



October 18, 2013

Dr. Ruth Lunn  
Director  
Office of the Report on Carcinogens  
National Toxicology Program  
National Institute for Environmental Health Sciences  
Research Triangle Park, NC 27709

Re: Nominations to the Report on Carcinogens, Request for Information – Methyl Isobutyl Ketone (78 *Federal Register* 57868, September 20, 2013)

Dear Dr. Lunn:

The Ketones Panel of the American Chemistry Council submits the enclosed information in response to the National Toxicology Program's request for information on methyl isobutyl ketone (MIBK). The Ketones Panel represents US manufacturers of MIBK and other ketones.

Please feel free to contact me at [steve\\_risotto@americanchemistry.com](mailto:steve_risotto@americanchemistry.com) or at (202) 249-6727 if you have questions on the enclosed information.

Sincerely,

[Redacted]

Stephen P. Risotto  
Senior Director

Enclosure



Comments of the American Chemistry Council's Ketones Panel  
On the Request for Information on the Nomination  
Of Methyl Isobutyl Ketone for Possible Review  
for the Report on Carcinogens

78 Federal Register 57868  
September 20, 2013

### Current Production, Use Patterns, and Human Exposure

EPA's Chemical Data Reporting (CDR) data base indicates that 107 million pounds of MIBK were manufactured or imported in 2010.<sup>1</sup> The CDR reports three US manufacturers and five importers. Most MIBK is produced by aldol condensation of acetone and its derivative intermediates, diacetone alcohol and mesityl oxide. Acetone is treated with barium hydroxide to yield diacetone alcohol, which is dehydrated to mesityl oxide, which in turn is hydrogenated to saturate the double bond and produce methyl isobutyl ketone. Another method is the hydrogenation of mesityl oxide over nickel at 160–190 °C. Methyl isobutyl ketone can also be prepared by reacting sodium acetoacetic ester with isopropyl bromide and treating the resulting 2-isopropyl acetoacetic ester with diluted acid to saponify the ester and decarboxylate the resulting keto acid (NTP, 2007).

MIBK is used as a denaturant for ethanol, in a variety of solvent applications, and in the manufacture of rubber anti-ozonants and non-ionic acetylenic surfactants. The largest solvent use is in surface coatings, primarily nitrocellulose lacquers and various solvent-borne coatings. MIBK is used as a reaction or extraction solvent for the production of antibiotics and pesticides and in the mining of various metals.

According to the 2012 Toxic Release Inventory, releases of MIBK are estimated to total 3.7 million pounds, of which about 3.4 million pounds were releases to air.<sup>2</sup> This represents a reduction by about 60 percent from the reported emissions of 9.8 million pounds in 2002.

The most probable routes of exposure in the workplace are by inhalation of vapors and by skin and eye contact during the production and use of methyl isobutyl ketone and products in which it is a constituent. In the National Occupational Exposure Survey (NIOSH, 1990) conducted from 1981 to 1983, the number of workers potentially exposed to methyl isobutyl ketone in the USA was estimated as 48 000. Exposure to methyl isobutyl ketone during spray painting was found to be 0.6 parts per million (ppm) as a time-weighted average (TWA) (Whitehead *et al.*, 1984).

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<sup>1</sup> Available at <http://www.epa.gov/oppt/cdr/>.

<sup>2</sup> Available at [http://iaspub.epa.gov/triexplorer/tri\\_release.chemical](http://iaspub.epa.gov/triexplorer/tri_release.chemical).

In a study among solvent-exposed workers, a mean exposure of 16.7 ppm was noted in an unidentified factory (Ogata *et al.*, 1995). Among a group of 27 furniture-makers exposed to a mixture of methyl isobutyl ketone and other solvents, the arithmetic mean exposure (TWA) to MIBK was 1.8 ppm (range, 0.1–15.1 ppm). A linear relationship between exposure and urinary concentration was found (Kawai *et al.*, 2003). Hanninen *et al.* (1976) reported a mean TWA concentration of 1.7 ppm (range 1–39 ppm) in the breathing zone of spray painters in car repair shops.

The Occupational Safety and Health Administration's (OSHA) Permissible Exposure Limit for MIBK is 100 ppm (410 mg/m<sup>3</sup>) for an 8-hour TWA. The National Institute of Occupational Safety and Health (NIOSH) has established recommended exposure limits of 50 ppm (205 mg/m<sup>3</sup>) as an 8-hour TWA and 75 ppm (300 mg/m<sup>3</sup>) for short-term (15-minute) exposure. Finally, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends Threshold Limit Values (TLVs)<sup>®</sup> of 50 ppm for an 8-hour TWA, 75 ppm for a 15-minute exposure, and 125 ppm (510 mg/m<sup>3</sup>) for a ceiling value.

The most probable routes of exposure to MIBK by the general population are ingestion of contaminated drinking-water and dermal contact with consumer products of which it is a constituent (Johnson, 2004). Dietary sources of exposure are: natural occurrence in food, addition to food as a flavoring, and migration into food from food packaging. MIBK has also been detected in human breast milk (Pellizzari *et al.*, 1982), and traces have also been detected in tap water in the USA (IPCS, 1990).<sup>3</sup>

MIBK is reported to occur naturally in a variety of fruits, meats, and vegetables. The following levels have been reported (IPCS, 1990): papaya, 8 micrograms/kilogram (µg/kg); beer, 10–120 µg/kg; and coffee, 6.5 milligrams/kg (mg/kg). MIBK is permitted as a flavoring agent in the USA, where it is considered as safe at current levels of intake.<sup>4</sup> Usual reported levels ranged from 2.6 mg/kg in meat products to 12.3 mg/kg in soft candy. Maximum reported levels were 25 mg/kg in frozen dairy and non-alcoholic beverages; other reported uses are in baked goods, gelatins and puddings (Burdock, 2005). The Council of Europe reported maximum levels of 11 mg/kg in beverages and 1 mg/kg in foods in general (Council of Europe, 2000).

Per-capita exposure to MIBK, estimated by the FAO/WHO Expert Committee on Food Additives based on poundage data provided by industry, is 7 µg per capita per day in Europe (based on a reported volume of 50 kg/year) and 2 µg per capita per day in the USA (based on a reported production volume of 8 kg/year) (FAO/WHO, 1999). More recently, individual intake was estimated at 0.02 µg/kg per day (Burdock, 2005).

MIBK is used in packaging materials that come into contact with food. Levels reported in foods from packaging are: baked goods, 10.9 mg/kg; frozen dairy products, 11.5 mg/kg; meat

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<sup>3</sup> More recent information on MIBK in surface and ground water is unavailable.

<sup>4</sup> 21 CFR § 172.515.

products, 2.6 mg/kg; soft candy, 12.3 mg/kg; gelatins and puddings, 10.9 mg/kg; and beverages, 10.2 mg/kg (IPCS, 1990).

## **Scientific Issues Important for Assessing Carcinogenicity of MIBK**

### Metabolism

Tests in human volunteers indicate that MIBK is rapidly eliminated from the body following exposure, primarily through exhaled breath. The relative pulmonary uptake in a study of eight volunteers exposed to 2 to 50 ppm MIBK during two hours of light exercise was observed to be ~60% and to increase with increasing dose (Hjelm *et al.*, 1990). In the study, levels in the blood rose rapidly after the onset of exposure, leveled off, and did not reach a plateau for 2 hours. At the end of the exposure, blood concentrations increased linearly with dose, with no tendency for saturation kinetics. In another study of 98 volunteers exposed to 100 ppm MIBK for 4 hours in an environmental chamber, steady-state blood concentrations of methyl isobutyl ketone were attained after 2 hours of exposure (Dick *et al.*, 1990). Blood and breath samples collected 90 minutes after exposure indicated that most of the absorbed compound had been eliminated from the body.

In studies with laboratory animals, MIBK has been found to be readily absorbed into the bloodstream after inhalation exposure and is likely to be widely distributed in the body (Duguay and Plaa, 1995). Metabolism occurs by reduction of the carbonyl group to a secondary alcohol (4-methyl-2-pentanol) and by oxidation to form a hydroxylated ketone (diacetone alcohol) (DiVincenzo *et al.*, 1976). The results are consistent with the metabolism of MIBK via alcohol dehydrogenase and cytochrome P450 enzyme activities.

### Subchronic Results in Laboratory Studies

Based on the results of acute and subchronic studies in laboratory animals exposed to MIBK, the major organ sites of toxicity and possible carcinogenicity were the kidney in rats and the liver in mice. Following exposure to up to 2000 ppm MIBK over 11 days, absolute and relative liver and kidney weights were increased in both F344 rats and B6C3F1 mice at various exposure concentrations (Phillips *et al.*, 1987). The primary microscopic findings were hyaline droplet formation (at 500 and 2000 ppm) and epithelial regeneration of the proximal convoluted tubule cells (2000 ppm) in the male rat kidney. In the liver, there were increased mitotic figures (qualitative assessment) in one female rat and two male rats, hepatic mitosis in one female mouse, and glycogen depletion in four female mice.

F344 rats and B6C3F1 mice exposed to up to 1000 ppm MIBK for 14 weeks also exhibited increases in absolute liver weight (male rats at 50 and 1000 ppm, male mice at 250 and 1000 ppm), relative liver weight (male mice at 1000 ppm), and absolute kidney weight (female rats at 250 ppm) (Phillips *et al.*, 1987). There also was an increase in both the incidences and extent of hyaline droplets in the kidneys of male rats exposed to 250 and 1000 ppm. There was also an increase in serum cholesterol levels in male rats exposed to 250 or

1000 ppm, in urinary glucose excretion in male rats exposed to 250 ppm and in male and female rats exposed to 1000 ppm, and in urinary total protein excretion in male rats exposed to 1000 ppm. There were no changes in serum or urine biomarkers of injury or in histopathology in mice.

Sprague–Dawley rats were exposed to up to 2000 ppm MIBK by inhalation for at least 70 days as part of a two-generation reproductive study (Nemec *et al.*, 2004). There were increases in both absolute and relative kidney weights in males. Histologic changes suggestive of chronic progressive nephropathy (CPN) were clearly present in male rats exposed to 1000 and 2000 ppm. There were also increases in absolute and relative liver weights of male and female rats exposed to 2000 ppm and associated exposure-related centrilobular hepatocellular hypertrophy in males exposed to 500, 1000, or 2000 ppm.

### Genotoxicity

The available data (O'Donoghue *et al.*, 1988; Zeiger *et al.*, 1992) suggest that MIBK is not genotoxic. In studies in *Salmonella* (Brooks *et al.*, 1988; Zeiger *et al.*, 1992), MIBK was not mutagenic in the presence or absence of metabolic activation in a variety of strains (TA98, TA100, TA1535, TA1537 and TA1538) in the pre-incubation assay in closed tubes. Similar negative results were also observed in the *S. typhimurium* assay with TA102 and TA104 (Zeiger *et al.*, 1992). Equivocal results were found in the L5178Y mouse lymphoma *Tk*<sup>+/-</sup> assay which was also performed in closed tubes (O'Donoghue *et al.*, 1988).

MIBK gave negative results for unscheduled DNA synthesis in rat hepatocytes, for micronuclei in the bone marrow of CD-1 mice (after intraperitoneal injection), for cell transformation in BALB/3T3 mouse embryo cells (O'Donoghue *et al.*, 1988), for mitotic gene conversion (Brooks *et al.*, 1988) and mitotic chromosome loss (Zimmermann *et al.*, 1989) in yeast and for chromosome damage in rat liver cells *in vitro* (Brooks *et al.*, 1988).

### Carcinogenicity

There are no human data available on the carcinogenicity of MIBK. The animal cancer data are limited to a 2-year bioassay conducted by the National Toxicology Program (NTP) in 2007 which exposed F344/N rats and B6C3F1 mice to concentrations of up to 1800 ppm for 6 hours per day on 5 days a week for 105 weeks (NTP 2007; Stout *et al.*, 2008). The NTP Technical Report concluded that, under the condition of the 2-year studies, MIBK exhibited -

- Some evidence of carcinogenic activity in male F344/N rats based on increased incidence of renal tubule neoplasms
- Equivocal evidence of carcinogenic activity in female F344/N rats based on the occurrence of renal mesenchymal tumors in the highest exposure group

- Some evidence of carcinogenic activity in male and female B6C3F1 mice based on increased incidence of liver neoplasms at 1800 ppm.

NTP also noted that MIBK exposure resulted in non-neoplastic lesions of the kidney characteristic of  $\alpha$ 2u-globulin ( $\alpha$ 2u) accumulation in male rats and CPN in female rats.<sup>5</sup> Neither  $\alpha$ 2u nephropathy nor CPN appear to have a counterpart in humans (Stout *et al.*, 2008) and, as a consequence, are generally not considered for human risk assessment. In considering whether a substance causes renal tumors in male rats through a response associated with  $\alpha$ 2u, both EPA (EPA 1991)<sup>6</sup> and the International Agency for Research on Cancer (IARC 1999)<sup>7</sup> have established criteria for assessing the potential relevance to human risks.<sup>8</sup> The IARC criteria include -

- Lack of genotoxic activity (agent and/or metabolites) based on an overall evaluation of *in vitro* and *in vivo* data,
- Male rat specificity for nephropathy and renal tumorigenicity,
- Induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory,
- Identification of the protein accumulating in the tubule cells as  $\alpha$ 2u,
- Reversible binding of the chemical or metabolite to  $\alpha$ 2u,
- Induction of sustained increased cell proliferation in the renal cortex, and
- Similarities in dose-response relationship of the tumor outcome with the histopathological end-points (protein droplets,  $\alpha$ 2u accumulation, cell proliferation)

The criteria for determining if a chemical induces  $\alpha$ 2u nephropathy include, but are not limited to: (1) evidence of an increased accumulation of protein droplets in renal proximal tubule epithelial cells in treated male rats, (2) confirmation that the protein droplets contain  $\alpha$  2u, and (3) demonstration of characteristic renal lesions consistent with  $\alpha$ 2u nephropathy, including granular casts at the OSOM–ISOM junction and linear mineralization within the renal papilla. It is also critical to show that these lesions are not present in female rats (U.S. EPA, 1991).

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<sup>5</sup> Among the NTP findings was an increase in linear papillary mineralization (LPM) in all of the treated male rat groups. LPM reflects the chronic outcome of preceding cell damage related to hyaline droplet formation which commences shortly after exposure begins and would be expected to persist until the levels of hepatic synthesis of  $\alpha$ 2u decline in old age (Roy *et al.*, 1983; Hard *et al.*, 1993).

<sup>6</sup> EPA.  $\alpha$ 2u-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. Prepared for the Risk Assessment Forum. EPA/625/3-91/019F. Washington, DC, (September 1991).

<sup>7</sup> IARC. Scientific Publications No. 147, Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis (1999).

<sup>8</sup> NTP references both the EPA and IARC criteria in its recent monograph for cumene. (NTP. Report on Carcinogens – Monograph on Cumene. September 2013)

Based on the NTP findings, an IARC Working Group concluded in early 2011 that the relevance of the tumor response in mice and rats to humans cannot be excluded and, consequently, designated MIBK as possibly carcinogenic to humans (Category 2B).<sup>9,10</sup> The IARC Working Group noted that mechanistic studies of MIBK provide evidence that some of the criteria that denote an  $\alpha$ 2u mode of action were met (*i.e.*, male rat-specific nephropathy, dose-response associations of end-points, dose-related increases in cell proliferation). They concluded, however, that -

recent NTP studies demonstrated inconsistencies with this proposed mechanism, including, in some cases, kidney tumor responses that were far weaker than expected based on the extent of  $\alpha$ 2u-globulin nephropathy. . . While tumor responses corresponded to some extent with a measure of cumulative  $\alpha$ 2u-globulin nephropathy (linear mineralization of the papilla) at the end of the 2-year studies, the severity of chronic nephropathy generally correlated best with the pattern of tumor response. These results suggest that, while  $\alpha$ 2u-globulin nephropathy may contribute to the renal tumor response, the critical component(s) of the nephropathy most closely associated with the development of tumors has not been identified. Thus, the strength of the evidence that male rat kidney tumors arose through a  $\alpha$ 2u-globulin nephropathy mechanism is weak.<sup>11</sup>

Regarding the mouse liver tumors, the IARC Working Group noted that “there was no evidence that the tumors arose from a cytotoxic-regenerative cell proliferation mechanism as no overt toxicity occurred in the livers of exposed mice.” The Working Group further explained that “only weak evidence exists that the tumors arose through a receptor-mediated mechanism, resulting from the induction of enzymes (CYP1A1 and CYP2B) that have been considered to be typical targets of the aryl hydrocarbon receptor and the constitutive activated receptor, respectively (Nebert *et al.*, 2000; Zelko & Negishi, 2000).” As a consequence, they concluded that the strength of evidence that male and female liver tumors arose through a nuclear receptor mechanism is weak.

#### Evaluation of Mode of Action for Rat Kidney Tumors

In evaluating the criteria identified by IARC for evaluating  $\alpha$ 2u-associated nephropathy, the IARC Working Group agreed that the criteria related to lack of genotoxic activity, protein droplet accumulation,  $\alpha$ 2u accumulation, and cell proliferation were met for MIBK. While the IARC Monograph concurs that the renal tubule neoplasm arose via the male-specific  $\alpha$ 2u-

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<sup>9</sup> IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Chemicals Present in Industrial and Consumer Products, Food and Drinking Water. Methyl Isobutyl Ketone. Volume 101:305-323 (2013).

<sup>10</sup> MIBK is not classified as a carcinogen under the European Commission’s Regulation No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation).

<sup>11</sup> *Id.*, at 318.

mediated mechanism, CPN was observed in both males and females “which suggests that an alternative mechanism may be involved.” The Working Group further noted that while Borghoff *et al.* (2009) did find  $\alpha$ 2u accumulation in tubule cells after oral administrations of 1000 mg/kg MIBK in male rats, “the experimental design did not allow for an evaluation of a dose-response relationship in the increases in  $\alpha$ 2u accumulation.” The Monograph noted that, while MacEwan *et al.*, 1971 had observed the reversibility of effects that could be attributed to  $\alpha$ 2u nephropathy, no direct evidence of reversible binding existed.

The study by Borghoff *et al.* (2009) observed increases in protein droplets, accumulation of  $\alpha$ 2u, and renal cell proliferation in male, but not female rats, comparable to those induced by d-limonene, an acknowledged inducer of  $\alpha$ 2u nephropathy. Along with an increase in protein droplets and mitotic figures in proximal tubules of the cortex, Borghoff *et al.* observed an accumulation of cell debris in the tubules at the junction of the OSOM and ISOM, lesions considered to be precursors of granular casts, which are typically observed in 90-day toxicology studies with inducers of  $\alpha$  2u nephropathy. This study further demonstrated that the concentration of  $\alpha$ 2u almost doubled in the kidney of both the d-limonene and the MIBK-administered male rats. Renal cell proliferation was also increased in MIBK administered male, but not female, rats and the increase was statistically significant.<sup>12</sup>

More recent work by Borghoff *et al.*<sup>13</sup> with F344/N rats exposed to 0, 450, 900, or 1800 ppm MIBK 6 hours/day for 1 or 4 weeks provides evidence for the exposure-related increase in measures of  $\alpha$ 2u nephropathy, sustained increase in renal cell proliferation along with an indication of reversible binding of MIBK to  $\alpha$ 2u. The researchers evaluated hyaline droplet accumulation (HDA),  $\alpha$ 2u staining of hyaline droplets, renal cell proliferation, and renal  $\alpha$ 2u concentration at both 1 and 4 weeks. They observed an exposure-related increase in all measures of  $\alpha$ 2u nephropathy in the male, but not female rat kidneys. The hyaline droplets present in the male rat kidney stained positively for  $\alpha$ 2u. The changes in HDA and  $\alpha$ 2u concentration were comparable to d-limonene. In a separate *in vitro* investigation using a two-compartment vial equilibration model to assess the interaction between MIBK and  $\alpha$ 2u, the dissociation constant (Kd) was estimated to be  $1.27 \times 10^{-5}$  M. This Kd is within the range similar to other chemicals known to bind to  $\alpha$ 2u and cause nephropathy. Taken together, and when combined previous findings, this new research provides compelling evidence to support the inclusion of MIBK in the category of chemicals exerting renal effects through a protein droplet  $\alpha$ 2u nephropathy-mediated mode of action.

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<sup>12</sup> Borghoff *et al.* conducting their study in young adult rats for a short (10-day) period of exposure in order to evaluate enhanced cell replication without interference from CPN. Evidence indicates that tubules involved in the spontaneous disease process of CPN have a high rate of cell replication (Konishi and Ward, 1989; Short *et al.*, 1989; Hard and Seely, 2006) and that the advanced disease is a risk factor for renal tubule tumor development (Hard, 1998; Hard and Khan, 2004).

<sup>13</sup> This research is ongoing and is described below.



## Chronic Progressive Nephropathy (CPN)

The incidence and severity of CPN was increased in the high-dose males in the NTP bioassay, while the incidences were significantly increased in all exposed groups of females, but the severity only slightly increased. Chemical exacerbation of CPN usually occurs in conjunction with the induction of  $\alpha$ 2u nephropathy (Hard *et al.*, 1993), so this is not a surprising observation in the males. However, exacerbation of CPN by chemicals is not confined to those causing toxicity through the  $\alpha$ 2u mechanism (Eustis *et al.*, 1994; Hard and Khan, 2004; Lock and Hard, 2004) or to male rats (Hard, 2002; Lock and Hard, 2004). The apparent increase in incidence of CPN in the MIBK-treated female rats would be quite independent of the  $\alpha$ 2u process occurring in the male rat kidney.

While exacerbation of CPN in rats should be regarded as an adverse effect, it should not be regarded as an indicator of chemically induced toxicity. The incidence and severity of this spontaneous disease can be influenced by several physiological factors (Hard and Khan, 2004). CPN can be modified by varying the protein content of the diet or the source of protein (Iwasaki *et al.*, 1988; Masoro and Yu, 1989; Rao *et al.*, 1993) or by varying caloric intake (Keenan *et al.*, 2000). Furthermore, CPN is characterized by a spectrum of histopathology and clinical features that set it apart from the main causes of chronic renal disease in humans (Hard and Khan, 2004). Thus, rat CPN has no strict counterpart in humans and therefore appears to have no relevance to human hazard assessment (Hard and Khan, 2004; Hard *et al.*, 2009).

Extensive statistical analysis of NTP studies shows a strong correlation between high-grade CPN, especially end-stage CPN, and renal tumor development (Hard *et al.*, 2013). In the absence of toxic effects elsewhere, Hard *et al.* conclude CPN does not constitute a carcinogenic effect of the chemical and that renal tumors developing as a result of CPN exacerbation in rats have no relevance for human risk assessment.

## Renal Mesenchymal Tumors

NTP reports renal mesenchymal tumors in two female rats exposed to the highest doses (1800 ppm). The Technical Report notes that these are rare neoplasms, and in fact, to date, no chemical in the NTP database has caused a statistical increase in their incidence (Hard, 1999; Lock and Hard, 2004). They have only been induced by chemicals that are potent genotoxic carcinogens (Hard, 1990; Hard, 1999), and clearly on the basis of its short-term testing, MIBK does not fall into this category.

The mesenchymal tumors in the high-dose female rats appear to represent a chance occurrence, based on the low prevalence of tumors of this broad group of diagnoses through control and treated rats in the NTP historical database. If these tumors were treatment related and not spontaneous, some precursor lesions in the form of interstitial cell foci might be expected (Hard and Butler, 1970; 1971). The NTP report makes no mention of such a finding.

## Mouse Liver Tumors – Mode of Action

In the NTP bioassay, significant positive trends were reported for hepatocellular adenomas in male and female mice; the increases were statistically significant at 1800 ppm in both males and females. A significant increase in multiple hepatocellular adenomas were also observed in females at 900 and 1800 ppm and in males at 1800 ppm. Hepatocellular carcinoma was elevated in females at 1800 ppm. While the increase was not statistically significant, it exceeded the historical control range. Combined adenomas and carcinomas were significantly increased in females at 900 and 1800 ppm and in males at 1800 ppm. Stout *et al.* (2008) note that the histologic appearance of the hepatocellular lesions was consistent with those that develop spontaneously in control mice.

Previous investigations of rodents exposed to MIBK suggest a phenobarbital (PB)-like signature of changes in the liver - increased enzyme activity and hepatocellular proliferation that is transient and not sustained, starting after 3-4 days and lasting as long as 14-28 days of treatment but no longer (Kolaja *et al.*, 1996; Whysner *et al.*, 1996). After 7 days of 1800 ppm MIBK exposure Geter *et al.* (2009) did not observe an increase in liver weight, while a study conducted by Dodd *et al.* (1982) reported significant increases in liver weight following 11 days. Additionally, a World Health Organization review of MIBK reported that exposure to concentrations as low as 250 ppm resulted in an increase in liver size and induced hepatic microsomal metabolism (WHO, 1991).

In examining data from a compound structurally related to MIBK, methyl ethyl ketone (MEK), exposure to a daily dose of 1.4 mL/kg for 3 days increased the amounts of ethanol- and PB-inducible cytochrome P-450 enzymes (Raunio *et al.*, 1990), while an inhalation exposure of rats to 600 ppm MEK (10 h/day for 7 days) did not cause an increase in 7-pentoxoresorufin-O-dealkylation (PROD) activity (Liira *et al.*, 1991). These results suggest a threshold for PB-like induction following MEK (and possibly MIBK) exposure.

Four receptors are primarily responsible for xenobiotic-induced liver weight increase; aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and the peroxisome proliferator activated receptor alpha (PPAR $\alpha$ ) (Graham and Lake, 2008). The increase in PROD liver activity and hepatocellular proliferation are key events following exposure to PB, a known agonist for CAR and PXR. Although CAR agonists induce hepatocellular proliferation in rodents, they do not appear to be mitogenic agents in humans (Lake, 2009). If a positive PB-like response is observed, it can be reasonably assumed that MIBK is activating CAR and/or PXR, and that the mouse liver tumors observed in the NTP bioassay are not relevant to human risk assessment.

Research planned by the MIBK Technical Consortium, described in the next section, is designed to evaluate if the mode of action for MIBK-induced liver tumors results from activation of CAR and/or PXR leading to hepatomegaly is a result of hepatocellular hypertrophy and hyperplasia.

## **Published, Ongoing, or Planned Studies Related to Evaluating MIBK's Carcinogenicity**

### Published Studies

Brott DA *et al.* Renal biomarker changes associated with hyaline droplet nephropathy in rats are time and potentially compound dependent. *Toxicology* 303:133-138 (2013).

Hard GC *et al.* Consideration of rat chronic progressive nephropathy in regulatory evaluation for carcinogenicity. *Tox Sci* 132(2):268-275 (2013).

Borghoff SJ *et al.* Methyl isobutyl ketone (MIBK) induction of  $\alpha$ 2u nephropathy in male, but not female rats. *Toxicology* 258:131-138 (2009)

### Ongoing Studies

#### **Reversible binding of MIBK or metabolites to $\alpha$ 2u**

Performing Laboratory – Battelle Pacific Northwest/Integrated Laboratory Systems, Inc.  
Richland, WA/Durham, NC

Study Sponsor MIBK Testing Consortium  
ACC Ketones Panel  
Washington, DC

The purpose of this study is to determine if MIBK or one of its metabolites interacts reversibly with  $\alpha$ 2u.

#### **Mode-of-Action Investigation into Methyl Isobutyl Ketone Induced Rat Kidney Tumors**

Performing Laboratory – Integrated Laboratory Systems, Inc.  
Durham, NC

Study Sponsor MIBK Testing Consortium  
ACC Ketones Panel  
Washington, DC

The purpose of this study is to evaluate the ability of MIBK to induce specific measures of  $\alpha$ 2u nephropathy (histological lesions associated with protein accumulation, increased concentration of  $\alpha$ 2u, and cell proliferation) in the kidneys of male and female F344 rats following exposure to MIBK via inhalation at the concentrations used in the 2 year bioassay.

### **Severity of spontaneous Chronic Progressive Nephropathy in F344 Rats; NTP-2000 versus NIH-07 Diet *ad libitum* for 17 Weeks**

Performing Laboratory – Integrated Laboratory Systems, Inc.  
Durham, NC

Study Sponsor Tox-Logic Consulting  
Petaluma, CA

The overall objective of this project is to evaluate the gene signature profiles within spontaneous and chemically exacerbated CPN lesions in male and female rats. This information will provide a molecular basis of this disease and allow for further understanding of the potential mode of action by which chemicals exacerbate this response in rats. Gene expression changes will be evaluated at ILS in samples collected from this study. This study protocol is focused on evaluating the gene expression changes in spontaneous CPN lesions and once the tools are developed to evaluate these changes from laser captured CPN lesions in FFPE tissue, the data will be presented to NTP scientists and tissue blocks from NTP study archives requested to evaluate and compare the changes in chemically exacerbated CPN lesions.

#### Planned Studies

### **Mode-of-Action Investigation into Methyl Isobutyl Ketone Induced Male and Female Mouse Liver Toxicity**

Performing Laboratory – Integrated Laboratory Systems, Inc.  
Durham, NC

Study Sponsor MIBK Testing Consortium  
ACC Ketones Panel  
Washington, DC

The purpose of this study is to evaluate if the mode of action for MIBK-induced liver tumors results from activation of CAR and/or PXR leading to hepatomegaly is a result of hepatocellular hypertrophy and hyperplasia.

#### **Scientists with Expertise or Knowledge about MIBK**

Susan Borghoff  
ToxStrategies  
Cary, NC

Samuel Cohen  
Dept of Pathology and Microbiology

University of Nebraska Medical Center  
Omaha, NE

Wolfgang Dekant  
Department of Toxicology  
University of Würzburg  
Würzburg, Germany

Gordon Hard  
Tairua, New Zealand

Torka Poet  
Summit Toxicology  
Richland, WA

## References

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Burdock GA. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed. Boca Raton, FL: CRC Press (2005). Cited in IARC Monograph Volume 101.

Council of Europe. *Chemically-defined Flavouring Substances*. Strasbourg, France: Council of Europe Publishing (2000). Cited in IARC Monograph Volume 101.

Dick R *et al.* Body burden profiles of methyl ethyl ketone and methyl isobutyl ketone exposure in human subjects. *Toxicologist* 10:112 (1990).

DiVincenzo GD *et al.* Characterization of the metabolites of methyl n-butyl ketone, methyl isobutyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance. *Toxicol Appl Pharmacol* 36:511–522 (1976).

Dodd DE *et al.* Methyl isobutyl ketone: Nine-day vapor inhalation study on rats and mice. Internal Study of The Dow Chemical Company. K-000607-026 (1982)

Duguay AB and Plaa GL. Tissue concentrations of methyl isobutyl ketone, methyl n-butyl ketone and their metabolites after oral or inhalation exposure. *Toxicol Lett* 75:51–58 (1995).

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