necessary for nasal epithelial cells to become susceptible to toxicity, and this depletion must be sustained for toxicity to occur. A transient 50% depletion of GSH that is reached only at the end of a 24-hour iodomethane exposure is an appropriate point of departure for the endpoint of nasal toxicity because the benchmark GSH depletion would only just be achieved, but not sustained.

The iodomethane PBPK models in this document for the nasal endpoint were used to identify HECs corresponding to a decrease in GSH of 50%. The HECs determined were 4.5 ppm for non-occupational risk assessments, and 5.8 ppm for occupational risk assessments.

In order to ensure that HECs for bystanders would protect children from potential effects on the nasal olfactory epithelium following acute exposure, HECs specifically for children were derived. Simulations were performed to derive child-specific HECs using age-specific nasal physiology and breathing parameters corresponding to a 3-month-old child that were collected by Kimbell et al. (2005). Kimbell et al. (2005) provided age-specific nasal volumes and surface areas, but did not further subdivide these volumes and areas into the nasal vestibule, olfactory and respiratory regions, and nasopharynx, as in Frederick et al. (1998, 2002). The relative fractional areas and volumes of the different regions were assumed to be the same in adults and children, and the ratio of tidal volume to body weight (TVOL/BW) is assumed to be constant across age groups. Data to describe the thickness of the nasal mucosa in children (age 0 – 1 year) were obtained from Inagi et al. (1992). Modeled adult exposure for bystanders at the HEC of 4.5 ppm for 24 hours resulted in a maximum GSH depletion of 50%. Modeled exposure to children for 24 hours at the HEC of 4.5 ppm resulted in maximum GSH depletions of 47% for those 3 months and 1 year of age, and 49% for children 5, 10, and 15 years old. Thus, the GSH depletion results are essentially the same across ages, with slightly more depletion predicted for adults; consequently, the HEC for adults is protective of children.

PBPK modeling of transient neurotoxicity

Peak concentration of iodomethane in the brain was used for interspecies extrapolation, yielding an HEC of 10 ppm. Because the brain concentration of iodomethane approaches steady state within a few hours, this HEC is applicable to both 24-hr and 8-hr (worker) exposures. Based upon a consideration of the MOA, the neurological effects of iodomethane in rats are best explained by the impact of the parent chemical on the central nervous system. The component of the human PBPK model for the parent chemical is based upon measured values for the partition coefficient in human blood and metabolism in quantitatively important human tissues (i.e. liver and kidney). The metabolic pathways for iodomethane are well understood and have been quantified in PBPK models in animals and humans for a number of halogenated solvents (methylene chloride, trichloroethylene, tetrachloroethylene). In addition, a human PBPK model has been developed for a structurally similar chemical, methyl chloride (Jonsson et al., 2001). The results of the Jonsson et al. (2001) analysis indicated that the tissue partition coefficients determined in rats were consistent with the values estimated for humans via Markov chain Monte Carlo evaluation of human kinetic data. Confidence in the PBPK model predictions for the neurological effects of iodomethane in rats is considered medium-to-high.

Derivation of HECs using RfC methodology

Short- and intermediate-term inhalation exposure

Non-occupational exposure A two-generation reproduction study in rats is the basis for the short- and intermediate-term HEC for bystander exposure. The critical endpoints identified in this study were in the offspring, and are: decreased pup weight and weight gain, decreased thymus weights, and delays in vaginal patency acquisition. The NOAEL exposure in rats is 5 ppm, based on effects observed at the LOAEL of 20 ppm.

The HEC for bystander exposure was determined using the following equation:

$$HEC = NOAEL_{study} \times D_{animal}\ (hrs/day)/D_{human}\ (hrs/day) \times W_{animal}\ (days/wk)/W_{human}\ (days/wk) \times RDGR$$

where RGDR = regional gas dose ratio for iodomethane = 1.

Bystander exposures to iodomethane were estimated assuming human exposure 24 hours/day, 7 days per week. Rats in the two-generation reproduction study were exposed 6 hours per day, 7 days per week. Incorporation of these exposure duration assumptions in Equation (1) above results in a bystander HEC of 1.25 ppm for the short- and intermediate-term iodomethane exposure.

This bystander HEC of 1.25 ppm is considered to be conservative because the ratio of rat to human blood:air partition coefficients for iodomethane (39/18, from Sweeney et al., 2009) indicates that at a given exposure concentration the blood level in rats will be approximately twice the blood level in humans. Thus, the actual HEC will be approximately twice that calculated using this default method. The duration of exposure in the multigenerational reproduction

toxicity is appropriate for short- and intermediate-term risk assessments and it yields the lowest HEC (i.e. most health protective exposure concentration) for these exposure scenarios. A total uncertainty factor (UF) of 30 defines the level of concern in accordance with guidance provided in the RfC methodology as described below.

Occupational exposure Separate worker HECs have been calculated for short- and intermediate-term iodomethane occupational exposures due to the time adjustments made for the exposure scenarios. Short- and intermediate-term iodomethane exposure for occupationally exposed workers were derived using Equation (1), and an assumption that workers were exposed for 8 hours per day, 5 days per week results in an HEC of 3.75 ppm.

Long-term inhalation exposure

Non-occupational exposure. The chronic inhalation study in rats is the basis for the long-term HEC for bystander exposure. The critical endpoint identified in this study was an increased incidence in salivary gland squamous cell metaplasia. The NOAEL exposure for this endpoint in rats is 5 ppm, based on the effect observed at the LOAEL of 20 ppm.

Bystander exposures were estimated assuming human exposure 24 hours/day, 7 days per week. Rats in the chronic inhalation study were exposed 6 hours per day, 5 days per week. The HEC for bystander exposure was determined using Equation (1) as described for derivation of short-term HECs. The bystander HEC for long-term exposure is 0.89 ppm.

Occupational exposure. Separate worker HECs have been calculated for long-term iodomethane occupational exposures due to the time adjustments made for the exposure scenarios. Long-term iodomethane exposures for occupationally exposed workers were derived using Equation (1), and an assumption that workers were exposed for 8 hours per day, 5 days per week results in an HEC of 3.75 ppm.

Dietary exposure

Although iodomethane is approved for use as an agricultural pesticide, it is considered a non-food-use chemical because it is quickly degraded or metabolized into non-toxic degradates and subsequently incorporated into natural plant constituents. The levels of iodide released from iodomethane degradation/metabolism are lower than those expected to cause toxic effects, and iodide is ubiquitous in the environment and a required nutrient. In addition, iodomethane residues are allowed to dissipate from the soil prior to planting. As a result, a risk assessment has not been conducted for this exposure scenario.

Dermal exposure

No dermal exposure to iodomethane of any significance is expected to occur for a variety of reasons. Potential dermal exposure is unlikely because: (1) iodomethane is not handled during the application process of soil injection or drip irrigation, (2) iodomethane is packaged in pressurized cylinders that limit the dermal exposure potential, and (3) emission reduction technologies such as tarping restrict potential for dermal exposure. The high vapor pressure of iodomethane also makes significant dermal exposure unlikely, and quantification of any potential low-level exposures would be uncertain. Personal protective equipment used by appliers to protect from dermal exposure includes loose fitting clothing worn to avoid trapping vapors at the skin surface.

Classification of carcinogenic potential

Carcinogenicity evaluation

Frank evidence of thyroid toxicity was reported in the combined chronic toxicity/carcinogenicity study in rats, the developmental toxicity study in rabbits, and the dietary carcinogenicity study in mice. Indications of thyroid toxicity included enlarged thyroids, increased thyroid weights, increased incidence of ultimobranchial thyroid cysts, follicular cell hyperplasia, follicular cell adenomas, and thyroid cytoplasmic vacuolation, as well as perturbations of the thyroid–pituitary axis (decreases in T3 and T4 in conjunction with increases in TSH and rT3). These results are consistent with reports in the open literature linking excess iodine to thyroid hormone perturbations and eventually thyroid tumor formation (Kanno et al., 1992; Zhu et al., 1995).

Several lines of research and analysis have been used to investigate and characterize the potential carcinogenicity and risks posed by chronic exposure to iodomethane. Long-term iodomethane exposure studies were conducted in rats and mice as part of the data package submitted to the US EPA to support registration. The two chronic studies were: (1) a 2-year inhalation study in male and female rats (Kirkpatrick, 2005), and (2) an 18-month dietary study (encapsulated iodomethane) in male and female mice (Harriman, 2005). An increase in follicular cell thyroid tumors compared to controls was observed in only the high-dose groups of male rats and male mice, which was statistically significant in male rats only. The follicular cell thyroid tumors have been evaluated in detail and determined to result from chronic alterations in thyroid–pituitary function due to excessive iodide exposure. Rodents are particularly sensitive to this non-genotoxic mode of thyroid tumor development. Mechanistic data characterize the effects of iodomethane on thyroid and pituitary hormones in rats, and a critical review of all available genotoxicity studies of iodomethane suggests that genotoxicity does not play a role in the thyroid tumors. Early carcinogenicity studies on iodomethane provide inadequate information and are inappropriate for assessing the carcinogenicity of iodomethane as determined by authoritative bodies such as the National Toxicology Program (NTP), which de-listed iodomethane from the report on carcinogens (NTP, 2005). Based on a thorough evaluation of the data that is summarized below, the classification for iodomethane of "Not Likely to Be Carcinogenic to Humans" at doses that do not alter rat thyroid hormone homeostasis appears to be appropriate based on the revised EPA *Guidelines for carcinogen risk assessment* (EPA, 2005).

Thyroid tumor evaluation

The EPA Risk Assessment Forum (RAF) developed guidance for interpretation of thyroid follicular cell tumors

in rodents (EPA Risk Assessment Forum, 1998). The EPA RAF guidance for interpretation of thyroid follicular cell tumors in rodents identified three types of tumors: those that result from alterations in thyroid–pituitary function, those that result from mutagenic changes in the DNA of the thyroid, and those that result from a combination of altered thyroid–pituitary function and genotoxicity (EPA Risk Assessment Forum, 1998). Alterations in thyroid–pituitary function that result in follicular cell tumors in rodents are characterized by a chronic reduction in circulating thyroid hormones and an increase in TSH. The RAF recommends that a margin of exposure (MOE) or similar procedure based on non-linearity of effects be used to conduct a risk assessment of thyroid tumors when the sole MOA is alterations in pituitary and thyroid hormones.

An evaluation of the thyroid follicular cell tumors based on the RAF criteria identified in rodents following chronic iodomethane exposure indicated that the sole MOA causing these tumors is alteration of pituitary and thyroid hormone levels. The MOA was characterized based on data from the chronic rat study and additional short-term studies performed on iodomethane. A summary of the evaluation is presented below.

Follicular tumor data. In the chronic rat exposure study, groups of 60 – 70 rats/sex/group were exposed via inhalation to 0, 5, 20, or 60 ppm iodomethane for 6 hours/day, 5 days/week for 104 weeks. Exposure of rats to iodomethane for 2 years resulted in a significant increase in benign thyroid follicular cell adenoma in the high-dose group of males only. No significant increases in thyroid gland carcinoma were observed in male rats, and no increases in tumor rates were observed among female rats. Neoplastic changes in the thyroids of high-dose male rats were accompanied by non-neoplastic changes including alterations in thyroid and pituitary hormone levels, follicular cell hyperplasia and cytoplasmic vacuolation, and increased thyroid weight at the 52-week sacrifice. Females appeared less sensitive to the thyroid effects from iodomethane exposure than males, based on smaller changes in thyroid hormone levels in exposed rats compared to controls and a lower incidence of histomorphologic findings. Incidences of thyroid tumors observed among male rats exposed for life are shown in Table 2.

Male and female CD-1 mice received encapsulated iodomethane in the diet for 18 months, resulting in a slight increase in a combined incidence of thyroid follicular cell tumors (benign and malignant combined) among male mice, significant by trend test only (Table 3).

Thyroid mechanistic data. In order to determine whether thyroid follicular cell tumors that occur in a rodent study are the result of alterations in the thyroid pituitary function or genotoxicity, mechanistic data identified by the RAF were evaluated. Included in the mechanistic data evaluated are: (1) knowledge of how and where the chemical affects thyroid function (including changes in thyroid and pituitary hormone levels), (2) reversibility of effects when test article exposure ceases, (3) progression of follicular changes following prolonged exposure, (4) characterization of potential genotoxicity, (5) effects of exposure to compounds of similar structure, and (6) correlations between doses that produce thyroid effects and tumors.

Briefly, statistically significant effects on pituitary and thyroid hormone levels of male and female rats in the chronic inhalation exposure study were observed only in the high-dose groups (Tables 4 and 5) (Kirkpatrick, 2005). Serum TSH levels were elevated above controls in male rats exposed to 60 ppm at the 26-, 52-, and 104-week time points, though the differences were statistically significant only at weeks 26 and 104. After 26 weeks of exposure, circulating TSH levels increased from 2.46 µg/mL in control male rats to 30.53 µg/mL in the 60-ppm-exposed male rats, an increase greater than 12-fold. This increase in TSH level was dose-dependent; a slight increase in TSH level was observed in the 20-ppm-exposed males at the same time point (26 weeks) but this increase was not statistically significant. TSH levels increased significantly in female rats exposed to 60 ppm iodomethane only after 26 weeks of exposure, and these levels were lower than the male TSH levels (Table 5).

The data on rat thyroid weights and morphology following chronic exposure to iodomethane demonstrate that iodomethane has a specific effect on the rat thyroid, increasing thyroid size through stimulation of cellular hyperplasia.

Additional thyroid–pituitary hormone data are available from a 2-day iodomethane exposure study in male rats (Table 6) (Himmelstein et al., 2009). In this study rats were exposed to 25 or 100 ppm iodomethane 6 hours/day for 2 days, and thyroid and pituitary hormone levels were measured 18 hours after the second day of exposure. Circulating TSH levels were increased significantly in a dose-dependent manner compared to controls in both exposure groups. Circulating triiodothyronine (T3) and thyroxine (T4)

Table 2. Thyroid follicular cell tumors among male Fischer F344 rats treated with iodomethane via inhalation for 2 years (Kirkpatrick, 2005).

Tumor type	Exposure concentration (ppm)				Trend[a]
	0	5	20	60	
Adenoma	2/60	2/60	4/60	13/70*	$p < 0.001$
Carcinoma	2/60	0/60	0/60	4/70	NS
Adenoma or carcinoma	4/60	2/60	4/60	15/70*	$p < 0.001$

Note. *Significantly different from controls in pair-wise comparisons with controls (Fisher exact test), $p < 0.01$. NS, not significant.
[a]One-sided trend test.

Table 3. Thyroid follicular cell tumors among male CD-1 mice treated with microencapsulated iodomethane in the diet for 18 months (Harriman, 2005).

Tumor type	Exposure concentration (ppm, mg/kg/day)				Trend[a]
	0, 0	60, 8	200, 28	600, 84	
Adenoma or carcinoma	0/50	0/50	1/50	3/49	$p = 0.0184$

[a]One-sided trend test.

levels were reduced in the 25-ppm-exposed group, and the decreases reached statistical significance in the 100-ppm-exposed group. The hormone changes observed in short-term and chronic rat exposure studies, and the alterations in thyroid weight and histology following chronic exposure, provide unequivocal evidence that iodomethane influences thyroid–pituitary function.

The action of iodomethane on the rat thyroid was identified as an effect due to iodide, based (at least in part) on data from the 2-day exposure study in male rats and an understanding of the effects of iodide on thyroid function (Table 7) (Himmelstein et al., 2009). Exposure to iodomethane increases the serum iodide level, and this increase in iodide is known to have a direct action on thyroid function.

A second potential site of iodomethane action on thyroid–pituitary function evaluated and discounted is altered thyroid hormone metabolism in the peripheral tissues. In the short-term exposure study on male rats, two endpoints were measured to determine whether iodomethane exposure alters the metabolism and clearance of T4 (Himmelstein

Table 4. Summary of thyroid hormone data from the 24-month inhalation study of iodomethane in rats (males) (Kirkpatrick, 2005).

Analysis group	0 ppm	5 ppm	20 ppm	60 ppm
Total T3 (ng/dL)				
Week 26	57.50 ± 5.8	51.40 ± 18.6	57.12 ± 21.2	38.08 ± 16.3
Week 52	43.23 ± 11.4	38.95 ± 15.6	51.34 ± 40.4	38.29 ± 11.4
Week 104	49.79 ± 21.0	52.77 ± 21.0	50.01 ± 20.8	44.28 ± 15.9
Total T4 (μg/dL)				
Week 26	3.87 ± 1.0	3.38 ± 0.4	3.24 ± 0.5	1.71 ± 1.4**
Week 52	2.56 ± 0.8	2.45 ± 0.9	3.44 ± 0.7	3.42 ± 0.8*
Week 104	2.25 ± 0.7	2.27 ± 0.7	2.24 ± 1.0	2.50 ± 0.6
(TSH μg/mL)				
Week 26	2.46 ± 1.2	3.78 ± 1.9	4.92 ± 3.9	30.53 ± 13.7**
Week 52	2.25 ± 0.9	2.26 ± 0.6	3.60 ± 2.8	9.11 ± 11.4
Week 104	2.38 ± 1.1	3.29 ± 1.6	3.48 ± 1.8	11.29 ± 14.9**
Reverse T3 (ng/dL)				
Week 26	0.13 ± 0.05	0.12 ± 0.05	0.11 ± 0.05	0.15 ± 0.03
Week 52	0.09 ± 0.03	0.09 ± 0.05	0.09 ± 0.04	0.19 ± 0.05**
Week 104	0.03 ± 0.03	0.04 ± 0.03	0.04 ± 0.03	0.07 ± 0.05**

Note. Weeks 26 and 52 total T3 and reverse T3 compared using the Kruskal–Wallis test. All total T4 and TSH and week 104 total T3 and reverse T3 compared using Dunnett's test.
*Significantly different from the control group at 0.05.
**Significantly different from the control group at 0.01.

Table 5. Summary of thyroid hormone data from the 24-month inhalation study of iodomethane in rats (females) (Kirkpatrick, 2005).

Analysis group	0 ppm	5 ppm	20 ppm	60 ppm
Total T3 (ng/dL)				
Week 26	67.54 ± 28.3	55.38 ± 17.1	80.12 ± 21.9	49.44 ± 19.7
Week 52	81.78 ± 33.1	78.70 ± 20.5	60.10 ± 9.8	72.55 ± 15.7
Week 104	72.72 ± 32.4	70.90 ± 19.3	65.93 ± 24.0	64.82 ± 22.2
Total T4 (μg/dL)				
Week 26	2.03 ± 0.6	1.68 ± 0.6	1.93 ± 0.5	1.78 ± 0.7
Week 52	2.02 ± 0.3	2.16 ± 0.4	1.74 ± 0.3	2.23 ± 0.6
Week 104	1.55 ± 1.0	1.56 ± 0.7	1.96 ± 0.8	2.47 ± 1.0**
(TSH μg/mL)				
Week 26	1.76 ± 0.6	1.76 ± 0.5	2.09 ± 0.7	12.92 ± 13.4**
Week 52	2.61 ± 0.7	3.33 ± 1.9	2.87 ± 1.3	5.49 ± 6.4
Week 104	2.52 ± 1.0	2.93 ± 1.8	3.78 ± 2.9	3.98 ± 6.3
Reverse T3 (ng/dL)				
Week 26	0.10 ± 0.05	0.11 ± 0.03	0.15 ± 0.05	0.19 ± 0.09
Week 52	0.12 ± 0.04	0.14 ± 0.06	0.09 ± 0.02	0.33 ± 0.16**
Week 104	0.05 ± 0.03	0.09 ± 0.04	0.20 ± 0.12**	0.24 ± 0.12**

Note. **Significantly different from the control group at 0.01 using Dunnett's test.

Table 6. Mean thyroid and pituitary hormone concentrations and UDP-glucuronyltransferase (UDPGT) activity from a 2-day iodomethane exposure study in male rats (Himmelstein et al., 2009).

Analysis group	0 ppm	25 ppm	100 ppm
Total T3 (ng/dL)[a]	74.1 ± 11.4	65.9 ± 9.2	50.8 ± 14.4[d, e]
Total T4 (μg/dL)[a]	3.4 ± 0.5	3.1 ± 0.8	2.1 ± 0.9[d, e]
TSH (ng/mL)[a]	5.9 ± 1.4	10.9 ± 7.7[c, d]	21.1 ± 11.2[c, d]
Reverse T3 (ng/mL)[a]	0.067 ± 0.049	0.119 ± 0.024	0.039 ± 0.037[f]
UDPGT (nmol/min/mg)[b]	16.1 ± 3.1	17.5 ± 2.0	17.8 ± 4.8

[a]Mean ± standard deviation; n = 10 for each group unless otherwise noted.
[b]n = 5 per group.
[c]Statistically significant difference from control ($p < 0.05$) by Dunn's test.
[d]Statistically significant difference from control ($p < 0.05$) by Jonckheere–Terpstra trend test.
[e]Statistically significant difference from control ($p < 0.05$) by Dunnett's test.
[f]n = 9 per group.

Table 7. Mean inorganic serum iodide concentration (ng/mL) in male rats for inhalation exposures 6 hours/day for 2 days, beginning at hour 0 the first day and hour 24 on the second day (Himmelstein et al., 2009).

Collection time (hr)	0 ppm	25 ppm	100 ppm
0	17 ± NA	NA ± NA	NA ± NA
1	17 ± NA	5070 ± 721	22,900 ± 1620
3	19 ± NA	9510 ± 3800	60,300 ± 2860
6	22 ± NA	25,600 ± 1940	53,800 ± 4480
9	39 ± NA	18,400 ± 1550	52,500 ± 8230
24	19 ± NA	1260 ± 83.9	8170 ± 1850
25	14 ± NA	5960 ± 576	27,200 ± 13,700
27	14 ± NA	10,800 ± 1100	55,200 ± 3050
30	4.1 ± NA	34,100 ± 8170	83,200 ± 7840
33	13 ± NA	24,700 ± 1310	58,300 ± 6520
48	14 ± NA	742 ± 141	4500 ± 396
0–48	17 ± 9	NA ± NA	NA ± NA

Note. NA, not available.

et al., 2009). The liver enzyme UDP-glucuronyltransferase (UDPGT), which inactivates T4 by conjugation, and reverse T3 (rT3), the inactive form of T3, which may result from deiodination of T4, were measured in rats exposed to 25 or 100 ppm iodomethane (Table 6). UDPGT and rT3 were unchanged compared to controls following 2 days of exposure to iodomethane. The lack of effects on UDPGT and rT3 in male rats exposed to iodomethane for 2 days indicates that T4 metabolism is not the primary site of iodomethane action on the thyroid-pituitary axis.

The rapid increases in serum iodide and the lack of an effect on UDPGT and rT3 following iodomethane exposure support the position that iodide is the active metabolite of iodomethane that alters the thyroid–pituitary function in male rats. Iodide is essential for normal thyroid function, but iodide deficiency and excess both inhibit thyroid function (Ganong, 2003). The effects of excess iodide on the thyroid are well documented because pharmacologic doses of iodides were the original antithyroid agents used clinically to treat hyperthyroidism before development of thioamide drugs for that purpose (Greenspan & Dong, 2004). The primary action of high doses of iodide is inhibition of thyroid hormone production and release.

The data demonstrate a dramatic increase in serum iodide following iodomethane exposure, followed by alterations in the thyroid and pituitary hormone levels. The alterations in thyroid and pituitary hormone levels are characteristic of exposure to excess iodide, suggesting that iodide released by the metabolism of iodomethane acts directly on the thyroid to reduce hormone release and thereby alter the function of the thyroid–pituitary axis.

Reversibility of thyroid effects. The effects of iodomethane on the thyroid are reversible after short-term exposure. Reversibility of the effects of iodomethane on the thyroid that ultimately result in tumor formation can be evaluated based on data from short-term studies conducted in rats, and the known effects of excess iodide on thyroid function. The putative active metabolite of iodomethane responsible for alterations in thyroid–pituitary function in rats is iodide. Serum iodide levels increased in a dose-dependent manner in rats exposed to 25 ppm or 100 ppm iodomethane, followed by a rapid decrease after cessation of exposure. Thus, the elevated serum iodide is readily reversed following termination of iodomethane exposure. The effects of iodide are reversible upon discontinuance (Greenspan & Dong, 2004).

Thyroid lesion progression. The histomorphological data from the chronic rat study provide evidence of thyroid lesion progression from primarily hyperplasia observed after 52 weeks of exposure to an increase in adenomas after 104 weeks of exposure (Kirkpatrick, 2005).

Structural similarity and potential thyroid carcinogenicity. Potential chronic toxicity and carcinogenicity of two iodinated compounds have been evaluated in rats following oral exposure and the results reported in the literature. The inorganic iodide salt, potassium iodide, was administered to rats in drinking water, while the organic compound, iodinated glycerol, was administered to rats daily by gavage (NTP, 1990; Takegawa et al., 2000). Chronic exposure of rats to iodinated glycerol resulted in an increase in thyroid follicular cell adenomas and an increase in squamous cell metaplasia in the salivary glands. The similarity of the results of these studies performed on iodinated compounds support the non-genotoxic MOA for iodomethane in the development of follicular cell tumors in rats.

Evaluation of the evidence for a mutagenic mechanism for thyroid effects. A critical review to compare the studies of genotoxicity under good laboratory practice (GLP) testing procedures to the studies of iodomethane genotoxicity published in the open literature suggests that iodomethane is not genotoxic following in vivo exposures. The data collected in vitro and in vivo confirm that genotoxicity is unlikely to be a MOA and explanation for the thyroid tumors observed in male rats and mice in the lifetime exposure studies.

The potential genotoxicity of iodomethane was evaluated in four sets of studies conducted under GLP to support registration. The four studies were conducted with iodomethane according to OPPTS guidelines. Specific precautions were taken in each assay to make certain that the test article did not volatilize, in order to ensure accurate dosimetry within the test system. The findings in the current GLP studies show that iodomethane was negative in the bacterial reverse mutation assay (Ames assay) with a full complement of strains of *Salmonella typhimurium* and *Escherichia coli*. Iodomethane did not cause mammalian cell gene mutations in vitro. It did not cause an increase in numerical chromosome aberrations in Chinese hamster ovary cells in vitro, but it did increase structural chromosome aberrations. Iodomethane was negative in the in vivo bone marrow micronucleus assay in mice, and thus iodomethane did not cause chromosome damage in the whole animal (Table 8).

Additional genotoxicity studies of iodomethane have been published in the open literature. Some published studies have reported positive findings of mutagenic and clastogenic activity in vitro at high concentrations of iodomethane. Older studies in the literature (1985 and earlier) are difficult to compare with the recent studies conducted according to EPA guidelines, because the raw data are rarely provided and different strains of cells or reagents may have been used than are optimal for the assay. There are often methodological deficiencies in the older studies, such as the use of cytotoxic doses or experimental procedures intended to evaluate or improve a then novel screening assay. Most of the early published mutagenicity studies (conducted in the 1970s and 1980s) that used iodomethane as a test article considered it to be a "known carcinogen" based on an evaluation from the International Agency for Research on Cancer (described in IARC, 1986) that recognized tumors at injection sites following subcutaneous dosing of iodomethane as positive for carcinogenicity. Thus, iodomethane was used in many cases as one of a battery of chemicals to evaluate and optimize new tests for genotoxicity to determine whether those tests could identify carcinogens, assuming that iodomethane would be positive for mutagenicity.

Table. 8 Summary comparison of genotoxicity findings in GLP and published studies of iodomethane.

Endpoint	GLP studies		Published literature studies		
	Finding	Citation	Interpretation	Citation	Comment
Bacterial mutagenicity	–	Wagner, 2001	CBD	Rosenkranz, 1979	Toxic doses likely, poor reporting
			–/CBD	Simmon, 1977, 1979a	Standard assay neg.; closed study pos.; survival unknown
			CBD	Hemminki, 1980	High toxic doses possible; survival questionable
			Inadequate	Takahashi, 1987	Very high doses used
Yeast recombinants	NA		CBD	Simmon, 1979b	Limited toxicity or survival data; technical problems; no dose response information
Fungi mutagenicity	NA		Inadequate	IARC, 1986	Reported neg.; no control for volatility
In vitro mammalian mutagenicity	–;	San, 2001	CBD	Amacher, 1984	Appears to be neg. based on current testing criteria
In vitro mammalian clastogenicity	+	Gudi, 2001	–/+	Clive, 1979	Mixed weak findings, no dose response
			+	Moore, 1982	Pos. dose response, low, moderate, and high toxicity
			+	Moore, 1985a, 1985b	Pos. dose response, low toxicity
			Probably –	Amacher, 1985	Appears neg. based on current testing criteria, data presentation makes evaluation difficult
In vitro mammalian	N/A		–	Oshiro, 1981	Iodomethane neg., other alkylating agents pos.
Cell transformation			–/+	Pienta, 1977	Author called pos., but no dose response
In vivo clastogenicity	–;	Gudi, 2001	NA		
In vivo DNA adducts	NA		Probably –;	Gansewendt, 1991	Radioactivity incorporated into the DNA bases; no confirmation of direct methylation

Note. GLP, good laboratory practice; CBD, cannot be determined; NA, not available; + or pos., positive; – or neg., negative.

Each study identified in the literature that investigated the potential mutagenicity or genotoxicity of iodomethane is reviewed briefly below. In general, given the limitations of the published studies, the results are generally consistent with the findings of the current GLP studies (Table 8). The studies in the open literature show the following: (1) iodomethane induces clastogenic effects in mammalian cells in vitro but does not appear to induce mutations in bacteria or mammalian cells at non-cytotoxic doses; (2) treatment of rats with radiolabeled iodomethane resulted in measurable levels of radioactivity associated with methylated DNA bases; however, gas chromatography-mass spectroscopy (GC-MS) analysis confirmed that the radiolabeled carbon atom was incorporated into the DNA base (from de novo synthesis) and was not the result of direct methylation of DNA.

Bacteria and yeast. In addition to the battery of bacterial mutagenicity tests run under current guidance and GLP procedures (Wagner & Dakoulas, 2001), several older studies have been published related to iodomethane's mutagenic potential in standard and non-standard assays in bacteria (*Salmonella typhimurium* and *Escherichia coli*) and yeast (*Saccharomyces cervesia*) (Moura Duarte, 1972; McCann et al., 1975; Simmon et al., 1977; Rosenkranz & Poirier, 1979; Simmon, 1979a, 1979b; Hemminki et al., 1980; Takahashi & Kawazoe, 1987a, 1987b) (Table 8). Substantial methodological and data reporting deficiencies occur throughout the reports in the public literature, such that the findings on the potential iodomethane mutagenicity presented in these studies are not completely reliable. Reporting deficiencies in many of the older studies make it difficult to compare the findings in the published studies to those of the modern study. As observed in the GLP studies, doses that are sufficiently high to overwhelm cellular defenses result in significant cytotoxicity. In several studies (Rosenkranz & Poirer, 1979; Simmon, 1979a; Hemminki et al., 1980; Takahashi & Kawazoe, 1987a, 1987b), it is likely that high cytotoxic test concentrations were employed (comparing doses used to the GLP study), although data on cell survival were not adequately reported. Methodological deficiencies in published studies include: (1) an insufficiently described assay in which high concentrations of iodomethane were applied to filter discs that were then dropped on plated bacterial cells (Rosenkranz & Poirier, 1979), (2) incubation of all yeast cells at the highest test concentration before serial dilution

(Simmon, 1979b), and (3) evaluation of cell survival based on different incubation conditions compared with evaluation of mutagenicity (Hemminki et al., 1980). Given the study limitations, the findings from the older published studies in bacteria do not necessarily conflict with the negative findings in the modern GLP studies.

Mammalian cell in vitro. Iodomethane has been tested in a variety of mammalian cell test systems; some systems examined mutagenicity and others examined clastogenic potential. These include two modern GLP studies (Gudi & Brown, 2001; San & Clarke, 2001) and nine earlier published studies (Pienta et al., 1977; Clive et al., 1979; Oshiro et al., 1981; Moore & Clive, 1982; Amacher & Zelljadt, 1984; Amacher & Dunn, 1985; Moore et al., 1985a, 1985b) (Table 8). The iodomethane studies conducted on mammalian cells in vitro indicate that iodomethane may cause clastogenic effects in cell culture but is not mutagenic. The findings of the GLP genotoxcity studies in vitro are reasonably consistent with the findings in the literature. The GLP study of San and Clark (2001, Table 8) was negative for induction of mutations in mammalian (Chinese hamster ovary, CHO) cells. A similar study by Amacher and Zelljadt (1984) examined HGPRT (hypoxanthine-guanine phosphoribosyl transferase) revertants in CHO cells. Although reported as positive by the authors, this result would probably be considered negative under current testing guidance due to the wide range of spontaneous mutant frequency, and as such, is consistent with the results of the guideline study of San and Clarke (2001).

The GLP study of Gudi and Brown (2001, Table 8) found a significant increase in structural chromosomal aberrations, but not numerical aberrations in CHO cells in vitro. This is generally consistent with published literature studies that suggest iodomethane may cause structural damage in mammalian cells in vitro. Mixed findings were observed by Clive et al. (1979) who suggested that the small colonies of L5178Y/TK+/– mouse lymphoma cells produced following iodomethane exposure reflected a clastogenic effect. The generally positive findings by Moore et al. (1985a, 1985b) and Moore and Clive (1982) of small colony formations in mouse lymphoma cells in vitro also were identified by the authors as indicative of a clastogenic effect, and would be consistent with the guideline study of Gudi and Brown (2001). Amacher and Dunn (1985) report a weakly positive effect of iodomethane as a mutagen in mouse lymphoma cells, but limitations in the data (e.g. no significant increases over controls at lower doses and low cell survival at higher doses) suggest that this study should not carry significant weight in the evaluation of the potential mutagenicity of iodomethane, and would likely be considered as negative based on current guidelines.

Oshiro et al. (1981) reported that iodomethane did not cause cell transformation in mouse embryo cells, while the other alkylating agents evaluated in this study were positive. In contrast, Pienta et al. (1977) asserted that iodomethane caused cell transformation in hamster embryo cells, despite the lack of dose response or statistical significance. The weight of evidence suggests that iodomethane exposure to mammalian cells in vitro may result in clastogenicity but not mutagenicity or numerical chromosome aberrations.

Mammalian in vivo. Two studies have been conducted examining the potential of iodomethane to cause DNA adducts or genetic damage in vivo. These are (1) a GLP micronucleus assay in mice following intraperitoneal administration (Gudi & Krsmanovic, 2001, Table 8) and (2) a DNA adduct study in F344 rats (Gansewendt et al., 1991a).

In the guideline study, treatment of male and female mice with 0, 25, 50, or 100 mg/kg (the maximum tolerated dose, MTD) of iodomethane via intraperitoneal injection did not increase the incidence of micronucleated polychromatic erythrocytes in bone marrow (Gudi & Krsmanovic, 2001). Thus, iodomethane exposure did not cause chromosomal damage in mice in vivo.

The second in vivo study was published by Gansewendt et al. (1991a) to evaluate the potential DNA binding of iodomethane in rats. Groups of male and female F344 rats were exposed to radiolabeled iodomethane by oral gavage or via inhalation. Radioactivity associated with DNA was examined in the liver, lung, stomach, and forestomach. ^{14}C-radiolabel was measured in DNA purified from all four tissues, with levels of total radioactivity predominating in the stomach and forestomach. The stomach and forestomach were not target tissues of iodomethane carcinogenesis among rats treated for 2 years via inhalation. The levels of measured radioactivity in the DNA of various tissues do not make intuitive sense if the radioactivity is assumed to be associated with DNA adducts. For example, radioactivity associated with DNA was essentially the same in the lung following oral exposure as following inhalation exposure, although if adducts formed due to tissue contact with the reactive chemical, more radioactivity would be expected at the portal of entry for each route of exposure, the lungs for inhalation, and perhaps the stomach for oral exposure. Moreover, radioactivity associated with DNA was highest in the stomach and forestomach from both oral and inhalation exposures. The authors noted that in previously published inhalation studies with [^{14}C]methyl chloride, researchers had found that significant levels of the ^{14}C-label was incorporated into the DNA during de novo synthesis of [^{14}C]methyl groups into the deoxynucleotides. The authors suspected that this might also have occurred with methyl bromide, since scientists had reported a remarkably long persistence (60 hr) of radioactivity in the stomach following inhalation exposures to [^{14}C]methyl bromide in rats. Based on these observations for structurally similar compounds, the authors attempted to determine whether the DNA binding of iodomethane was a result of direct methylation or de novo synthesis of radiolabeled carbon into the nucleotide base.

Gansewendt et al. (1991a) took two approaches to try to differentiate the location of the radiolabel. The first approach was to hydrolyze the DNA (either enzymatically or with acid hydrolysis), followed by high performance liquid chromatography (HPLC) separation of the bases. The collected fractions corresponding to the methylguanine

adducts were then analyzed by GC-MS in an attempt to confirm the radiolabeled adducts.

The authors observed increases in radioactivity in the HPLC fractions that corresponded to N7-methylguanine and O^6-methylguanine retention times, and estimated the DNA adduct levels based on the radioactivity in the fractions. However, the authors also noted that radioactivity in the fractions corresponding to guanine, cytosine, thymidine, and adenosine clearly indicated that de novo synthesis of the radiolabel was occurring. In addition, Gansewendt et al. (1991a) observed that methylated DNA bases formed as a result of enzymatic hydrolysis and HPLC preparation of untreated calf thymus DNA. Importantly, the authors confirmed with GC-MS that the radiolabeled carbon from [^{14}C] iodomethane-treated rats was incorporated into the purine bases (i.e. de novo synthesis) in N3-methyladenine and N7-methylguanine[1] in liver. Levels of the O^6-methylguanine were too low to measure by GC-MS.

In summary, Gansewendt et al. (1991a) measured increased radioactivity associated with methylated DNA bases, N7-methylguanine, N3-methyladenine, and O^6-methylguanine. They confirmed by GC-MS that the carbon-14 radiolabel was present in the guanine portion of the molecule for N7-methylguanine and the adenine portion of the N3-methyladenine, whereas levels of O^6-methylguanine were too low to measure by GC-MS. The authors stated that a portion of the methylated DNA molecules likely resulted from direct methylation of DNA by iodomethane (i.e. true DNA adducts), but no evidence was presented in the paper to confirm that assertion. A more likely explanation of the results is that the radioactivity levels measured in the lung, liver, stomach, and forestomach of rats are a result of de novo synthesis, and the levels of incorporation of the label reflect the background rates of cell synthesis in those tissues. This explanation is consistent with the observation that approximately the same levels of radioactivity per unit dose were observed in the four tissues following oral versus inhalation exposures—because higher relative levels would be expected at the portal of entry if the label were the result of DNA methylation. It is also consistent with the fact that the highest levels were observed in the stomach and forestomach where cell turnover is much higher than in the liver or lung. This explanation is also consistent with the rapid breakdown of iodomethane in vivo. Very similar findings of radio-incorporation were observed with methyl bromide, which did not cause tumors in long-term inhalation studies (Gansewendt et al., 1991b). As such, genotoxicity is unlikely to be a MOA that explains the thyroid tumors observed in male rats and mice in the lifetime exposure studies of iodomethane.

Thus, a weight of evidence evaluation of the available genotoxicity data in both the current EPA guideline genotoxicity studies and those in the open literature indicate that iodomethane does not cause mutations in bacteria or in mammalian cells in vitro. In mammalian cells in vitro, iodomethane may cause clastogenic effects in cell culture but it is not mutagenic. Clastogenicity was not observed following high-dose exposures in vivo. A study conducted to examine the potential for formation of DNA adducts of iodomethane in vivo did not yield evidence that iodomethane causes DNA adducts, but did demonstrate that degraded iodomethane adds to the carbon pool which is incorporated into DNA during DNA synthesis. The data collected in vitro and in vivo confirm that genotoxicity is unlikely to be a MOA and explanation for the thyroid tumors observed in male rats and mice in the lifetime exposure studies.

The review of the genotoxicity data concludes that iodomethane is not genotoxic following in vivo exposures; consequently, genotoxicity is unlikely to be the cause of iodomethane-induced thyroid tumors in animals.

Dose correlations with thyroid effects. A clear dose response effect is observed in serum iodide level, alterations in thyroid hormone levels, thyroid histomorphology, and thyroid follicular cell tumor incidence in male rats exposed to iodomethane. In male rats exposed to 25 ppm or 100 ppm iodomethane for 6 hours per day for 2 days, serum iodide levels increased in a dose-dependent manner (Table 7). A dose response effect was observed in circulating hormone levels in the chronic rat study, such that no significant changes in thyroid or pituitary hormone levels were identified in male rats exposed to 5 or 20 ppm iodomethane compared to controls, but alterations in thyroid hormone levels were significant in rats exposed to 60 ppm iodomethane. A similar dose response pattern was observed in the thyroid follicular cell tumor incidence. Tumor incidence was elevated in the male rats exposed to 60 ppm iodomethane but not in the low- and mid-dose groups compared to controls. Thyroid tumor incidence was elevated only in male rats exposed to 60 ppm iodomethane, the only dose that produced significant changes in thyroid hormone levels (Kirkpatrick, 2005).

Thyroid tumor evaluation conclusion. The thyroid tumor response following chronic iodomethane exposure is mediated by the sustained stimulation of cell proliferation by TSH, consistent with the increase in thyroid follicular cell tumors. The data on thyroid weights and morphology following exposure to iodomethane demonstrate that iodomethane has a specific effect on the rat thyroid, increasing thyroid size through stimulation of cellular hyperplasia. Alterations in circulating thyroid and pituitary hormones observed in short-term and chronic studies provide unequivocal evidence that iodomethane exposure at high concentrations influences thyroid–pituitary function in rodents. Collectively the mechanistic data suggest that iodomethane metabolized to iodide exerts actions centrally on the thyroid gland rather than peripherally to decrease thyroid hormone levels. In addition, the data collected in vitro and in vivo confirm that genotoxicity is unlikely to be a MOA and explanation for the thyroid

[1]A full mass spectrum of the internal standard, N7-methyl-D_3-guanine, which was labeled with three deuterium atoms on the guanine moiety, is shown in Figure 4 of Gansewendt et al. (1991a). The derivatized molecular ion has as a mass of 312 amu, while the largest ion fragment is 297 amu, corresponding to loss of the methyl group leaving the deuterated guanine ion. In the middle frame of Figure 4 of the paper, the authors measured the presence of N7-methylguanine adduct with a guanine moiety of 296 amu, which corresponds to a radiolabeled guanine ion containing a ^{14}C in place of an unlabeled carbon with a molecular weight of 12. Thus, this spectrum confirms that the location of the ^{14}C is in the guanine not the methyl group.

tumors observed in male rats and mice in the lifetime exposure studies. The sole MOA identified for the development of thyroid tumor development in rats exposed to iodomethane is alteration in pituitary and thyroid hormones.

The EPA OPP Cancer Assessment Review Committee (CARC) evaluated the rodent bioassays and mechanistic data available for iodomethane. Evidence of carcinogenicity in the iodomethane database manifested as an increased incidence of thyroid follicular cell tumors observed in both the inhalation chronic toxicity/carcinogenicity study in rats and the carcinogenicity study in mice. The committee concluded that the key event influencing the thyroid tumor response is the sustained stimulation of cell proliferation by TSH, consistent with the increase in thyroid follicular cell tumors *only*. Based on the evidence that rats are substantially more sensitive than humans to the development of thyroid follicular cell tumors in response to thyroid hormone imbalance, the CARC classified iodomethane as not likely to be carcinogenic to humans at doses that do not alter rat thyroid hormone homeostasis. This corresponds to a standard hazard descriptor from the revised EPA *Guidelines for carcinogen risk assessment* (EPA, 2005).

Evidence for carcinogenicity from the literature.

Early carcinogenicity studies on iodomethane included subcutaneous and intraperitoneal injection studies (described in International Agency for Research on Cancer, 1986). Injection of iodomethane test article in the animal would result in high local concentrations in the tissues that overwhelm the detoxification ability of the cells and result in significant necrosis as observed in the cell survival data from in vitro mutagenicity studies performed at high doses. In one study, iodomethane was injected intraperitoneally in Strain A mice, a strain that develops lung tumors by an early age. Iodomethane produced no statistically significant increases in tumor formation at any dose group (by pairwise comparisons or two-sided trend test), whereas positive controls (urethane) resulted in high incidences of lung tumors. These data provide inadequate information and are inappropriate for assessing the carcinogenicity of iodomethane. Authoritative bodies (IARC and NTP) did not classify iodomethane carcinogenicity based on these data, due to inadequacy. We now have two chronic exposure studies conducted by appropriate routes of administration (rats by inhalation, and mice by dietary administration). These data, along with significant mechanistic data and new genotoxicity studies, demonstrate that iodomethane does not pose a carcinogenic hazard to humans.

Uncertainty factors

When conducting inhalation risk assessments, the magnitude of the uncertainty factors (UFs) applied to the HECs depends on the methodology used to calculate the risk. This risk assessment is based on PBPK modeling and the RfC methodology developed by the EPA ORD for the derivation of inhalation reference concentrations (RfCs) and HECs for use in margin of exposure (MOE) calculations. Since the PBPK models and the RfC methodology take into consideration the pharmacokinetic differences between test species and humans, the traditional UF of 10 for interspecies extrapolation may be reduced to 3, while the UF for intraspecies variation is retained at 10. The UF of 3 for interspecies extrapolation is retained to account for the pharmacodynamic differences between animals and humans which are not accounted for in the RfC methodology or PBPK models. Thus, the overall UF applied to HECs derived using the RfC methodology or PBPK modeling is customarily 30. The UF appropriate for application to the HECs for iodomethane is 30.

Summary of HECs and acceptable exposures

A summary of the HECs for the potential iodomethane exposures to bystanders and workers is provided in Tables 9 and 10. HECs were derived taking into account the differences in exposure duration between the animal studies and the anticipated human exposure scenarios. Bystander exposures were evaluated assuming that human exposure occurs for 24 hours over 1 day. Worker exposure was estimated assuming exposure could occur 8 hours per day, 5 days per week. Rabbits in the developmental toxicity study and rats in the two-generation reproduction study were exposed 6 hours per day, 7 days per week. Rats in the 13-week and chronic inhalation studies were exposed to iodomethane 6 hours/day and 5 days per week.

Summary and conclusions

The current iodomethane database provides an abundance of information to assess potential risks to the human population from iodomethane exposure via the inhalation route. The pattern of toxicity attributed to iodomethane exposure via the inhalation route includes developmental toxicity,

Table 9. HECs derived for potential iodomethane exposure to bystanders.

Endpoint	Study	NOAEL (ppm)	Method	HEC (ppm)	Dose metric
Acute exposure: bystanders					
Fetal toxicity	Developmental rabbit	10	PBPK	7.4	Fetal plasma iodide AUC
Nasal degeneration	13-week rat	21	PBPK	4.5	50% GSH depletion NOE
Transient neurotoxicity	Acute neurotoxicity	27	PBPK	10	Peak brain iodomethane
Short- and intermediate-term exposure: bystanders					
Offspring effects	Two-generation rat	5	RfC	1.25	RGDR = 1
Long-term exposure: bystanders					
Salivary gland squamous metaplasia	Chronic inhalation rat	5	RfC	0.89	RGDR = 1

Table 10. HECs derived for potential iodomethane exposure to workers.

Endpoint	Study	NOAEL (ppm)	Method	HEC (ppm)	Dose metric
Acute exposure: workers					
Fetal toxicity	Developmental rabbit	10	PBPK	23	Fetal plasma iodide AUC
Nasal degeneration	13-week rat	21	PBPK	5.8	50% GSH depletion NOE
Transient neurotoxicity	Acute neurotoxicity	27	PBPK	10	Peak brain iodomethane
Short- and intermediate-term exposure: workers					
Offspring effects	Two-generation rat	5	RfC	3.75	RGDR = 1
Long-term exposure: workers					
Salivary gland squamous metaplasia	Chronic inhalation rat	5	RfC	3.75	RGDR = 1

nasal lesions, neurotoxicity, and thyroid toxicity. Three critical endpoints were evaluated to characterize the potential risk from acute exposure to workers and bystanders. These endpoints are: fetal losses in a developmental toxicity study in rabbits, nasal histopathology in a subchronic inhalation toxicity study in rats, and transient neurotoxicity in rats. The lowest HEC for non-occupational, or bystander exposure was 4.5 ppm for the nasal histopathology endpoint. The EPA Health Effects Division (HED) CARC has identified iodomethane as not likely to be carcinogenic to humans at doses that do not alter rat thyroid hormone homeostasis. This is consistent with the revised EPA *Guidelines for carcinogen risk assessment* (EPA, 2005).

The iodomethane PBPK models were developed to derive human exposures that are equivalent to the animal NOAELs by predicting the human internal dose to the target tissue that is equivalent to the animal internal dose at the target tissue following the NOAEL exposure. The PBPK models are the product of extensive physiologic and compound-specific research, and application of sophisticated model design methods that are described in all the accompanying articles in this journal issue. These tools allowed determination of HECs for bystanders and workers for each of three endpoints of potential concern for acute exposures based on internal dose metrics rather than on external exposures alone. The PBPK models for iodomethane provide more certain estimates of risk from potential exposure than can be obtained using default risk assessment methods that generally rely on external exposure divided by an uncertainty factor, and the default methods do not incorporate metabolism. The results of PBPK modeling of the endpoints from acute exposure to iodomethane were unexpected in that the endpoint with the lowest animal NOAEL did not result in the lowest HEC. The lowest HEC for an acute duration endpoint was determined for the effect on the portal of entry nasal degeneration, which had a NOAEL of 21 ppm. The developmental endpoint had the lowest NOAEL for an effect attributed to an acute exposure, 10 ppm. The observation relevant to the developmental endpoint, that fetal rabbits concentrate iodide while the human fetus does not concentrate iodide, was captured in the PBPK model and this is reflected in a higher HEC for that endpoint than would be determined using default methodology. The fact that humans are less sensitive than rabbits to the critical effect that causes developmental toxicity in rabbits resulted in a lower HEC for the developmental endpoint than for the nasal degeneration endpoint. When default assumptions are used for risk assessment of effects from acute inhalation exposure to a given chemical, the endpoint with the lowest animal NOAEL generally results in the lowest HEC, but that is not the case for iodomethane evaluated using PBPK modeling. The effect of nasal olfactory degeneration is the primary effect of concern for risk assessment of acute exposure to iodomethane.

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References

Amacher, D. E., and Dunn, E. M. 1985. Mutagenesis at the ouabain-resistance locus of 3.7.2C L5178Y cells by chromosomal mutagens. *Environ. Mutagen.* 7:523–533.

Amacher, D. E., and Zelljadt, I. 1984. Mutagenic activity of some clastogenic chemicals at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. *Mutat. Res.* 136:137–145.

Arrington, L. R., Taylor, R. N.Jr., Ammerman, C. B., and Shirley, R. L. 1965. Effects of excess dietary iodine upon rabbits, hamsters, rats, swine. *J. Nutr.* 87 (4):394–8.

Balster, R. L. 1998. Neural basis of inhalant abuse. *Drug Alcohol Depend* 51 (1–2):207–214.

Chamberlain, M. P., Lock, E. A., Gaskell, B. A., and Reed, C. J. 1998a. The role of glutathione S-transferase and cytochrome P450-dependent metabolism in the olfactory toxicity of methyl iodide in the rat. *Arch. Toxicol.* 72:420–428.

Chamberlain, M. P., Lock, E. A., and Reed, C. J. 1998b. Investigations of the pathways of toxicity of methyl iodide in the rat nasal cavity. *Toxicology* 129 (2–3):169–181.

Clive, D., Johnson, K. O., Spector, J. F., Batson, A. G., and Brown, M. M. 1979. Validation and characterization of the L5178Y/TK+/– mouse lymphoma mutagen assay system. *Mutat. Res.* 59:61–108.

Corley, R. A., Minard, K. R., Trease, L. L., Trease, H. E., Harkema, J. R., Kimbell, J. S., Gargas, M. L., and Kinzell, J. H. 2009. Magnetic resonance imaging and computational fluid dynamics (CFD) simulations of rabbit

nasal airflows for the development of hybrid CFD/PBPK models. *Inhal. Toxicol.*

EPA. 1991. *Guidelines for developmental toxicity risk assessment*. Federal Register. 56 (234):63798–63826. EPA/600/FR-91/001, December 5.

EPA. 1994. *Methods for derivation of inhalation reference concentrations and application of dosimetry*. Office of Health and Environmental Assessment, Office of Research and Development, US Environmental Protection Agency. EPA/600/8-90/066F, October.

EPA. 2005. *Guidelines for carcinogen risk assessment*. EPA/630/P-03/001F, March.

EPA OPP Health Effects Division (HED). 2007. *Iodomethane: revised human health risk assessment which incorporates results of human iodine monitoring*, DP Barcode D339055, PC Code: 000011. Memorandum from Dr. Elizabeth Mendez, EPA HED to Mary Waller, EPA, RD, August 23.

EPA Risk Assessment Forum. 1998. *Assessment of thyroid follicular cell tumors*. EPA/630/R-97/002, March.

FDA. 2003 *Amiodarone Pacerone product label.* Food and Drug Administration approved, 08/2003.

Frederick, C. B., Bush, M. L., Lomax, L. G., Black, K. A., Finch, L., Kimbell, J. S., Morgan, K. T., Subramaniam, R. P., Morris, J. B., and Ultman, J. S. 1998. Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. *Toxicol. Appl. Pharmacol.* 152:211–231.

Frederick, C. B., Lomax, L. G., Black, K. A., Finch, L., Scribner, H. E., Kimbell, J. S., Morgan, K. T., Subramaniam, R. P., Morris, and J. B. 2002. Use of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry comparisons of ester vapors. *Toxicol. Appl. Pharmacol.* 183:23–40.

Frederick, C. B., Potter, D. W., Chang-Mateu, M. I., Andersen, and M. E. 1992. A physiologically based pharmacokinetic and pharmacodynamic model to describe the oral dosing of rats with ethyl acrylate and its implications for risk assessment. *Toxicol. Appl. Pharmacol.* 114:246–260.

Ganong, W. F. 2003. The thyroid gland. In: J. Foltin, J. Matragrano, J. Ransom, and K. Davis (eds.), *Review of medical physiology*. New York: Lange Medical Books/McGraw-Hill, pp 320–335.

Gansewendt, B., Xu, D., Foest, U., Hallier, E., Bolt, H. M., and Peter, H. 1991a. DNA binding of methyl iodide in male and female F344 rats. *Carcinogenesis* 12:463–467.

Gansewendt, B., Foest, U., Xu, D., Hallier, E., Bolt, H. M., and Peter, H. 1991b. Formation of DNA adducts in F-344 rats after oral administration or inhalation of [14C]methyl bromide. *Food Chem. Toxicol.* 29:557–563.

Greenspan, F. S., and Dong, B. J. 2004. Thyroid and antithyroid drugs. In *Basic and clinical pharmacology*, 9th ed., ed. B. G. Katzung, . New York: Appleton & Lange, Lange Medical Books/McGraw-Hill, pp. 625–640.

Gudi, R., Brown, C. 2001. *In vitro mammalian chromosome aberrations test with iodomethane, performed at BioReliance, Rockville (MD)*. BioReliance, Laboratory Study No. AA38UL.331.BTL, DPR Doc. No. 52875-021. MRID# 45593814.

Gudi, R., Krsmanovic, L. 2001. *Mammalian erythrocyte micronucleus test with iodomethane, performed at BioReliance, Rockville (MD).* BioReliance, Laboratory Study No. AA38UL.123.BTL, 021. MRID# 45593816.

Harriman, J. F. 2005. *An 18 month dietary carcinogenicity study of microencapsulated iodomethane in mice*. Unpublished report prepared by WIL Research Laboratories, Inc. Lab. No. WIL-4180125, DPR Doc. No. 52875-099. MRID# 46582801.

Hemminki, K., Falck, K., Vainio, H. 1980. Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals: epoxides, glycidyl ethers, methylating, ethylating agents, halogenated hydrocarbons, hydrazine derivatives, aldehydes, thiuram, dithiocarbamate derivatives. *Arch. Toxicol.* 46:277–285.

Hermouet, C., Garnier, R., Efthymiou M-L., Fournier, PE. 1996. Methyl iodide poisoning: report of two cases. *American J. Indust*. Med. 30:759-764.

Himmelstein, M. W., Kegelman, T. A., DeLorme, M. P., Everds, N. E., O'Connor, J. C., and Kemper, R. A., Nabb, D. L., Mileson, B. E., and Bevan, C. 2009. Two-day inhalation toxicity study of methyl iodide in the rat. *Inhal. Toxicol.* 21:480–487.

Inagi, K. 1992. Histological study of mucous membranes in the human nasal septum. *Nippon Jibiinkoka Gakkai Kaiho* 95:1174-89.

International Agency for Research on Cancer. 1986. Methyl iodide. In *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans*, vol. 41, pp. 213–227. Lyon: IARC working group.

Jonsson, F., Bois, F. Y., and Johanson, G. 2001. Assessing the reliability of PBPK models using data from methyl chloride-exposed, non-conjugating human subjects. *Arch. Toxicol.* 75:189–199.

Kanno, J., Onodera, H., Furuta, K., Maekawa, A., Kasuga, T., Hayashi, and Y. 1992. Tumor-promoting effects of both iodine deficiency and iodine excess in the rat thyroid. *Toxicol. Pathol.* 20(2):226–235.

Kimbell, J. S., Kalisak, D. L., Conolly, R. B., Miller, F. J., Jarabek, A. M. 2005. *A mechanistic model of lifetime cancer risk for inhalation exposures to reactive gases.* Poster #1304, Society of Toxicology Annual Meeting, March 2005.

Kirkpatrick, D. 2001. *A 13-week inhalation toxicity study (with a four-week interim necropsy) of iodomethane in albino rats: Final report*. Unpublished study prepared by WIL Research Laboratories, Inc. Lab. Project Number: WIL-418015. EPA MRID# 45593810.

Kirkpatrick, D. 2005. *A 24-month inhalation combined chronic toxicity/carcinogenicity study of iodomethane in rats: Final report.* Project Number: WIL-418019. Unpublished study prepared by WIL Research Laboratories, Inc. MRID# 46512401.

Kirman, C. R., Sweeney, L. M., Gargas, M. L., and Kinzell, J. H. 2009. Evaluation of possible modes of action for acute effects of methyl iodide in laboratory animals. *Inhal. Toxicol.* 21:537–551.

Lee, M. S., Faller, T. H., Kreuzer, P. E., Kessler, W., Csanaday, G. A., Putz, C., Rios-Blanco, M. N., Pottenger, L. H., Segerback, D., Osterman-Golkar, S., Swenberg, J. A., and Filser, J. G. 2005. Propylene oxide in blood and soluble nonprotein thiols in nasal mucosa and other tissues of male Fischer 344/N rats exposed to propylene oxide vapors—relevance of glutathione depletion for propylene oxide-induced rat nasal tumors. *Toxicol. Sci.* 83:177–189.

Logothetopoulos, J., and Scott, R. F. 1956. Active iodide transport across the placenta of the guinea pig, rabbit, rat. *J. Physiol.* 132:365–371.

McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc. Natl. Acad. Sci. U.S.A*. 72:5135–5139.

Moore, M. M., and Clive, D. 1982. The quantitation of TK–/– and HGPRT-mutants of L5178Y/TK+/– mouse lymphoma cells at varying times post-treatment. *Environ. Mutagen.* 4:499–519.

Moore, M. M., Clive, D., Howard, B. E., Batson, A. G., and Turner, N. T. 1985a. In situ analysis of trifluorothymidine-resistant (TFTr) mutants of L5178Y/TK+/– mouse lymphoma cells. *Mutat. Res.* 151:147–159.

Moore, M. M., Clive, D., Hozier, J. C., Howard, B. E., Batson, A. G., Turner, N. T., and Sawyer, J. 1985b. Analysis of trifluorothymidine-resistant (TFTr) mutants of L5178Y/TK+/– mouse lymphoma cells. *Mutat. Res.* 151:161–174.

Moura Duarte, F. A. 1972. Mutagenic effects of some esters of inorganic acids in *Aspergillus nidulans (Eidam)* winter (Spanish). *Cienc. Cultura* 24:42–52.

Nemec, M. 2001a. *An inhalation prenatal developmental toxicity study of iodomethane in rats: Final report.* Unpublished study prepared by WIL Research Laboratories, Inc. Lab. Project Number: WIL-418010. EPA MRID# 45593812.

Nemec, M. 2001b. *An inhalation two-generation reproductive toxicity study of iodomethane in rats: Comprehensive.* Unpublished study prepared by WIL Research Laboratories, Inc. Final Report. Lab. Project Number: WIL-418004, WIL-418004F, WIL-418004M. EPA MRID# 45710301.

Nemec, M. D. 2002. *An inhalation prenatal developmental toxicity study of iodomethane in rabbits.* WIL-418002. WIL Research Laboratories, Ashland, OH. January 28, DPR Doc. No. 52875-018. MRID# 45593811.

Nemec, M. D. 2003. A phased-exposure prenatal developmental toxicity study of iodomethane in rabbits. WIL-418023. WIL Research Laboratories, Ashland, OH. February 3. DPR Doc. No. 52875-045. MRID# 46077001.

Nemec, M. D., Sloter, E., Stump, D., Holson, J., Kirkpatrick, D., and Kinzell, J. 2009. Prenatal developmental toxicity studies of inhaled methyl iodide vapor in rabbits reveal a susceptible window of exposure inducing late gestational fetotoxicity. *Inhal. Toxicol.* 21:449–461.

NTP. 1990. *Toxicology and carcinogenesis studies of iodinated glycerol (Organidin®) (CAS No. 5634-39-9) in F344/N rats and B6C3F1 mice (gavage studies)*. TR-340. National Toxicology Program, Department of Health and Human Services. http://ntp.niehs.nih.gov/index.cfm?objectid=07084288-BB13-395C-EB6D19F1901E5F5B.

NTP. 2005. *Report on carcinogens*, 11th ed., Appendix B. National Toxicology Program, Department of Health and Human Services. January. http://ntp.niehs.nih.gov/?objectid=035E5806-F735-FE81-FF769DFE5509AF0A.

Oshiro, Y., Balwierz, P. S., and Molinary, S. V. 1981. Morphological transformation of C3H/10T1/2 CL8 cells by alkylating agents. *Toxicol. Lett.* 9:301–306.

Phimister, A. J., Lee, M. G., Morin, D., Buckpitt, A. R., and Plopper, C. G. 2004. Glutathione depletion is a major determinant of inhaled naphthalene

respiratory toxicity and naphthalene metabolism in mice. *Toxicol. Sci.* 82:268–278.

Pienta, R. J., Poiley, J. A., and Lebherz, W. B., 3rd, 1977. Morphological transformation of early passage golden Syrian hamster embryo cells derived from cryopreserved primary cultures as a reliable in vitro bioassay for identifying diverse carcinogens. *Int. J. Cancer* 19:642–655.

Plopper, C. G., Van Winkle, L. S., Fanucchi, M. V., Malburg, S. R. C., Nishio, S. J., Chang, A., and Buckpitt, A. R. 2001. Early events in naphthalene-induced acute Clara cell toxicity II. Comparison of glutathione depletion and histopathology by airway location. *Am. J. Respir. Cell. Mol. Biol.* 24:272–281.

Poet, T. S., Wu, H., Corley, R. A., and Thrall, K. D. 2009. In vitro GSH conjugation of methyl iodide in rat, rabbit, and human blood and tissues. *Inhal. Toxicol.* 21:524–530.

Rayburn, W., Robinson, A., Braverman, L. E., He, X. M., Pino, S., Gargas, M. L., and Kinzell, J. H. 2008. Iodide concentrations in matched maternal serum, cord serum and amniotic fluid from preterm and term human pregnancies. *Reprod. Toxicol.* 25:129–132.

Reed, C. J., Gaskell, B. A., Banger, K. K., and Lock, E. A. 1995. Olfactory toxicity of methyl iodide in the rat. *Arch. Toxicol.* 70 (1):51–56.

Rios-Blanco, M. N., Yamaguchi, S., Dhawan-Robl, M., Kessler, W., Schoonhoven, R., Filser, J. G., and Swenberg, J. A. 2003. Effects of propylene oxide exposure on rat nasal respiratory cell proliferation. *Toxicol. Sci.* 75:279–288.

Rosenkranz, H. S., and Poirier, L. A. 1979. Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J. Natl. Cancer Inst.* 62:873–892.

Roti, E., Gnudi, A., and Braverman, L. E. 1983. The placental transport, synthesis, and metabolism of hormones and drugs which affect thyroid function. *Endocr. Rev.* 4:131–149.

San, R. H. C., Clark, J. J. 2001. *In vitro mammalian cell gene mutation test (CHO/HGPRT assay) with iodomethane.* BioReliance, Rockville (MD), Laboratory Study No. AA38UL.782.BTL, DPR Doc. No. 52875-021. MRID# 45593815.

Schaefer, G. J. 2001. *An acute neurotoxicity study of iodomethane in rats: Final report.* Unpublished study prepared by WIL Research Laboratories, Inc. Lab. Project Number: WIL-418008. EPA MRID# 45593817.

Simmon, V. F. 1979a. In vitro mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. *J. Natl. Cancer Inst.* 62:893–899.

Simmon, V. F. 1979b. In vitro assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J. Natl. Cancer Inst.* 62:901–909.

Simmon, V. F., Kauhanen, K., Tardiff, R. G. 1977. Mutagenic activity of chemicals in drinking water. In *Progress in genetic toxicology* eds. D. Scott, B. A. Bridges, F. H. Sobels, pp. 249–258. Elsevier, North Holland Biomedical Press.

Sloter, E. D., Nemec, M., Stump, D., Holson, J., Kirkpatrick, D., and Gargas, M., Kinzell, J. 2009. Methyl iodide induced fetal hypothyroidism implicated in late-stage fetal death in rabbits. *Inhal. Toxicol.* 21:462–479.

Snyder, R., and Andrews, L. S. 1996. Toxic effects of solvents and vapors. In *Casarett and Doull's Toxicology: The basic science of poisons*5th ed., ed. C. D. Klasssen. New York:McGraw-Hill, pp. 737–771.

Sved, D. 2002. *A comparative oral (gavage) and inhalation metabolism and toxicokinetic study with iodomethane in male rats.* Unpublished study prepared by WIL Research Laboratories, Inc. Lab. Project Number: WIL-418007. EPA MRID# 45641401.

Sweeney, L. M., Kirman, C. R., Gannon, S. A., Thrall, K. D., Gargas, M. L., and Kinzell, J. H. 2009. Development of a physiologically based pharmacokinetic (PBPK) model for methyl iodide in rats, rabbits, humans. *Inhal. Toxicol.* 21:552–582.

Takahashi, K., and Kawazoe, Y. 1987a. Potent induction of the adaptive response by a weak mutagen, methyl iodide, in *Escherichia coli*. *Mutat. Res.* 180:163–169.

Takahashi, K., an Kawazoe, Y. 1987b. Methyl iodide, a potent inducer of the adaptive response without appreciable mutagenicity in *E. coli*. *Biochem. Biophys. Res. Commun.* 144:447–453.

Takegawa, K., Mitsumori, K., Onodera, H., Shimo, T., Kitaura, K., Yasuhara, K., Hirose, M., and Takahashi, M. 2000. Studies on the carcinogenicity of potassium iodide in F344 rats. *Food Chem. Toxicol.* 38(9):773–781.

Thrall, K. D., Morris, J. E., Sasser, L. B., Creim, J. A., Gargas, M. L., Kinzell, J. H., and Corley, R. A. 2009a. Studies supporting the development of a PBPK model for methyl iodide: the pharmacokinetics of sodium iodide (NaI) in pregnant rabbits. *Inhal. Toxicol.* 21:519–523.

Thrall, K. D., Woodstock, A. D., and Soelberg, J. J. 2009b. A real-time methodology to evaluate the nasal absorption of volatile compounds in intact animals. *Inhal. Toxicol.* 21:531–536.

Trevor, A. J., White, P. F. 2004. General anesthetics. In *Basic and clinical pharmacology*, 9th ed.ed. B. G. Katzung New York: Lange Medical Books/McGraw-Hill, pp. 401–417.

Wagner, V., DakoulasE. 2001. *Bacterial reverse mutation assay (Ames) with iodomethane: Final report.* Lab. Project Number: AA38UL.504004.BTL: SPGT504004. Unpublished study prepared by BioReliance. DPR Doc. No. 52875-021. MRID# 45593813.

Zhu, Y., Bilous, M., Boyages, S. C. 1995 . Excess iodine induces the expression of thyroid solid cell nests in lymphocytic thyroiditis-prone BB/W rats. *Autoimmunity* 20:201–206.