Amended Safety Assessment of MIBK as Used in Cosmetics

Status:	Draft Final Report for Panel Review
Release Date:	March 4, 2024
Panel Meeting Date:	March 28-29, 2024

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Regina Tucker, M.S., former Scientific Analyst/Writer, and Thushara Diyabalanage Ph.D. Senior Scientific Analyst/Writer, CIR.

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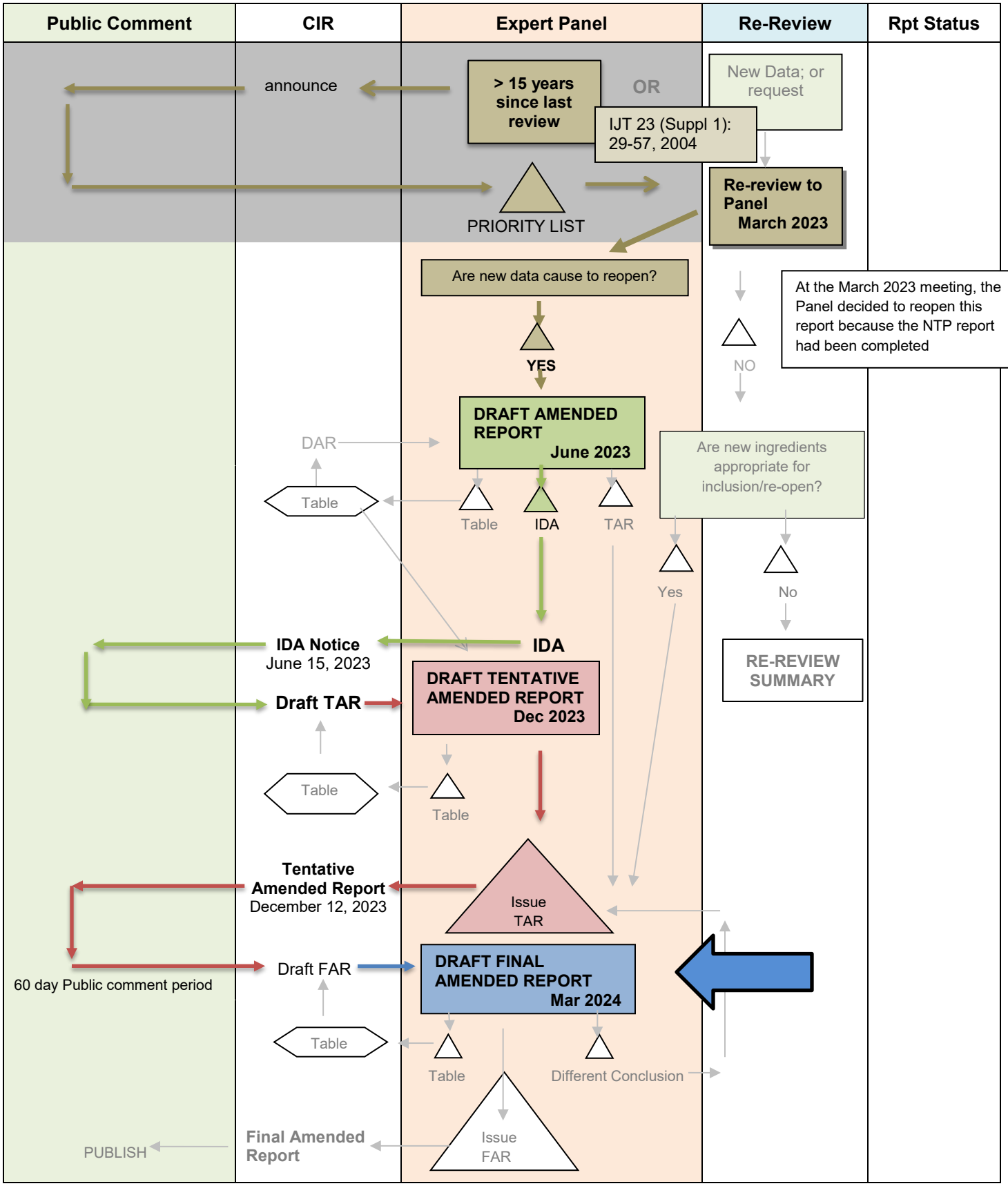
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY MIBK

MEETING March 2024



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Thushara Diyabalanage Ph.D.
Senior Scientific Analyst/Writer, CIR
Date: March 4, 2024
Subject: Final Amended Report of the Safety Assessment of MIBK

Enclosed is the Draft Final Report of the Amended Safety Assessment of MIBK. At its March 2023 meeting, the Panel decided to reopen the safety assessment of MIBK because a carcinogenicity study had since been completed by the NTP. After reviewing the Draft Amended Report submitted at the June 2023 meeting, the Panel issued an Insufficient Data Announcement for more information regarding concentration of use, irritation and sensitization data, and the confirmation of its use only as a denaturant. Although no new data were received, the Panel was comfortable with issuing a Tentative Amended Report at the December 2023 meeting (for reasons outlined in the Discussion), reaffirming their original conclusion that MIBK is safe as used in nail care products and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment. Because current concentrations of use are not reported; the expectation is that this ingredient would be used at concentrations comparable to that reported in the 2004 safety assessment.

Since the December meeting, CIR has not received any new unpublished data. Comments from the Council on the draft Tentative Amended Report that was reviewed in December *(PCPCcomments1_MIBK_032024*) and on the Tentative Amended Report issued following that meeting (*PCPCcomments2_MIBK_032024)* have been addressed *(response-PCPCcomments1_MIBK_032024; response-PCPCcomments2_MIBK_032024*, respectively).

Also included in this package, for your review, are:

- A flow chart (*flow_MIBK_032024*)
- Literature search strategy (*search_MIBK_032024*)
- Data profile (*dataprofile_MIBK_032024*)
- Transcripts from the previous meetings at which this re-review has been discussed (*transcripts_MIBK_032024*)
- Report history (*history_MIBK_032024*)
- Original report *(originalreport_MIBK_032024)*
- Minutes of the meeting at which the original report was discussed (*originalminutes_MIBK_032024*)

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. If these are satisfactory, the Panel should issue a Final Amended Report.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: November 30, 2023

SUBJECT: Draft Tentative Report: Amended Safety Assessment of MIBK as Used in Cosmetics (draft prepared for the December 2023 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft tentative report, Amended Safety Assessment of MIBK as Used in Cosmetics.

Abstract – It would be helpful to include the full name (methyl isobutyl ketone) in the Abstract.

Non-Cosmetic Use – Although the specifications for denatured alcohol may have been established by the Bureau of Alcohol, Tobacco, and Firearms (now Bureau of Alcohol, Tobacco, Firearms and Explosives under the Department of Justice), it would be helpful to give the name of the agency currently responsible for these specifications, the Alcohol and Tobacco Tax and Trade Bureau (under the Department of Treasury).

Short-Term, Dermal, old report summary – Please add the units for "5-12".

Short-Term, Oral, old report summary – What were the oral doses used in the mouse study in which 9 of 10 mice died?

Carcinogenicity, Mode of Action – A word is missing from the following: "The mode of action (MOA) underlying MIBK-induced liver tumors was [?] in male and female B6C3F1, …." (the Summary uses "investigated"). It would be helpful to state some of the results of this study, rather than just the conclusion.

Neurotoxicity, old report summary – How long were the rats treated with the "doubled" doses? Although the old report said the median duration of immobility was "803 ppm", units of ppm do not make sense for a "duration". Please check the original reference to see if this is correct.

Nephropathy – Please describe the "dissociation constant of MIBK" further (e.g., MIBK and alpha 2u-globulin dissociation constant).

Ocular Irritation, old report summary – Please revise: “The cornea, iris, and conjunctiva were scored at days 1, 2, 3, 7, 10, 14, and 21 d post-instillation.” (days does not need to be before and after the numbers).

MIBK – March 2024 – Thushara Diyabalanage	
Comment Submitter: Alexandra Kowcz, MS MBA; Industry Liaisons to the CIR Expert Panel **Date of Submission: November 30, 2023**	
Comment	**Response/Action**
Abstract – It would be helpful to include the full name (methyl isobutyl ketone) in the Abstract.	Addressed
Non-Cosmetic Use – Although the specifications for denatured alcohol may have been established by the Bureau of Alcohol, Tobacco, and Firearms (now Bureau of Alcohol, Tobacco, Firearms and Explosives under the Department of Justice), it would be helpful to give the name of the agency currently responsible for these specifications, the Alcohol and Tobacco Tax and Trade Bureau (under the Department of Treasury)	Addressed
Short-Term, Dermal, old report summary – Please add the units for "5-12"	Addressed
Short-Term, Oral, old report summary – What were the oral doses used in the mouse study in which 9 of 10 mice died?	Addressed
Carcinogenicity, Mode of Action – A word is missing from the following: "The mode of action (MOA) underlying MIBK-induced liver tumors was [?] in male and female B6C3F1, …." (the Summary uses "investigated"). It would be helpful to state some of the results of this study, rather than just the conclusion.	Addressed
Neurotoxicity, old report summary – How long were the rats treated with the "doubled" doses? Although the old report said the median duration of immobility was "803 ppm", units of ppm do not make sense for a "duration". Please check the original reference to see if this is correct.	Addressed
Nephropathy – Please describe the "dissociation constant of MIBK" further (e.g., MIBK and alpha 2u-globulin dissociation constant)	Addressed
Ocular Irritation, old report summary – Please revise: "The cornea, iris, and conjunctiva were scored at days 1, 2, 3, 7, 10, 14, and 21 d post-instillation." (days does not need to be before and after the numbers)	Addressed

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: January 5, 2024

SUBJECT: Tentative Report: Amended Safety Assessment of MIBK as Used in Cosmetics (release date December 12, 2023)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Amended Safety Assessment of MIBK as Used in Cosmetics.

Abbreviations; Occupational Exposure – NIOSH is the National Institute for (not "of" as stated in the report) Occupational Safety and Health

Cosmetic Use – As there is only one ingredient in this report, the airbrush paragraph needs to be revised from "some of these ingredients" to "this ingredient" or "MIBK".

Non-Cosmetic – Please delete "an" in: "MIBK is an approved for direct addition to food…"

ADME, old report summary – Rather than just saying "MIBK in human blood samples collected immediately after delivery were examined." It should state that MIBK was found in human blood samples immediately after delivery. It would have been helpful if the original CIR report stated the levels of MIBK found in maternal blood.

ADME, Animal, Oral, old report summary – It would be helpful to know the different routes that were examined in the study that found differences in MIBK metabolite concentrations. Based on the information in the report, it appears that it was an oral and inhalation study.

Acute, Dermal – Please correct "wss" (should be "was"). Please add the word "patch" to: "A semi-occlusive [patch] with…"

Acute, Inhalation, old report summary – A guinea pig study is described in which it says the animals were exposed in a "1 cm^3 inhalation chamber". Unfortunately, this is what it says in the original CIR report. A guinea pig could not fit into a chamber that small. If it says this in the

original paper, it was probably an error. Please do not repeat this chamber size in the current CIR report.

Short-Term, old report summary – In the following, please add the word "to": "rats were exposed [to] 100, 500, or 2000 ppm MIBK…" Please change "in" to "at" in the following: "rats exposed to MIBK in [at] 4.53 mg/l air for 6 h/d, 5 d/wk, for 4 wk."

Carcinogenicity; Table 2; Table 3 – In the text and in Tables 2 and 3, please make it clear that in mice, eosinophilic foci were observed in the livers.

Mode of Action, Inhalation; Summary – Because reference 12 was completed in mice, it is not clear why this MoA is also extended to rats (or rodents as it says in the discussion). Perhaps more information from reference 13 should be stated.

Neurotoxicity, old report study – In many of the described studies, the endpoints examined are not stated. It says: "no "neurologic alterations", "no "neurotoxicity", or "no signs of neurological dysfunction". If possible, please state the endpoints examined. The ID_{50} must be the concentration of MIBK that caused a 50% decrease in the duration of immobility. This is not clear from how it is currently stated ("A decrease in the duration of immobility in the swimming test was reported after exposure to MIBK; the duration of immobility (ID_{50}) was 803 ppm.")

Nephropathy – It is not clear why the studies described in the Nephropathy section are not included in the Mode of Action section.

Irritation, old report summary – Please correct "albino rabbit" to "albino rabbits". If they studied "neuropathy" in the dermally exposed guinea pigs, that should be descried in the neurotoxicity section.

Occupational Exposure – Is it necessary to state the occupational exposure limits as they were in the original report? Only the ACGIH values have changed. One paragraph on occupational limits should be sufficient.

Occupational Exposure; Summary – For reference 18, it is not clear if the value 21.9 ± 15 ppm is an air concentration or urinary concentration. It says: "TWA concentration of the urine of workers…" but TWA generally refers to air concentrations. If it is a urinary concentration, it would more likely be expressed as mg/g creatinine as is done later in the paragraph. What were the urinary levels of unmetabolized MIBK in workers in the study described in reference 19? The description appears to be stating air concentrations but not urinary concentrations. Were urinary concentrations considered a good marker of occupational exposure to MIBK?

Discussion – The male rat-specific MoA for kidney tumors should also be mentioned in the Discussion.

Table 2, Results – Please indicate that the eosinophilic foci were observed in the livers of mice. Did the female mice really have "decreased body weight" or was it "decreased body weight gain"? Was the difference in body weight gain statistically significant? Because the results in

the table are given for males and females, it is not necessary to state “in males” under the Male rat results.

Table 3 – Either change the title of the table to “Incidence of neoplastic and non-neoplastic lesions of the liver in mice and kidneys in rats”, or indicate that the eosinophilic foci were in the livers of mice.

MIBK – March 2024 – Thushara Diyabalanage	
Comment Submitter: Alexandra Kowcz, MS, MBA; Industry Liaison to the Personal Care Council **Date of Submission: January 5, 2024**	
Comment	**Response/Action**
Abbreviations; Occupational Exposure – NIOSH is the National Institute for (not "of" as stated in the report) Occupational Safety and Health	Addressed
Cosmetic Use-As there is only one ingredient in this report, the airbrush paragraph needs to be revised from "some of these ingredients" to "this ingredient" or "MIBK"	Addressed
Non-Cosmetic – Please delete "an" in: "MIBK is an approved for direct addition to food…"	Addressed
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ADME, Animal, Oral, old report summary – It would be helpful to know the different routes that were examined in the study that found differences in MIBK metabolite concentrations. Based on the information in the report, it appears that it was an oral and inhalation study	Correct
Acute, Dermal – Please correct "wss" (should be "was"). Please add the word "patch" to: "A semi-occlusive [patch] with…"	Addressed
Acute, Inhalation, old report summary – A guinea pig study is described in which it says the animals were exposed in a "1 cm3 inhalation chamber". Unfortunately, this is what it says in the original CIR report. A guinea pig could not fit into a chamber that small. If it says this in the original paper, it was probably an error. Please do not repeat this chamber size in the current CIR report.	Addressed
Short-Term, old report summary – In the following, please add the word "to": "rats were exposed [to] 100, 500, or 2000 ppm MIBK…" Please change "in" to "at" in the following: "rats exposed to MIBK in [at] 4.53 mg/l air for 6 h/d, 5 d/wk, for 4 wk."	Addressed
Carcinogenicity; Table 2; Table 3 – In the text and in Tables 2 and 3, please make it clear that in mice, eosinophilic foci were observed in the livers	Addressed
Mode of Action, Inhalation; Summary – Because reference 12 was completed in mice, it is not clear why this MoA is also extended to rats (or rodents as it says in the discussion). Perhaps more information from reference 13 should be stated	Addressed, This MoA is very specific to rodents and extending it to rats further elaborates it.
Neurotoxicity, old report study – In many of the described studies, the endpoints examined are not stated. It says: "no "neurologic alterations", "no "neurotoxicity", or "no signs of neurological dysfunction". If possible, please state the endpoints examined. The ID50 must be the concentration of MIBK that caused a 50% decrease in the duration of immobility. This is not clear from how it is currently stated ("A decrease in the duration of immobility in the swimming test was reported after exposure to MIBK; the duration of immobility (ID50) was 803 ppm.")	Addressed
Nephropathy – It is not clear why the studies described in the Nephropathy section are not included in the Mode of Action section.	Need the view of the panel

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Discussion – The male rat-specific MoA for kidney tumors should also be mentioned in the Discussion	Need the view of the panel
Table 2, Results – Please indicate that the eosinophilic foci were observed in the livers of mice. Did the female mice really have "decreased body weight" or was it "decreased body weight gain"? Was the difference in body weight gain statistically significant? Because the results in the table are given for males and females, it is not necessary to state "in males" under the Male rat results.	Addressed
Table 3 – Either change the title of the table to "Incidence of neoplastic and non-neoplastic lesions of the liver in mice and kidneys in rats", or indicate that the eosinophilic foci were in the livers of mice	Addressed

MIBK History

2007– The Expert Panel for Cosmetic Safety (Panel) published a Final Report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products.

March 2023 – Review of the available published literature since 2005 was conducted in accordance with CIR Procedures regarding re-review of ingredients after ~15 years. The Panel re-opened the safety assessment for this ingredient, due to new toxicological and carcinogenicity study data provided by the National Toxicology Program (NTP). The Panel would also like to review the function of MIBK in aftershave lotions.

June 2023 – The Panel issued an IDA for MIBK. The additional data needed to determine safety for this cosmetic ingredient are:

- Concentration of use and function in aftershave formulations
- Confirmatory sensitization studies at maximum use concentration

December 2023 - A draft amended report was submitted, and the panel issued a Tentative Amended Report.

March 2024 - Final amended report is being presented to the panel.

MIBK Data Profile* – March 2024 – Thushara Diyabalanage																														
				Toxicokinetics			Acute Tox			Repeated Dose			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies		
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Inhalation	In Vitro	In Vivo	Inhalation	Mode of Action	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Inhalation Studies	Case Reports	Occupational
MIBK	XO	O	O	O	O	XO	XO	O	O	O	O	O	O	XO	O	O	X	X		O			X				XO	O	O	XO

* "X" indicates that new data were available in a category for the ingredient. "O" indicates data were reported in the orginal safety assessment.

MIBK

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
MIBK	90052-75-8	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy (from 2000 on)

PubMed

(((“MIBK”) OR (108-10-1[EC/RN Number])) AND (("2000"[Date - Publication]: "3000"[Date – Publication]))) – 151 hits; 7 useful hits

ECHA

(“4 methylpentan-2-one)-8 useful hits

Internet searches using trade names and other technical names. No relevant hits.

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

MARCH 2023 PANEL MEETING-REREVIEW CONSIDERATION

Belsito Team– March 6, 2023

DR. BELSITO: Moving onto MIBK. So we looked at this in 2004, concluded that it was safe as used in nail polish removers and as an alcohol denaturant in cosmetic products. It's been 15 years, so we're looking at it again to see if we want to reopen it. Extensive search was performed from 2000 and on. Historical review, there were new tox data found for several endpoints. A carcinogenicity study was completed by NTP. At the time of the original, what we reviewed, no studies on carcinogenicity potential were found. But it was noted in the discussion that the NTP study was ongoing. And we now have sort of -- lots of new data to look at here. The historical concentration of use was 21 percent. Paul, the carcinogenicity study, is that not relevant?

DR. SNYDER: Well, they were all at the highest dose, 1,800 parts per million --

DR. BELSITO: You broke up, or I broke up.

DR. SNYDER: I said they were all at the highest dose tested, 1,800 parts per million.

DR. BELSITO: Okay.

DR. SNYDER: Those positive endpoints on that Car study, the lower doses were negative.

DR. BELSITO: That would go in the discussion?

DR. SNYDER: Yes.

DR. KLAASSEN: Also, the kidney toxicity or the kidney cancer, Berghoff has shown that that's due to an alpha2u mechanism which is only relevant for male rats. So that's another reason to disregard it. It turns out that, while no --

DR. BELSITO: Which PDF page are you on, Curt? You're on the general tox?

DR. KLAASSEN: Yeah. Well, actually, carcinogenicity and mechanism of the carcinogenicity.

DR. BELSITO: Of the large kidneys?

DR. KLAASSEN: Yeah. So the kidney mechanism has been shown by Berghoff that it's due to alpha2u. And this is a mechanism of kidney injury that only occurs in male rats. It doesn't occur in female rats, doesn't occur in mice or any other species.

DR. SNYDER: Most notably humans.

DR. BELSITO: Yeah. We've dealt with that before --

DR. KLAASSEN: Exactly.

DR. BELSITO: -- in these studies.

DR. KLAASSEN: Exactly.

DR. SNYDER: There were still liver tumors in males and females at the high dose, so.

DR. BELSITO: Right.

DR. KLAASSEN: Yeah. I'd like to talk about that. It is known that this also causes liver tumors, as was just mentioned. Now it turns out that these chemicals are Car agonists and Pxr agonists. And chemicals in rodents that are Car and Pxr agonists cause liver cancer, especially the Car agonists. And, so, that's probably why we have this cancer in rodents in the livers that probably doesn't have relevance to humans as well. So neither one of these cancers, because of dose and because of mechanism or potential mechanisms, we do not need to be concerned.

MR. GREMILLION: When you say potential mechanism, is that because of the physiology of the mice is different than humans?

DR. KLAASSEN: Yes, in some regards. Mice are not identical to humans. And, so, there's a few responses that we've learned, actually, in the last 30 years that some of these responses occur in laboratory animals that do not occur in humans. And this is just a couple of examples. For your question the answer is yes.

DR. RETTIE: The nuclear receptors themselves don't translate very well across species, Pxr in particular. So maybe that's another piece.

DR. BELSITO: So does that answer your question, Tom?

MR. GREMILLION: Yeah, I think so. It seems like that would affect a lot of data. Maybe you mentioned this, but I just want to confirm there's no max concentration. Is that concentration of use?

DR. KLAASSEN: Well, for right now, for methyl isobutyl ketone, we might not have any uses, if I read this document correctly.

DR. RETTIE: I think there's a single use.

DR. KLAASSEN: Okay. In 2003, it was used at 21 percent. I don't know how many uses today, but it's not used very much.

MR. GREMILLION: Yeah. Sorry, it says no uses.

DR. BELSITO: Yeah. But that would be the same as what we had before. We're just deciding whether to reopen it or not based upon new data. So based upon the new data, we need to go back and look at what the historical concentration of use was and in what kind of product. It was used on nails.

MS. TUCKER: It was used in a nail preparation. The concentration that we had for 2000, it was used in a nail correction pen. And there was one use for 2022. Let me pull it.

DR. SNYDER: Well, the narrative says no uses, but the table says one use.

DR. KLAASSEN: Right.

DR. SNYDER: So that was my question; which one's right?

DR. BELSITO: Yeah.

MS. TUCKER: Okay. One second.

DR. SNYDER: On Page 2, your memo says, in April 2022, PCPC concentration use survey, no uses were reported. But, in the table, it says one use.

MS. TUCKER: So we should defer to the table. That may have been a typographical error in the memo because what is on the table is correct.

DR. SNYDER: Okay. All right.

MS. TUCKER: Okay.

DR. SNYDER: Yeah. Thank you.

MS. TUCKER: No problem.

DR. RETTIE: So this is a solvent prohibited in Europe --

MS. TUCKER: Yes.

DR. RETTIE: -- just recently?

MS. TUCKER: I believe it was as of 2009.

DR. RETTIE: Okay. So we have a prohibited solvent in Europe and a bunch of new tox endpoints, even if we would argue about the relevance of them, as we've done already. Is that (audio skip)?

DR. BELSITO: So, in the old report, we really approved it for nails only. And, if you look at the discussion, we've allowed it as a denaturant because it was allowed by the U.S. at up to four percent, so we went with that. But we also said we'd review the NTP data when available. The current use is in a nail pen. Is that correct, Regina?

DR. SNYDER: It says dermal contact in aftershave lotion for 2022 on Page 8.

MS. TUCKER: So, for 2022, it's the aftershave lotion, and it's a pen. In the years previous, it was for the nail correction pen, where we have the 21 percent concentration of use reported.

DR. BELSITO: So, presumably, it's in dermal contact in an aftershave lotion. So, presumably --

DR. SNYDER: So no concentration of use?

DR. BELSITO: -- it's probably being used as an alcohol denaturant, right, in an aftershave lotion? I mean, it's not going to be used as a nail correction pen. We don't know the concentration of use. So I think we probably need to reopen it. I don't know, Bart, because we don't know what that use is for. Presumably, I mean, I would think it's being used as an alcohol denaturant. But we would need to reopen to determine what specifically was the reason it was used there and what the concentration of use was. And we'd have to know what the current U.S. regulations for MIBK are; whether they said it also should not be used, or whether they still allow it up to 4 percent. Because if it's being used as an alcohol denaturant and it's less than four percent, we can't override government regulations, can we?

DR. HELDRETH: No, we certainly can't do anything regulatory, but the Panel can still weigh in whether or not they feel it's safe for use or not.

DR. BELSITO: Okay. I think we should reopen it.

DR. KLAASSEN: Yes. I think we should reopen it even though the conclusion probably won't be that different. We say in the last document that the NTP is doing that study. And we could make this document up to date with what's known, especially in regard to the carcinogenicity, and bring everything else up to date.

DR. RETTIE: That sounds good to me.

DR. BELSITO: So we're going to reopen. And the particular question we have, Regina, is what's its function in this aftershave lotion and what's the concentration?

DR. SNYDER: What does the Annex 2 prohibition mean in regards to cosmetics?

DR. BELSITO: That there's a hazard, probably. I don't know when that -- what was (audio skip).

DR. ANSELL: No, Annex 2 would be a restriction on its use. It may be based on toxicity, but it also can be for other reasons. For example, if it's not being used, and no one supports it, it will go on Annex 2.

DR. SNYDER: Okay.

DR. ANSELL: So you'd have to --

DR. SNYDER: Would the alcohol denaturant be included in that use?

DR. ANSELL: That's a good question. I was looking at the U.S. denaturants, whether it would be allowed. I'm not sure whether Europe would decide it's not allowed to be a denaturant and exclude it from the list of approved denaturants, or whether they would restrict it by addition to Annex 2 or potentially Annex 3. So I'm not sure that I know.

DR. SNYDER: I guess we would just reopen, Don, to find out the clarification of use in the aftershave and the use purpose, what it's used for.

DR. BELSITO: Right. That's what I said. We need to reopen. What's the function in the aftershave lotion and the concentration?

DR. SNYDER: Yeah.

DR. BELSITO: And make sure that the current U.S. regulations, that it can be used up to 4 percent as a denaturant, are still in effect.

DR. HELDRETH: And, Regina, so most up-to-date frequency of use information we have is 2022 in here, correct?

MS. TUCKER: Yes. I was just looking at the memo, and I wanted to make sure that I was clear that it's the concentration of use that was not reported for this year.

DR. HELDRETH: Right. But in 2022, the VCRP information we got from FDA showed this one reported use.

MS. TUCKER: Yes.

DR. HELDRETH: I'm looking at the 2023 data that we got this year, and it's not reported. So, essentially, it's showing that this ingredient is not in use anymore.

DR. ANSELL: As an ingredient.

DR. HELDRETH: Right.

DR. ANSELL: And, apparently, SDA 1 and SDA 23 use MIBK as denaturants to the extent of one gallon of MIBK per 100 gallons. So that would be one to 100 for SDA 1, and one and a half gallons in SDA 23h.

DR. HELDRETH: If we go by the general rule of thumb of when an ingredient name makes it onto the label, MIBK wouldn't make it onto the label of any products that use a denaturant alcohol that you're talking about, correct?

DR. ANSELL: Correct.

DR. BELSITO: Where did you see 2023 uses, Bart? I don't have that.

DR. HELDRETH: Well, we do a FOIA request every year with FDA VCRP and get the frequency of use. Regina has the 2022 values here. I just went and looked at our data download that we got from FDA this year, and MIBK is not listed there. So, as an ingredient by itself, it has no uses at this point. But, as Jay said, it may be used as a denaturant. But, if it's really used at that small of a part of another ingredient, it wouldn't make it onto the label.

DR. ANSELL: Yeah. The label could disclose it as denatured alcohol, as opposed to defining which denaturant, or alternatively disclose it by its use number.

DR. BELSITO: That would be U.S. regulations for denaturants, right, not us to decide?

DR. ANSELL: Right.

DR. HELDRETH: But the Panel has looked at things like alcohol denat and considered the different common denaturants that would be used in there to consider if the Panel was worried about them at all. But, yeah, at least from the current FDA database snapshot that we have, it looks like this chemical is not used as an ingredient in the U.S. at this moment.

DR. BELSITO: So what do we do? Europe has banned it. We can't really review it because we have no uses or concentration of use. Do we not reopen it? Do we reopen it?

DR. HELDRETH: No, of course, that's the Panel's prerogative, if you feel like there is a potential concern if someone were to use it, you could arrive at an insufficient data conclusion. But if (audio skip) you need to know about (audio skip) concentration of use. Then, ultimately, it'll get moved to the zero-use category in two years if nobody comes forward with that data.

MR. GREMILLION: I suggest maybe another option would be to table it until the end of the year when the deadline for the mandatory reporting sets in.

MS. TUCKER: I just have a quick question, Bart. When you searched, did you search under MIBK or methyl isobutyl ketone?

DR. HELDRETH: I searched under MIBK.

MS. TUCKER: Okay. So, under methyl isobutyl ketone, we have two uses.

DR. HELDRETH: Okay.

MS. TUCKER: There's two for other manicuring preparations and for aftershave. But that's when I searched under methyl isobutyl ketone.

DR. HELDRETH: Yes, you're right. I didn't do that.

DR. BELSITO: Okay.

DR. HELDRETH: Let me do that.

DR. BELSITO: The 2023 data, you said, Regina, there was one nail, other nail, and one aftershave?

MS. TUCKER: Yes. So, according to the 23 under methyl isobutyl ketone, it's other manicuring preparations and aftershave lotion.

DR. BELSITO: Okay.

MS. TUCKER: So that would just be a -- I think, last year it was on -- let me just make sure here.

DR. BELSITO: Okay. Then, the nail, I'm not that concerned about. It'd be nice to have concentration of use, but I think we really need to know the function in the aftershave lotion and the concentration in that.

MS. TUCKER: Okay. Okay.

DR. SNYDER: Yeah. We can reopen, try to get that, and then just close it again. We don't need to -- yeah.

DR. BELSITO: Yeah. I mean, we're reopened things before and gotten the information and decided that based upon the new information, there was no need to pursue the reopening. Correct, Bart?

DR. HELDRETH: Correct. Yes. I mean, there are situations where that has worked. Here, where we have a very different use than we saw before, I'm not sure we could say we've reaffirmed the original conclusion, when the original conclusion was only for nail products.

DR. BELSITO: Well, no. We said that it was safe as an alcohol denaturant.

DR. HELDRETH: Okay. So, if this aftershave use is an alcohol denaturant use --

DR. BELSITO: Then we're fine.

DR. HELDRETH: Right. But, if it's not or we don't get information back describing it --

DR. BELSITO: Then we reopen.

DR. HELDRETH: Yeah. Then it stays reopened.

Cohen Team – March 6, 2023

DR. COHEN: Okay. MIBK. Methyl isobutyl ketone. All right, so this has been reviewed in 2004, when it was considered safe as used in nail polish removers and as an alcohol denaturant in cosmetic products. It's been 15 years. There's been some new data about this that was provided. Frequency of use decreased from two to one. And we have no maximum use in the current report or in the past, it was up to 21 percent. It's used as an aftershave.

So, this is a question about reopening.

DR. BERGFELD: Do you want to mention what the EC has done? It's prohibited in Annex 2.

DR. COHEN: It's prohibited -- yeah, it's an IARC-2B as well. The dilemma here is --

DR. SLAGA: The cancer studies.

DR. COHEN: Yeah.

DR. ROSS: Yeah.

DR. SLAGA: We can remember this where we heard, at the time we were reviewing this in the past, that NTP already started a study. I don't know if some of the new people, if they don't know but NTP studies take a long, long time. They're done right, they're done very well. They're done always with two species, rats and mice, 50 males and 50 females in both cases.

It's after the studies are done and they summarize everything, they have a peer review panel that looks at it and discusses it. So, there's a lot of heavy weight put on NTP studies.

In this case, there was one study in mice for not long, long term, I think it was only 13 weeks. But it had some indications, and this is one of the reasons that NTP went forward with their studies. And so, it's always very nice to include NTP studies in these evaluations because they carry a lot of weight.

The dose that was used in the initial mouse study was very high -- inhalation study. And NTP followed that and used different -- from no dose to low dose, medium dose and very high dose. And there was no cancer in the low doses. There was only cancer in the high dose. The very, very high inhalation dose by inhalation exposure.

And in the rats, there was cancer both in the rats as well as the mice at that high dose, which was also -- well, in the rats it was the male rats had kidney cancer. In mice, both males and females had liver cancer.

And there was indications in the previous study, at the high dose, that someone else did that there was some liver problems. Liver weight went up, et cetera, and some precancerous kind of things.

The problem is at such a high dose, it's really hard to relate it back to something that's used as a preservative or used as a denaturant, you know, which is -- then it's all used up. So, I'd like to see -- there's other studies that reported, too. So there's a lot of database to put in.

So, here's the weight. Do we reopen to add all this data, which makes it a very nice report, then. And we can justify in the heavy discussion why the high dose doesn't relate to this particular treatment for nail polishes or alcohol denaturant.

So, I don't know. I'd like to see all that data together so, in a way, I would say let's reopen it. And if we don't reopen it, we'll be criticized, I guarantee you that -- well, you didn't really consider the cancer data. You know, we are considering the cancer data.

But, you know, it was a very high, high dose. There was only that one dose. So, there's really no dose response, there's only that one dose. So, maybe if you went higher than that there would be another increase, a dose response. Anyway, I suggest we reopen it.

DR. BERGFELD: How available is that information, the NTP study?

DR. SLAGA: Come again?

DR. BERGFELD: How available is the information on the NTP study?

DR. SLAGA: Well, it's pretty easy once it's published, yeah.

DR. BERGFELD: Is it not published yet?

DR. SLAGA: Huh?

DR. BERGFELD: It's not published yet?

MS. TUCKER: It's published. We have the NTP report with the carcinogenicity studies available now.

DR. SLAGA: Yeah. No, no, we can get the data. The data that we put.

DR. COHEN: Yeah. I had it as a reopen. And remember the 2000 max use concentration was 21 percent. It was high. The question is, how does that relate in real use over time?

DR. SLAGA: Right.

DR. COHEN: The other dilemma is we're opening this up and there's one reported use.

DR. SLAGA: Yeah.

DR. ROSS: Sorry. Go ahead.

DR. TILTON: Yeah, so that 21 percent was for a nail correction pen and that's it.

DR. SLAGA: Yeah.

MS. TUCKER: That's it, yeah. The 21 percent was for the nail correction pen. But also for the 2023 data, I think, we have two usage.

DR. COHEN: Ah.

MS. TUCKER: In the aftershave lotion and in the nail preparation.

DR. COHEN: Okay. I thought it was one. Okay, good.

MS. TUCKER: So, it was one for 2022, but for the 2023, which you guys don't have yet, it has gone up.

DR. ROSS: I was negative on the number of uses. I do not reopen. But I would echo everything Tom just said on the cancer studies. So, I'm not actually sure you can reopen it when you've got some positive cancer data out there.

DR. SLAGA: Well, the one reason, too, we had a discussion about how the data when we don't reopen it, it's not tied back to the original publication. In this case, you can put all its data together and no one would think you're hiding anything. I think it would be important to reopen it.

DR. BERGFELD: Reopen.

DR. ROSS: That was my advice.

DR. COHEN: You're on mute, Susan.

DR. TILTON: Thank you. I was trying to keep everyone away from the construction sounds so I was trying to stay silent. I agree. I was on the fence going through this one. Because I noted that the new sub-chronic tox data and the carcinogenicity data really were reported at highest concentrations tested. And we don't have a lot of use, but the reported uses in an aftershave lotion and there's no reported concentration. And so, it sounds like in the new information there might be additional use. I don't know if we have reported concentrations there, or if it would be possible to get that information.

DR. COHEN: It would be certainly helpful to have it. And I think, Tom, your discussion was very motivating. And I think if you're reading the popular press about the presence of carcinogens in cosmetics -- and there's been a number of articles about that -- we're looking at something that has that potential. And the frustrating part is it's going to be hundreds of collective man hours for two uses. But I don't think we have a choice, and number two, we really don't know what the real use is.

We won't know for two years when it's mandatory to report all these things what we're looking at. And we'll probably be happy we did it, if we get real use data back two or three years from now and we reopened it. And if there's not a lot of use we won't remember we did it two years earlier anyway.

DR. SLAGA: Right.

DR. ROSS: I agree. And just one thing in the tables, what carcinogenicity in vitro. I'm not sure you can have carcinogenicity in vitro.

DR. SLAGA: Yeah.

DR. ROSS: Yeah. I mean, I would more like --

DR. SLAGA: I have that in my report.

DR. ROSS: It's proliferation and transcriptomic.

DR. SLAGA: Anti-carcinogenicity of cancer cells, you can't call it that. Anti-carcinogenicity -- carcinogenicity is the generation or development of cancer from a normal cell. If you already have a cancer cell, you're looking at the effects of inhibiting that cancer growth through a cytotoxic effect or some specific effects.

So, I'd have that changed. It's really not anti-carcinogenicity, it's anticancer that you're looking at. And so, it has a negative effect, or it inhibits the growth of those breast cancer cells in culture. Okay?

So, I'd have that to be changed to call it something different.

DR. ROSS: My comment was on the tables where you have carcinogenicity in vitro. Just beneath the NTP studies. You know, I mean, to me I think that's more proliferation and transcriptomic, not carcinogenicity in vitro.

DR. SLAGA: Yeah.

DR. ROSS: So, I mean, you could --

DR. SLAGA: In vitro, it's called transformation, not carcinogenicity. Carcinogenicity is in a whole animal, so to speak.

MS. FIUME: On that subject, can I ask? Because in our current format for reports, we had in vitro cell transformation presented under carcinogenicity studies. I thought --

DR. SLAGA: Yeah, it's under carcinogenicity study but it's transformation of cells in culture. Once you put cells in culture, to me, they're not normal anymore, so you can't really call it a normal cell. And that's why transformation of that altered cell to a cancer cell, if you will, is --

DR. COHEN: I think it's a bit of semantics because in one of them it wrapped appropriately carcinogenicity, in vitro trial. The other one, it looked like it's in line. So, I think they are carcinogenicity studies, but they're in vitro studies that are looking at the potential for that, right?

DR. SLAGA: Well, yeah. The potential for being like carcinogenicity. But it's true they call it transformation.

MS. FIUME: So, I'm just trying to make sure we captured it correctly in the report as far as placement.

DR. SLAGA: Yeah.

DR. ROSS: I mean --

DR. COHEN: It looks like you did.

DR. ROSS: -- in vitro transformation.

MS. FIUME: Yes. And that's actually what the subtitle will be in the report. The subsection name would be In Vitro Cell Transformation. Thank you.

Full Panel – March 7, 2023

DR. BERGFELD: Moving onto the third one in this group, the MIBK. Dr. Belsito.

DR. BELSITO: Yeah. So the MIBK, this is another report that's coming back to us because it's been 15 years or more. We felt that we needed to reopen it to understand the function of this material in an aftershave lotion and a concentration in the aftershave lotion.

DR. COHEN: Second.

DR. BERGFELD: Any other discussion regarding this ingredient? Was there discussion about the NTP studies?

DR. COHEN: Yeah. Tom gave us a very nice discussion about the NTP data that we might be able to have here.

DR. SLAGA: It would give us a chance to look at it in more detail.

DR. COHEN: Don, quick question on the MIBK. When you look at the original report, there didn't seem to be HRIPT or sensitization data on there in that report. It looked like mostly a couple of animal studies. But maybe that'll give us an opportunity to see some of that if we reopen.

DR. BELSITO: Yeah. I mean, we punted to the U.S. regulation that it could be used up to 4 percent as a denaturant in alcohol. I think that's why you're seeing that lack of data if you read the discussion.

DR. COHEN: But now as an aftershave, it might change things?

DR. BELSITO: Well, we don't know how it's used in the aftershave. Whether it's used -- you know, because the aftershave contains alcohol and it's the denaturant there. And the U.S. had said that, at least, that it can be used up to 4 percent as an alcohol denaturant. So, we need to get further information on that aftershave.

DR. COHEN: Okay.

DR. BERGFELD: Any other discussion or things that need to come forward next time we look at this? Okay. All those opposing? Abstaining? This ingredient, MIBK, is reopened.

JUNE 2023 PANEL MEETING – INITIAL REVIEW OF AMENDED REPORT

Belsito Team– June 12, 2023

DR. BELSITO: MIBK. QRSTUVW. We have a Wave 2 on this.

DR. SNYDER: From Women's Voices of the Earth.

DR. BELSITO: It's PCPC comments. I said, "Address Wave 2 PCPC comments." I agree with all. Team? Wave 2. MIBK. Okay. So, this is about specifying their application in nail activator products. And, yeah, Women's Voice of the Earth. They provided artificial nail activator products containing MIBK, suggesting a draft amended report should explicitly mention the common presence of MIBK in nail activator products. The current version of the cosmetic use of MIBK has been categorized under manicuring preparations and shaving preparations, with one reported use in each category. 2004, it was two nail polish and enamel remover formulations.

DR. RETTIE: Can I ask what a nail activator is?

DR. BELSITO: So, it probably is used in like acrylate-based nail polishes where you need to activate the polymerization of the acrylate. So, they'll have activators and inhibitors. So MIBK would be added as an activator, so when it's painted on the nail the process of polymerization begins.

DR. RETTIE: Thank you. I didn't know anything about that.

DR. BELSITO: Well, you should go to a nail salon in Seattle, Allan, and get your nails done once.

DR. SNYDER: It sounds like a team bonding event.

DR. BELSITO: Yeah. No pun intended with the bonding. Dan would've had fun with that. Okay, I mean, how far do we go? There's no nail activation category, so I don't think we can do anything with it. It's another manicuring product, right? So, I think it falls under the proper -- it wasn't clear to me why they'd want that very detailed specification.

MS. FIUME: I'm not sure either and, Jinqiu, you've found that some of those products are no longer in use, right?

DR. BELSITO: Can't hear you Jinqiu.

DR. ZHU: I'm not sure why they put that comment down because that's specifically, not categorically VCRP, and not related to the safety. I don't know why.

DR. BELSITO: Again, yeah. I mean, I just think noted but it's not a VCRP category and we do list the nail use or other manicuring use. Okay. So then, let's go into the report on MIBK. So basically, the initial assessment that we had was safe as used in nail polish removers and as an alcohol denaturing cosmetic products. We reopened the safety assessment in this ingredient to consider new carcinogenicity and tox data, because we had said in the original that we would look at it when it became available and somehow, we didn't. So that's the whole purpose for reopening this ingredient. And we have the NTP study. It was found to be possibly carcinogenic, but not relevant to humans because the induction pathways in rodents are not active in humans. Discussion, otherwise, essentially the same with the addition of the air brush exclusion or do we go back and decide not to reopen it? I mean, we reopened it so do we -- I wasn't sure how we do it. Or is it insufficient for concentration of use, especially because it's used in an aftershave, and we don't know if it's used there as a denaturant. So, there are lots of possibilities here. The carcinogenicity study, which is the reason we reopened it, is not relevant to humans. But now we have it used in an aftershave and we don't have the concentration of use.

DR. SNYDER: I said up to 4 percent as an alcohol denaturant.

DR. BELSITO: Yeah. But we don't know the concentration of use in the aftershave to even suggest whether it's -- I mean, presumably, it's being used as a denaturant in the alcohol in that aftershave. But we don't know the concentration of use. So now do we have an insufficiency for that concentration of use?

I mean, this is weird because we reopened it to look at the carcinogenicity, and now we have a report that it's used in aftershave, and we don't know what it's used for in the aftershave or the concentration of use.

DR. SNYDER: Did we have concentration of use in the original report?

MS. FIUME: Twenty-one percent in nails.

DR. BELSITO: In nails.

DR. SNYDER: But now we have a new use in the aftershave.

DR. BELSITO: Yes.

DR. SNYDER: Without a concentration.

DR. BELSITO: Without a concentration of use.

DR. SNYDER: Why do people make this so hard? Let's send it out insufficient. We have to. We're obligated to -- we have to document that we reviewed the NTP's data and we cleared that. But in the process of doing that, we got this new use.

DR. BELSITO: Right. So, we document that. We clear that and we say that it's insufficient for concentration of use in the aftershave.

DR. SNYDER: Yeah. Yeah.

DR. BELSITO: To allow us to determine whether presumably it's used as an alcohol denaturant. Or what its function --

DR. SNYDER: Or we can say safe as used as a nail product and insufficient for --

DR. BELSITO: Yeah. Exactly. That's where we are right now.

DR. SNYDER: Yeah. That's where we are.

DR. BELSITO: Safe as used in nail formulations. Insufficient for use in an aftershave. Data that's needed is concentration of use. Although its function -- what's the cosmetic ingredient dictionary define its function as?

MS. FIUME: So, it's denaturant, fragrance ingredient and solvent.

DR. BELSITO: Okay. So, it could be used as a solvent in an aftershave above the level of a denaturant. Yeah, so we need the concentration of use.

MS. FIUME: And that's the only data need?

DR. BELSITO: Yeah. Safe as used in nail formulations as described. Insufficient for aftershave lotion. Data need, concentration of use.

MS. FIUME: So, as a IDA?

DR. SNYDER: Yep.

DR. KLAASSEN: In regard to the carcinogenicity studies, I think we need more detail there. Like, how many animals were positive. It's just too brief. Both in the -- what were the real concentrations. Was it just one concentration, 1,800 parts per million? And we just need more data in regard to the number of animals that had tumors, et cetera, there. And also in the carcinogenicity studies, there's also kidney tumors, right, and that should be put in here. And the explanation that you gave down below, in regard to nephrotoxicity, the business about the alpha2u mechanism should also be brought up to the carcinogenicity.

Because the main concern, theoretically, is this carcinogenicity. So, a little bit more explanation of what the data really was in the liver, then the rest of that paragraph is okay. And then make a new paragraph here and say what did the NTP study say about the kidney tumors, and then bring up the rest of the paragraph in regard to the alpha2u. Now, in the paragraph that you do have about the alpha2u, you discuss the limonene. And it doesn't come across why they use limonene. But the reason they use limonene is that that is a positive test for -- I mean, we know that alpha -- that limonene causes these kidney tumors by this mechanism.

So that's kind of the gold standard. So, they're comparing this saying, oh, it's similar to when you give limonene, and it's the same mechanism. So just clarify that a little bit, it'll make the report read better.

DR. BERGFELD: So, you want Regina to put in that limonene reduces a positive control, given its known effect on alpha2u nephropathy, or something to that extent?

DR. KLAASSEN: Right.

DR. SNYDER: It attributed to the mechanisms due to the rat male, rat-specific alpha2u, and have the appropriate controls or something like that. That's fine.

DR. BELSITO: Okay. So just an explanation as to why limonene was control, since usually we bring it up only as a fragrance material?

DR. KLAASSEN: Right.

DR. BELSITO: Okay.

DR. RETTIE: Going back to the alcohol denaturant use, the question of 4 percent concentration. It seems the EPA approves methyl Isobutyl ketone as a denaturant than a maximum concentration of 4 percent, so that would seem to all tie up.

DR. SNYDER: Yeah, it's a denaturant but what Don raised was there's also a solvent use.

DR. BELSITO: Right. We don't know its concentration or its function in the aftershave lotion.

DR. RETTIE: But I thought you were saying that it was most likely that it was being used as a denaturant in the aftershave.

DR. BELSITO: Well, that's what I would presume but we don't know. And therefore, it's insufficient for that information.

DR. RETTIE: I just bring up the coincidence that it's 4 percent and 4 percent maximum. Also, the rather interesting point here, that it's used as an alcohol denaturant in rum.

DR. BELSITO: I don't drink rum so I could care less.

DR. RETTIE: I do. I'm very concerned now.

DR. SNYDER: Hide the rum.

DR. KLAASSEN: It's so you won't drink the rum.

DR. BELSITO: But, Allan, as long as you're not a rat you don't need to worry.

DR. RETTIE: Okay.

DR. SNYDER: You've got to quit drinking that low-end stuff.

DR. RETTIE: Well, you know me.

DR. BELSITO: Okay. So, wait a minute. Allan may have a point that I may have overlooked here. But we don't have a concentration of use. Were you implying that it says that it's used at four percent in Table 3? We don't have concentrations.

DR. SNYDER: In our old report. We said it was safe as used up to 4 percent as a denaturant.

DR. BELSITO: Oh, yeah, based upon the FDA definition, right. But we don't have a concentration of use here.

DR. SNYDER: Right.

DR. BELSITO: Right. I thought Allan said we did but he was --

DR. RETTIE: No. No, no. I was just trying to link up some concentrations and likely uses and they seemed to gel.

DR. BELSITO: Okay. So, we're going to beef up the carcinogenicity. I have some other just editorial comments and we're going to go with a safe as used in nail products, insufficient for concentration of use in the aftershave.

MS. FIUME: Can I just ask a question for a point of clarification? Because earlier you had talked about bringing some of the conclusions up to current terminology. And I can't remember, it may be because different information is given.

Typically, we don't name functions in the conclusion. Is this a different type of compound that those functions should stay in the conclusion? Or since you're looking at it, is it something where you would modernize the entire conclusion to just safe as used, if you get the information on what the concentration is and how it's used in shaving creams?

Because the uses are listed in the report, does the function need to be tied to the conclusion? Because that's different than what's normally done.

DR. SNYDER: That's a good point.

DR. BELSITO: Well, yeah. But sometimes when we've been concerned -- I mean, but this goes back to 2001, right, because we were doing the acrylates in nail products on 9/11, if you re- --

MS. FIUME: I was not here.

DR. BELSITO: You were not here. Well, we were. We were in the middle of doing it when Doug Shone (phonetic) walked in and told us about the World Trade Tower. So, yeah. So that was --

DR. SNYDER: Could we say insufficient for salt use as a solvent?

DR. KLAASSEN: Or insufficient for 9/11.

DR. BELSITO: For what, the acrylates?

DR. SNYDER: No, this one.

DR. BELSITO: This one?

DR. SNYDER: Instead of aftershave, for solvent use. Because we don't know what cosmetic use.

DR. BELSITO: We don't know. Right.

DR. SNYDER: Because that goes back to a use rather than a product.

MS. FIUME: Right. So, yeah. So, saying insufficient for that reason is still tying it to a function.

DR. BELSITO: Yeah. I think in this case, at this point, it's going out -- this is not a final, right? This is --

MS. FIUME: It would be an IDA.

DR. BELSITO: Yeah. Insufficient data announcement. I think it is clearer to everyone that we're not concerned about the nail use. And that what we want to know is what the concentration of it is in the aftershave. We can always change the conclusion later and just say safe as used or whatever. But right now, I think that what we're specifically asking for is concentration of use and/or function in the aftershave. If they come back and they say it's used as an alcohol denaturant, or the concentration is 2 percent, then we're fine.

DR. SNYDER: Okay.

DR. BELSITO: Okay.

Cohen Team - June 12, 2023

DR. COHEN: Okay. So, methyl isobutyl ketone. We have a draft amended report. In the initial assessment, the Panel found that it was originally safe in nail polish removers as an alcohol denaturant in cosmetic products. And in March of this year, we reopened the safety assessment of this ingredient. And we considered some new carcinogenicity and toxicology data by the NTP. And this study was in progress at the time of the original report.

So we got in -- we have 2023, VCRP data. It's reported in two formulations. It also has reported use as a fragrance. And this was IARC, to be possible human carcinogen. The second wave had some discussions about its use as a nail activator. And I think, at least, at the very get out we have IDA -- we need concentration of use, irritation and sensitization in humans. But that's just basic stuff, opening it up to the group.

DR. BERGFELD: Can we get Tom to talk about the NTP?

DR. SLAGA: Yeah. One of the main reasons we were waiting on the NTP study, and it turns out that the one that is listed in the document is more for a mode of action related to rats, but it's not really relevant to the humans. So, there's no concern with the NTP.

DR. ROSS: I would agree. I had no concern with that.

DR. SLAGA: But we reopened it because there was so much new data, including the NTP. So it really will have, in my eyes, the same conclusion as it was before. So, I don't know how to handle it. I mean, you know, you can add the data and have the same conclusion, but --

DR. COHEN: I kind of looked at this as a draft report now. Like once it comes back to us like this, it's a draft report, and we can use components of the old report as data points. But if this was coming to us for the first time, wouldn't we be asking for some of these things like irritation and sensitization in people?

DR. SLAGA: Yes. Yeah.

DR. BERGFELD: I think we have to respond to the Women's Voices for the Earth. This is one of those that they spoke on.

DR. COHEN: Yeah, it was included in Wave 2.

DR. ROSS: They wanted it included as nail activator? Was that in there?

DR. BERGFELD: Yeah.

DR. COHEN: Yeah. I was hoping you could clarify this for us. Nail activator usually allows a powdered process to move forward faster, right?

DR. ROSS: Yeah.

DR. BERGFELD: Maybe dry faster.

DR. COHEN: It just speeds it up.

DR. ROSS: I had no problem including that. But I think the response was it was in other uses. Wasn't that the response that came back from --

DR. HELDRETH: Jinqiu?

DR. ROSS: Jinqiu. That it was already included in other uses.

DR. TILTON: Other uses, yeah.

DR. ROSS: So do we need to add it? That's the question. I don't mind adding it.

DR. COHEN: Yeah.

DR. ROSS: It seems like a reasonable request. But if you've already got it in another category -- I think that's an administrative question really. What do you think, Bart?

DR. HELDRETH: Right. It doesn't change your concerns about how consumers are exposed?

DR. BERGFELD: Yeah. I think it's a nail product period. But I think that with the women's voices for the earth, I mean, they're working hard to respond to us, we need to respond back.

DR. ROSS: Yeah.

DR. BERGFELD: And it's included under this, blah, blah, blah.

DR. COHEN: Well, in this particular case, is there any reason not to just mention it?

DR. BERGFELD: You can mention it.

DR. COHEN: In uses?

DR. ROSS: There's no reason not to.

DR. COHEN: Yeah, I thought it was a pretty well done comment to us. And I think under cosmetic uses, we could just make a comment about it in there. No?

DR. BERGFELD: Yep.

DR. COHEN: Any reason we can't?

DR. HELDRETH: No.

DR. ROSS: That seems like a way to go. Can someone tell me why this is in Annex 2?

DR. TILTON: Two B, which I think it is probable.

DR. ROSS: Oh no, that's IR. This is Annex 2 --

DR. TILTON: Prohibited.

DR. ROSS: Prohibited in cosmetics. And I mean, we -- it can be used here at 4 percent as an alcohol denaturant. It's Annex 2 in Europe. The carcinogenicity study, as Tom said, two years, I mean, you see some tumors, but there's no plausible mechanism in humans. Lots of very nice studies, including from a friend of mine, Brian Lake.

The tox looks pretty much, okay. There's some acute inhalation tox, which is one I wanted to ask you about, but that's a pretty high dose. Took a look at that. The DART is okay. The genotox is negative. The dermal all-animal data, slight irritation with neat compound. Sensitization is okay, David, with 30 percent in guinea pigs, but there's no human data.

DR. COHEN: No. Yeah.

DR. ROSS: So we get back to the same point,

DR. COHEN: Yeah. I mean, all we have is guinea pig data on this, then we can wrap -- I can -- I don't even know if you need me here.

I mean, if all we had was guinea pig data for all of our information and was negative in that, why would we even need -- that's not enough, I don't think.

DR. BERGFELD: Well, I think that should be an item of discussion for a couple ingredients then.

DR. ROSS: Yeah.

DR. BERGFELD: Animal data validity versus adding human. I mean, it's an overriding question for all the ingredients.

DR. COHEN: It is and I don't have an answer for it. I'm hoping that some of these in vitro and in silico models will start really helping and build that bridge for us. Because I don't think we're going to, in the future, get all that -- we're not going to get the animal data anymore in the future. So we're going to have to build bridges within perfect human data. And I'm open to that, it's going to be fine.

DR. ROSS: We have no concentrations of use.

DR. COHEN: We have no concentration of use.

DR. ROSS: Of the aftershave or the -- I mean, historically it was used in nail manicuring at 21 percent. There's nothing in the most recent survey. But I didn't see anything with the aftershave.

MS. TUCKER: So to address why with Annex 2, my notes indicate that it was due to its CMR status, carcinogenic Category 2.

DR. ROSS: So the IR 2b is --

DR. ANSELL: Yeah, I would have to go to the SCCS to get permission to review to continue its use. So Annex 2 does include materials which are unsafe, but it also includes unsupported materials.

DR. COHEN: Yeah, yeah.

DR. ROSS: Yes.

DR. ANSELL: And the only application we have is the use as a denaturant, you know, in denatured alcohol. So we would definitely think we have enough data to continue its use as a denaturant, a special denaturant alcohol used in cosmetic ingredients.

DR. ROSS: Up to 4 percent. Yeah, I agree. I mean, it can go into Annex 2 for cause, for data, or it can go into Annex 2 because of a lack of data with SCCS. So, it's a bit tricky sometimes to figure out which.

DR. COHEN: We saw that with some hair dyes.

DR. ROSS: We did. I have this same comment in one of the hair dyes. Yeah.

DR. COHEN: So, can we harmonize on our IDA, concentration of use, irritation and sensitization in humans? Anything else?

DR. ANSELL: You want sensitization data on denatured alcohol?

DR. COHEN: If you're dipping your fingers in denatured alcohol, I'm not worried about the alcohol so much, I'm worried about the denaturant. You might be in there for a bit of time. Why wouldn't I want that? Like, walk me through that.

DR. ANSELL: They are -- well, they're formulations are defined by Europe. I don't --

DR. COHEN: It's a 100 Daltons, right? It's could be a con- -- I mean, it may not be a contact sensitizer, probably, possibly not, but at 21 percent in alcohol, right? So it could be used up to 21 percent in a denaturant --

DR. ANSELL: No, it can be used in one gallon, one and a half gallons. It's a defined formulation. So, when it's used as SDA, I guess it's SDA one and 23H, it would be present to the extent of 1.5 gallons in every hundred gallons of alcohol.

DR. ROSS: As a denaturant. Yeah.

DR. ANSELL: Yeah.

DR. BERGFELD: I'm sorry, what does that translate to concentration then?

DR. ANSELL: Well, that would be 1.5 percent, one gallon and a hundred gallons.

DR. COHEN: Yeah. So, then where is this 2000 data coming with 21 percent in a nail correction pen, right. I'm just reading the table, I'm not challenging your concentration.

DR. ROSS: At different uses for different products.

DR. COHEN: Isn't it always being used as an alcohol denaturant?

DR. BERGFELD: There are other uses.

DR. COHEN: Or in this case it's not being used as an alcohol denaturant?

DR. ROSS: Well, they have to be different. If the maximum concentration of alcohol denaturant is 4 percent, and then you've got a 21 percent concentration of use for the nail product, those products have to be different.

DR. COHEN: Yeah. These are irreconcilable numbers. Your description, I buy at face value. But 21 percent from the old report, that's more than a denaturant, no?

DR. ANSELL: So, this was open to consider the NTP data.

DR. ROSS: Yeah.

DR. ANSELL: The NTP data is not a concern. The only use we see for this material today, including considering the Annex 2 listing in the EU, is as an alcohol denaturant. And so, we would support just approving it for used as a denaturant.

DR. ROSS: But how do we reconcile these two concentrations, Jay, that we've got? One is, you know, 4 percent max alcohol denaturant, the other one is 21 percent in -- and, I mean, maybe it'll come in if we ask for concentrations, it'll come in at 4 percent and that'll be fine.

DR. COHEN: Right?

DR. ROSS: And maybe that 21 percent is truly historical and it's not used at that concentration anymore, and that's an easy way of solving it.

DR. COHEN: I would think we could reopen a report just for, like, Paragraph 3, and just look at Paragraph 3 and keep every -- unless you're telling us all that data is expired on the concentration of use.

We have to base it off of what we have left. And we've gotten no data from industry on its concentration of use, and probably not even on frequency of use. Is it correct to assume when we're reopening, we have a new report?

DR. ANSELL: Yes.

DR. COHEN: It's a new report.

DR. HELDRETH: It's a draft report.

DR. COHEN: So, if this was the report that you got, right, and the only concentration of use data is 21 percent, would you assume that it was a denaturant?

DR. ANSELL: No, no, I think that's fine. I think that's fine. You know, we've opened it. The concern was the carcinogenicity report.

DR. COHEN: Yes.

DR. ANSELL: If additional questions come up, you know, ask them.

DR. COHEN: Look, the concern will be if we don't get concentration of use data, right, and we're stuck with this 21 percent in this old report, this could really get hung up.

DR. ANSELL: Well, or we just say safe as used as the denaturant.

DR. BERGFELD: And be done with it.

DR. ANSELL: And exclude the applications which are not supported.

DR. COHEN: I have to go look at this.

DR. ROSS: You have to acknowledge safe as used when non-sensitizing, safe as used when used for a denaturant. You just added a new use there.

DR. COHEN: In the old days it might have concluded like that actually. Right? Because I think that's how it was concluded, right, as a denaturant.

DR. ANSELL: Right.

DR. COHEN: Then how did it clear with 21 percent concentration in the old report?

DR. HELDRETH: Right.

DR. COHEN: Right. I don't get that.

DR. ROSS: Yeah, I looked at that. I just felt it was, you know, small amounts of exposure with the conclusion of the old report.

DR. HELDRETH: So you could put out your Insufficient Data Announcement for concentration of use, irritation, sensitivity in humans at maximum use concentration. And then if industry provides data showing that denaturant use is the only one they're using at -- concentration is at 4 percent, put that 4 percent in our table, you can disregard the old 21 percent use.

DR. COHEN: Yeah.

DR. HELDRETH: And maybe irritation and sensitization are not as much of a concern.

DR. ROSS: In fact, in the old conclusion, which is one sentence, they separate use, nail polish remover and as alcohol denaturant. So, it's two separate things.

DR. COHEN: I'm still -- maybe me. Why don't I want to know about the irritation and sensitization potential at 4 percent? If you're dipping your fingers in it.

DR. ROSS: I think you need it.

DR. COHEN: Right. I'm still -- I haven't been assuaged as to that. Even if it's a denaturant. The 4 percent it's still a decent concentration, but we will see what other data we can glean from this.

DR. BERGFELD: So you're basically opening it as a new document?

DR. COHEN: It's been open as a new document.

DR. BERGFELD: And the real hangup is the actual use, whether it's just a nail product?

DR. COHEN: Yeah.

DR. BERGFELD: And the other is the concentration, what is it?

DR. ANSELL: Mm-hmm.

DR. ROSS: Exactly. And not just as a nail product, but as an aftershave.

DR. BERGFELD: Aftershave. Okay.

DR. ROSS: Suspect that might be 4 percent.

DR. COHEN: Yeah, that's a reasonable concentration to want to know about sensitization. You're shaving the skin, you're taking the top part of stratum cornea off, you're putting this on. It's not rinsed off, it's a leave-on.

DR. BERGFELD: But we've had other nail products that we concluded only for use in nail.

DR. COHEN: Yeah, yeah. No, that may be how we finish this. But, yeah, I think --

DR. BERGFELD: So, the insufficient -- and what are you going to ask for now? Why don't you clarify that?

DR. COHEN: Concentration of use. I guess, method of use. Irritation and sensitization in humans at max use.

DR. BERGFELD: Or in vitro? I mean, are you going to ask for other human or?

DR. COHEN: You know what, I wasn't going to ask for that. I guess I would deal with it if it came in. That's a very interesting question, though, Wilma, right.

DR. BERGFELD: Yeah, if there are some pieces in here in the contact sensitization in documents on the in vitro.

DR. COHEN: Yeah.

DR. BERGFELD: I think you could ask for either one.

DR. HELDRETH: Sensitization data as opposed to specifying a specific study.

DR. BERGFELD: Yeah, you could do it that way too. And the minutes reflect that you have this discussion with what that means.

MR. ANSELL: Mm-hmm.

DR. COHEN: Right? But, they disappear in the final report.

DR. BERGFELD: No, they do, but they're still on record, as you saw.

DR. COHEN: No, I know.

DR. BERGFELD: Everybody wants to see it.

DR. COHEN: Okay. Do we feel okay with MIBK now?

DR. BERGFELD: Mm-hmm.

Full Panel – June 13, 2023

DR. COHEN: Methyl Isobutyl Ketone. We have a draft amended report. In its initial assessment of MIBK in 2004, the Panel found that it was safe as used as a nail polish remover and as an alcohol denaturant in cosmetics. In March of 2023 we reopened the safety assessment, primarily for consideration of new carcinogenicity and toxicologic data provided by the NTP.

According to the 2023 VCRP, MIBK is reported to be used in two formulations, a manicuring prep and an aftershave lotion. Our discussions of the NTP data suggested that it was not an issue of concern for us here. There was a request from the Women's Voices for the Earth regarding a description of its use as a nail activator, and we thought we could include that in cosmetic uses.

We propose a motion of insufficient data with the following data needs: concentration of use, irritation and sensitization in humans at max use or suitable surrogate, confirmation that it's used only as a denaturant. In the original report, this max use of concentration of 21 percent and there's reported use in an aftershave. So that's our motion, and I just have a question for Don after.

DR. BERGFELD: And is there a second to that, Belsito Team?

DR. BELSITO: No.

DR. BERGFELD: Comment then?

DR. BELSITO: We thought it was safe as used in nail products. A nail activator is a nail product. There's no specific category for nail activation. And we thought it was insufficient for concentration of use in aftershave, or clarification that we thought it probably was a denaturant in the alcohol of the aftershave, but we don't know that, so safe as use in nail products, insufficient for concentration of use in the aftershave.

DR. COHEN: I think we're kind of splitting hairs. I mean, in the report it's 21 percent, so it's not a denaturant at 21 percent.

DR. BELSITO: It was a nail product at 21.

DR. COHEN: Right, all right.

DR. BELSITO: Denaturants were defined as up to four percent.

DR. COHEN: So, your IDA is -- you want concentration of use?

DR. BELSITO: We want to know the concentration of use in the aftershave or the function in the aftershave. If they come back and say it's a denaturant, then we know that according to FDA regulation it can't be more than 4 percent. If they come back and say it's 2 percent, then we presume it's a denaturant but it's less than the 4 that we allowed before.

DR. COHEN: But what if it's 21 percent?

DR. BELSITO: It's insufficient.

DR. COHEN: So, that's why we're asking for irritation and sensitization on that.

DR. BELSITO: We don't even know the concentration yet.

DR. COHEN: Well, it would just -- we're putting it in a basket of insufficient data that we're asking for. I mean, we're not saying something very very different here.

DR. BELSITO: I understand, but what is your insufficiency in nail products?

DR. COHEN: So, if it's in a nail product and it's used as an activator, you could dip your fingers in this material. At 21 percent, we don't have irritation and sensitization.

DR. BELSITO: That's not the consumer use for a nail activator. A nail activator is used to activate the polymerization of acrylates in a nail polish.

DR. COHEN: To dry them. To dry the material. Right, that's kind of my take on it, is it could accelerate the dryer, the dryness.

DR. BELSITO: Activator, right, it's to polymerize. It's to activate the polymerization. So it's in a nail manicuring product.

DR. COHEN: I think that you could probably --

DR. BELSITO: It's not intended for application to the skin.

DR. ROSS: So isn't it danger applying it to the skin by mistake?

DR. BELSITO: You know, again, that would not be the intended use. We went through this when we were looking at the use of acrylates in nail polish. We went through this extensively in 2001, about the risk of sensitization to acrylates. And basically it comes down to the intended use, and the intended use is for application to the nail which is dead. I mean, essentially, right. I mean, it's -- so.

DR. COHEN: Yeah. So, we have it as in use as an aftershave. Right?

DR. BELSITO: And, it's insufficient.

DR. COHEN: And, so in that regard, you have no concern of irritation or sensitization in its use as an aftershave even as a denaturant?

DR. BELSITO: We looked at that before in the old report, did we not?

DR. COHEN: I have to go back and look.

MS. FIUME: There's sensitization data in this report, a guinea pig maximization test and OECD test guidelines 406, a guinea pig maximization test.

DR. BELSITO: Right.

DR. COHEN: In the original report -- I might be missing it -- I see mice, rabbits --

MS. FIUME: PDF Page 26.

DR. BELSITO: Yeah.

DR. COHEN: What is there?

DR. BELSITO: Dermal irritation.

DR. COHEN: We have sensitization?

DR. BELSITO: We have new data on sensitization. Guinea pig maximization test undiluted, challenged with 30 percent, no reaction.

DR. COHEN: Yeah, this all animal data.

DR. ROSS: Yeah, it was guinea pig --

DR. BELSITO: David, you've got to get over the idea that you need human data to clear sensitization.

DR. COHEN: I'm not having a problem with that concept, but we don't have any current concentration of use, and the last one's 21 percent and that's pretty high. And if it's winding up on someone's face at 21 percent --

DR. BELSITO: Right, that's why it's insufficient in an aftershave lotion. We're not saying it's sufficient in an aftershave.

DR. COHEN: Can you reiterate what your motion was?

DR. BELSITO: Safe as used in nail products, insufficient in aftershave lotions for concentration of use and/or function of use.

DR. COHEN: Okay.

DR. BERGFELD: So you're retracting your motion?

DR. COHEN: Okay, I'm reading mine and it's not terribly different other than the irritation and sensitization, which you're trying to persuade me that the current data is enough, from the guinea pigs.

DR. BELSITO: For nails.

DR. COHEN: No, for faces.

DR. BELSITO: No.

DR. BERGFELD: For nails.

DR. ROSS: They're going insufficient on the aftershave.

DR. BELSITO: Yes.

DR. COHEN: Okay.

DR. ROSS: Yeah. Which I think is fine.

DR. BERGFELD: So you're retracting your motion?

DR. COHEN: I'll amend my motion to safe as used in a nail product, insufficient for aftershave. We need concentration of use, and confirmation that it's a denaturant?

DR. BELSITO: And/or confirmation it's a denaturant, yeah.

DR. BERGFELD: Bart, go ahead.

DR. HELDRETH: Procedurally, since we're at the draft report stage, you do have the opportunity to kind of kick the can down the road a little bit. You don't really have to give conclusion here. You just essentially should issue an insufficient data announcement with your data needs on it.

DR. COHEN: This is starting from scratch again.

DR. HELDRETH: An IDA would be the appropriate more than anything.

DR. BELSITO: Okay, insufficient data, concentration of use and/or function in aftershave.

DR. COHEN: You don't want to put in sensitization and irritation for the first stage of the draft, if it's something on this as an aftershave?

DR. BELSITO: We have sensitization.

DR. COHEN: You're telling me that that's guinea pig again?

DR. BELSITO: Yeah.

DR. COHEN: Don, I think you're digging your heels in. If we just ask for --

DR. BELSITO: You want to ask, fine.

DR. COHEN: Yeah.

DR. BELSITO: But, you know, I mean, the point is is that Europe thinks human testing is unethical, and they ban animal testing. That's why we're moving to in vitro testing. So if we have a guinea pig test at a 100 percent that's clear, to be asking for human testing I think, granted we're only a US regulatory body, but we affect companies that manufacture and sell in Europe.

DR. COHEN: Agreed.

DR. SNYDER: And no clinical reports and your clinical experiences are also relevant.

DR. BELSITO: Right.

DR. COHEN: We don't test for this, right.

DR. BELSITO: Right.

DR. COHEN: I mean, so, if you don't test for it, it doesn't exist. And, you know, many times when we've had IDAs and we ask for materials, it's not like we're getting -- they're not running the tests between our meetings, we're getting stuff from like 1987 that they did.

DR. BELSITO: I understand.

DR. COHEN: That may come up --

DR. BELSITO: But, on the other hand, you can't blame companies for waiting until the last minute when we ask for data and then we make a decision that we really didn't need that data.

DR. COHEN: You guy felt you didn't need it. We thought we might find it helpful. So, let's meet at just adding that IDA on. And then next round we could have a greater discussion because, Don, it goes to our modernization of our analyses, which may not have animal and human data in the future.

DR. BERGFELD: Are you agreeing? Bart?

DR. HELDRETH: I just wanted to make a comment on the ethics with the HRIPT. I presented at a conference a few months ago, and received some feedback from some industry members.

And I understand the European viewpoint that if we're doing an HRIPT, there's a potential to permanently make one of the test subject allergic to that material where they weren't before. But the response I received was that nobody in the industry uses the HRIPT as a range-finding study, they use it as a confirmatory test. So if they already have guinea pig maximization test, they have a direct peptide reactivity assay, LLNA, everything, and then they do the HRIPT at a much conserved dose. So there's really no ethics there, at least from the industry's standpoint.

DR. BELSITO: All right. And, in fact, the name, HRIPT, by RIFM, has been change to, CNIH, Confirmation of No Induction in Humans. And you're right about the way it's used, but the Europeans still think it's unethical, pointblank.

DR. COHEN: Did RIFM look at this, Don?

DR. BELSITO: Did RIFM look at it? No.

DR. COHEN: Because I saw some kind of a comment that its reported use as a fragrance.

DR. BELSITO: No, we haven't looked at it.

DR. COHEN: Okay. All right.

DR. BERGFELD: All right. Will you summarize where we are right now?

DR. BELSITO: It's insufficient, and David wants sensitization and irritation data.

DR. COHEN: Did you get all that what we had?

MS. TUCKER: Yes.

DR. BERGFELD: All right. No other comments on this, I'll call the question. All those in favor of an insufficient report going out. Okay. Unanimous.

DECEMBER 2023 PANEL MEETING - SECOND REVIEW/DRAFT TENTATIVE AMENDED REPORT

Belsito Team– December 4, 2023

DR. RETTIE: Yep. MIBK. I think.

DR. BELSITO: Okay. Yeah. Let's see if I can save this. Okay. Okay. So here we're dealing with the fact that suddenly this has been reported to be used in a nail polish remover and an aftershave lotion whereas before we were told that it was used in other nail care products and as an alcohol denaturant. And then we don't have concentrations of use for either of these two uses that we're being told about. But as I thought about this, isn't a nail polish remover another nail preparation? And if its use in the aftershave lotion is as an alcohol denaturant, then we've already dealt with it.

And if it's not used as an alcohol denaturant in an aftershave lotion then it's being used in a misbranded way or whatever the FDA would say about it. So, do we really need to reopen this document?

DR. HELDRETH: Well, it's already open.

DR. BELSITO: Okay.

DR. HELDRETH: So, we're sitting at the draft tentative amended report stage.

DR. BELSITO: So, could we go ahead with our same conclusion and then in the discussion just say if it's used in the aftershave lotion as not as an alcohol denaturant it would be misbranded or whatever the FDA word is when it's not an approved use?

DR. HELDRETH: I mean, I would leave stating that to the FDA, but we could say that if it's used in such a way where it's not as an alcohol denaturant, the data are insufficient to determine the safety of that use, we would need the concentration of use and so forth.

DR. BELSITO: Okay. And what about the nail polish remover?

DR. HELDRETH: I mean, that's up to you. I mean, we had the two data needs, and nothing came in. No concentration of use and I guess that was just asking --

DR. BELSITO: Wouldn't the concentration of use be determined by the prior? I mean, we've done this for other ingredients that we don't have current concentration of use. Wouldn't it be guided by the last concentration of use?

DR. HELDRETH: Yeah, we could certainly do that but then there's also confirmatory sensitization at max use.

DR. BELSITO: Yes, yes. Yes. Yes. Yes. Yes, I'll be there. I'm sure 12:00 is fine. Okay. Very good. Yeah, bye-bye. Sorry about that. I missed it.

DR. HELDRETH: No worries. So, for saying that it's the same as a nail polish and we're accepting the 21 percent as the max use concentration, are we still comfortable with the fact that we did not get confirmatory sensitization studies at max use?

DR. BELSITO: So, I mean, we weren't concerned about the sensitization at max use before because it was -- we were considering it as a nail polish.

DR. HELDRETH: Okay.

DR. BELSITO: Right? Now, do we think that women are messier with nail polish removers and more likely to get it on skin, I suppose that's possible -- or men too. Sorry, that was very -- a lot of guys in New York are using nail polish now, believe me.

DR. HELDRETH: Sure.

DR. BELSITO: That individuals who use nail polish are messier with their removers and would get more on their skin?

DR. HELDRETH: Well, I mean, I know when you go to some nail salons, and they just dip the whole hand in a bowl of remover.

DR. BELSITO: Oh, really? Okay.

DR. HELDRETH: I don't know if we can say if that's intended use or not but anecdotally, I believe it happens all the time.

DR. BELSITO: So, what do we have for sensitization here in terms of data? Sensitization. We have guinea pig max, so the number of animals is good. Intradermal induction with five percent. Cutaneous induction was undiluted, and the challenge was with 30 percent. I think we can clear 24 percent based upon the guinea pig data.

DR. HELDRETH: I agree.

DR. BELSITO: So, I mean I think we can go ahead and continue to say that it's safe as used in nail products and as an alcohol denaturant which is defined as up to four percent, I think? Yeah. Four percent is defined as a denaturant and then in the discussion say that if it's used in the aftershave lotion as not as an alcohol denaturant would be insufficient for concentration of use, really, at this point. Right?

DR. HELDRETH: Yeah, absolutely. I mean, even if you just are making the conclusion specific to only as a denaturant, I think you've met what's expected. But, yeah, I mean, there's no reason not to add a little extra in the discussion as well.

DR. BELSITO: Okay, so guys, are you happy with that? Safe as used in nail care products and as an alcohol denaturant?

DR. SNYDER: I agree.

DR. BELSITO: And then in the Discussion we'll add about the aftershave lotion. Anything else need to go in the Discussion?

DR. RETTIE: Do we need to refer to the fact it was banned in Europe in 2022?

DR. BELSITO: Probably banned in Europe in 2022 because there was no data presented.

DR. RETTIE: I got a note that said it was banned on the back of concerns about carcinogenicity, mutagenicity, and repro tox.

DR. BELSITO: Okay. And then what data do we have?

DR. RETTIE: Well, there was a lot of data on kidney injury specific to rats, and I think that has all been gone through before. Different mechanism in rats compared to humans.

DR. BELSITO: Yeah. Alpha (inaudible). So, I mean, all these, the mode of action is non-genotoxic and is not pertinent to both the liver and the kidney are non-genotoxic and not pertinent to humans. That's very well proven.

DR. SNYDER: Yeah, not relevant to humans. We have that well covered.

DR. BELSITO: Yeah. Yeah. I mean, I suspect that it was not approved in Europe because they just didn't have data on concentration and you know, I mean, we don't either. If we were looking at this for the first time and we had no concentration guidelines we'd do the same thing, but this is an old report where there are concentrations reported and we're just going ahead and saying if those are the concentrations, we're fine.

DR. RETTIE: Okay.

DR. BELSITO: Okay.

Cohen Team– December 4, 2023

DR. COHEN: MIBK. So, this is a draft tentative amended report on MIBK and that was originally published in 2004 with a conclusion that based on the animal and clinical data included in the report MIBK is safe as used in nail polish removers as an alcohol denaturant. In March 2023, we reopened the safety assessment. This was in light of some NTP data which was in progress at the original review. After reviewing the draft amended report in June, we issued an IDA with the following needs: concentration of use and function in aftershave formulations and confirmatory sensitization studies at max use concentration.

Of note the 2023 VCRP data showed MIBK reported in two formulations, one, manicuring preparation and one in an aftershave lotion. Since our IDA no new data was received or found. Comments or questions on this remaining insufficient?

DR. BERGFELD: Well, you could confirm the nail and say the insufficient is for the other products.

DR. ROSS: I agree, Wilma. Yeah, you could do the nail. I think we went that way last time; I think.

DR. BERGFELD: Yeah. Yeah.

DR. ROSS: And then it's insufficient for the aftershave because no concentrations, no testing and we don't know what con- -- yeah. It's okay as a denaturant up to four percent but we have no idea what the concentration is. So, yeah, I think it's insufficient for the aftershave.

DR. COHEN: What? So, wait. But when have we split the decisions based on use like that? I mean, in final product. I mean, we split the decision on other things but am I -- maybe I'm --

DR. BERGFELD: You don't have to split the decision you just say conclusion is safe in nail products but in your discussion talk about there's no evidence to support the aftershave and possibly the manicuring other products.

DR. ROSS: I think last time the discussion of the nail focused around further guinea pig maximization was okay without a human HRIPT. And I think we came down that it was okay. I think that was the conclusion and that we went out asking for data on the aftershave which we didn't get so it's still insufficient on the aftershave.

DR. BERGFELD: Was it only the aftershave?

DR. ROSS: Yeah.

DR. BERGFELD: I don't remember that. Okay.

MS. TUCKER: It was only the aftershave.

DR. COHEN: Say that again.

MS. TUCKER: I'm sorry, this is Regina. It was only the aftershave.

DR. ROSS: Yeah. Thanks, Regina.

MS. TUCKER: You're welcome.

DR. COHEN: So, what are we clearing? We're clearing this.

DR. ROSS: We're clearing --

DR. BERGFELD: We're confirming.

DR. COHEN: In nail polish removers as a nail polish remover an alcohol denaturant?

DR. ROSS: I think we already did that. I think we did that last time and asked for more data on the aftershave, and we didn't get the aftershave so we're consistent with what we said last time that it's okay in further nail use and the aftershave is still insufficient.

DR. COHEN: But that can't be the conclusion.

DR. BERGFELD: No, no. It's a discussion. The conclusion doesn't change, I don't think, unless we put the manicuring portion in it.

DR. COHEN: Well, it was originally safe as used in nail polish removers and as an alcohol denaturant. If it's me, I'm getting wrapped around the axel please let me know, cut the hair free, but how are we concluding this?

MS. FIUME: David, I might be able to help. So, for an example, formaldehyde, the conclusion is very long and is talking about the methylene glycol versus formaldehyde and the amounts of formalin but then it specifically says additionally formaldehyde and methylene glycol are safe in the present practices of use in concentration in nail hardening products. However, formaldehyde and methylene glycol are unsafe in the present practices of use in concentration in hair smoothing products. So, there have been conclusions where it has been --

DR. BERGFELD: Mixed.

MS. FIUME: -- split -- yeah, mixed based on function or based on what products it's used in.

DR. COHEN: So, I guess that because there's a lot of knowing, we understand a lot of those uses and we know they're in Brazilian hair straightening products but this one, like we have one, perhaps, rogue use and maybe it's not even -- maybe it's just even mischaracterized. Like it may have just been a data error, right? We don't know.

MS. FIUME: But it hasn't been corrected and is data in the report so it would be appropriate if the panel found the data insufficient to be able to conclude on the safe use of it to state that use in the aftershave lotion -- the data are insufficient to for the use in the aftershave lotion -- unless your point is looking at it as a larger, more expanded view of the type of use. But if you want to there is precedence for stating that it's insufficient for the specific use reported.

DR. COHEN: Yeah. No, no. I certainly get the formaldehyde thing. It's a very huge issue and MIBK has got two uses, and it doesn't rise to that occasion, I don't think. So, perhaps maybe it's safe as used in nail polish removers.

DR. ROSS: Well, I mean --

DR. COHEN: Or in nail polish -- in nail products.

DR. BERGFELD: Nail products.

DR. COHEN: Listen, I just wanted to make sure there's clarity tomorrow when we come together on this and we issued an IDA, right? We issued an IDA. The IDA was not fulfilled and then we're just going out with the safe as used, like, that doesn't make any sense to me, right? So, we either have to split the conclusion because we have insufficient data and, the question is how do we do that?

DR. TILTON: So, I guess, I thought we had already agreed previously with the motion as safe as used in nail products and as a denaturant in cosmetic products up to four percent max concentration and that we had requested additional information about the function and use in aftershaves.

DR. ROSS: Yeah, Susan, it's my sense of it also. I mean, we already concluded it was okay in nail polish removers and then the only issue was this aftershave issue. So, I didn't have a problem again with insufficient on the aftershave. I think to your safe as used in nail polish removers.

DR. BERGFELD: Nail products.

DR. COHEN: In nail products, right?

DR. BERGFELD: And as a denaturant, yeah.

DR. ROSS: As a denaturant, yeah.

DR. COHEN: Okay. So, as the denaturant we're okay with, because that's what it may be used for in the aftershave.

DR. ROSS: It may be, but we just don't know.

DR. COHEN: Right. Okay. All right, I'm sorry for the hangup on that. Any other comments or questions on it? Okay.

Full Panel- December 5, 2023

DR. BELSITO: In 2004 we concluded that based on animal and clinical data in the report MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products. In March 2023 we reopened the safety assessment of the ingredient and considered new carcinogenicity and toxicology data provided by NTP. The study was actually in progress when we issued our initial report, and we said we would look at it when it became available.

After reviewing the Draft Amended Report in June 2023 we issued an IDA because there were reports of this being used in a nail polish remover, but more importantly reports of it being used in an aftershave lotion. We really got no information from the IDA, but in rethinking this we do have pretty good sensitization data. And we felt that the difference between a nail polish remover and a nail polish was probably diminutive. And thought that perhaps the use in an aftershave was an alcohol denaturant, but if it wasn't then it wouldn't be an approved use. So we felt we could stay with our original conclusion, somewhat modified and say safe as used in nail care products and as an alcohol denaturant.

DR. BERGFELD: And that's a motion?

DR. BELSITO: That's a motion.

DR. BERGFELD: And, David, is that a second, or not?

DR. COHEN: It's a second. We agree with the Belsito's Team assessment.

DR. BERGFELD: All right, any comments?

DR. SNYDER: Don, did you want to put an upper limit on that denaturant up to four percent?

DR. BELSITO: It's defined as four percent.

DR. SNYDER: Okay.

DR. ROSS: I was going to echo Paul's comment. And I know it's defined up to four percent, but do we need to do it again in the conclusion, that's the only thing.

DR. BERGFELD: Can do it in the Discussion.

DR. ROSS: Okay.

DR. BERGFELD: Any other comment? I'm going to call the question then. All opposed? Abstaining? It's approved. Thank you very much.

SEVENTY-THIRD MEETING OF THE EXPERT PANEL

December 20-21, 1999

Dr. Schroeter stated that his Team had discussed the possibility of limiting the concentration of this ingredient in cosmetics to 50 ppm, and, if agreed upon, the rationale for this limitation could be incorporated into the report discussion. He also indicated that Andrew Jaques, with CMA, gave a presentation on MIBK at yesterday's Team meetings. A large amount of data was presented in summary form, and Mr. Jaques stated that these data will be submitted, in detail, to CIR for incorporation into the Draft Report. Some of the comments that were made by Mr. Jaques during his presentation are included in the section on Team Meeting Minutes at the end of this document.

Dr. Schroeter recalled from the current Draft Report that a two-year NTP bioassay on MIBK will be initiated in the year 2000. He said that his Team would like to know the justification for this study, i.e., exactly why the decision to initiate this study was made.

Dr. Belsito asked Dr. Schroeter to repeat his earlier comments on limiting the concentration of MIBK in cosmetics to 50 ppm.

Dr. Schroeter noted that the inhalation toxicity data on MIBK in the Draft Report were considered adequate by his Team and that this position should be documented in the report discussion.

Dr. Andersen stated that the proposed 50 ppm limitation on MIBK is consistent with the threshold limit value for inhalation exposure.

Dr. Bergfeld said that the expert from industry who addressed the Panel on MIBK yesterday presented a lot of information, but references were not provided. She noted that the information presented represented either the presenter's interpretation of data or that of a group of toxicologists. Dr. Bergfeld emphasized that anyone who is going to make a presentation to the Panel should provide the references for the information presented.

The Panel issued the following informal data request:

1) Concentration of use
2) Skin sensitization at concentration of use
3) UV absorption spectrum, if there is significant absorption in the UVA or UVB region, then a phototoxicity study may be needed
4) An explanation for the NTP decision to conduct a 2-year carcinogenicity study on this non-genotoxic chemical

Dr. Carlton wanted to know if the study on MIBK and alpha-2u-globulin nephropathy that was mentioned in the presentation by Mr. Jaques will be provided.

Dr. Belsito said that Mr. Jaques agreed to provide current impurities data and the reference indicating that the nephropathy in male rats was related to alpha-2u-globulin formation, which is unique to that species.

Dr. Andersen said that, according to his notes, Mr. Jacques will provide details on the large amount of data that he presented in summary form at yesterday's Team meetings.

Dr. Bergfeld noted that a special request that presenters provide the documentation for all studies mentioned in their presentations is being made.

SEVENTY-FIFTH MEETING OF THE EXPERT PANEL

May 18-19, 2000

Dr. Schroeter recalled that the following Informal Data Request was issued at the December 20-21,1999 Panel meeting:

1) Concentration of use
2) Skin sensitization at concentration of use
3) UV absorption spectrum; if there is significant absorption in the UVA or UVB region, then a phototoxicity study may be needed
4) An explanation for the NTP decision to conduct a 2-year carcinogenicity study on this non-genotoxic chemical

Dr. Schroeter indicated that the data needed in order for the Panel to conclude that MIBK is safe as used have not been received. He added that in reviewing the safety of MIBK, his Team focused on the limitation of its cosmetic use as a nail polish remover. With this in mind, it was determined that the Panel could conclude that MIBK is safe as used in products that are applied to the

nail, thereby eliminating the need for data to establish the safety of MIBK in products applied to the skin. Dr. Schroeter's Team concluded that MIBK is safe as used as a nail polish remover, and agreed that a report discussion limiting the application of products containing MIBK to the nail (for the purpose of nail polish removal) needs to be developed. It was also agreed that the report discussion should contain a caution statement regarding accidental inhalation exposure as well as a no-effect-concentration of MIBK, a neurotoxic impurity of MIBK.

Dr. Bergfeld noted that the Panel is aware of an NTP carcinogenicity study on MIBK that is underway, and that the results will be reviewed by the Panel upon completion of the study, perhaps four years from now.

Ms. Fise asked the Panel to adopt the policy of reviewing NTP study results after they have been made available. This relates to ingredients in the CIR review process as well as those for which the review process has been completed.

Dr. Bergfeld concurred with the recommendation made by Ms. Fise.

Dr. Belsito said that MIBK is also used as a denaturant and that his Team determined that the Panel could arrive at a conclusion on the safety of MIBK relative to this use. He noted that MIBK is used as a denaturant at concentrations up to 4% in ethanol.

Dr. McEwen stated that MIBK is used as a denaturant for alcohol, subject to the regulations of the Bureau of Alcohol, Tobacco, and Firearms.

Dr. Bergfeld recommended that the report discussion contain a statement regarding the use of MIBK as a denaturant, based on comments made by Drs. Belsito and McEwen.

Regarding the statement made by Mr. Jacques, with the Chemical Manufacturers Association, indicating that neuropathy in rodents can result from walking around in wire cages, Dr. Belsito requested that, in the absence of any documentation of this finding, that this statement be deleted from the report text.

The Panel voted unanimously in favor of issuing a Tentative Report with the following conclusion: Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that MIBK is safe as used in nail polish removers and as a denaturant in cosmetic products.

SEVENTY-SEVENTH MEETING OF THE EXPERT PANEL

December 4-5, 2000

Dr. Schroeter noted that MIBK, a very irritating chemical, is used in a nail correction pen (nail polish remover) and that a Tentative Report with the following conclusion was issued at the May 18-19, 2000 Panel meeting: Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that MIBK is safe as used in nail polish removers and as a denaturant in cosmetic products.

Dr. Schroeter also indicated that his Team agreed that one of the statements in the report discussion should be revised to indicate that MIBK could be used safely as a solvent in nail polish removers in a controlled application system. The terminology, controlled application system, is not included in the current report draft.

Concerning the ongoing NTP carcinogenicity study on MIBK that is mentioned in the report discussion, Ms. Fise recommended a subsequent review of the CIR report on MIBK by the Panel as soon as the NTP study results are available, perhaps, five years from now.

Dr. McEwen recommended that the report conclusion (stated above) be modified to indicate that MIBK is safe as used as an alcohol denaturant in cosmetics.

Dr. Belsito requested that the statement in the report discussion relating to the importance of avoiding inhalation exposure to MIBK be modified to indicate that this concern is also based on evidence of hepatic injury in animal studies.

The Panel agreed with the proposed revisions for the report discussion and conclusion and voted unanimously in favor of issuing a Final Report on MIBK with the following conclusion: Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products.

Amended Safety Assessment of MIBK as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: March 4, 2024
Panel Meeting Date: March 28-29, 2024

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Regina Tucker, M.S., former Scientific Analyst/Writer, and Thushara Diyabalanage Ph.D. Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

α2u	α2u-globulin
α2u-N	α2u-globulin-neuropathy
ACGIH	American Conference of Governmental Industrial Hygienists
AhR	aryl hydrocarbon receptor
BrdU	bromodeoxyuridine
BROD	benzyloxyresorufin-*O*-dealkylase
CAR	constitutive androstane receptor
CIR	Cosmetic Ingredient Review
CNS	central nervous system
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CYP	cytochrome P450
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EROD	ethoxyresorufin-*O*-deethylase
EU	European Union
FDA	Food and Drug Administration
GC/MS	gas chromatography/ mass spectroscopy
ID_{50}	duration of immobility
LD_{50}	median lethal dose
MOA	mode of action
4-MPOL	4-methyl-2- pentanol
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
Panel	Expert Panel for Cosmetic Ingredient Safety
PND	postnatal day
PPAR-α	peroxisome proliferator-activated receptor-α
PROD	pentoxyresorufin-*O*-dealkylase
PXR	pregnane X receptor
RD_{50}	50% decrease in the respiratory rate
RDS	replicative DNA synthesis
STEL	short-term exposure limit
TG	test guideline
TLV	threshold limit value
TWA	time-weighted average
US	United States
VCRP	Voluntary Cosmetic Registration Program
VOR	vestibulo-oculomotor reflex

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of MIBK (aka methyl isobutyl ketone) as used in cosmetic formulations. MIBK is reported to function in cosmetics as a denaturant, fragrance ingredient, and solvent. The Panel considered the available data and concluded that MIBK is safe as used in nail care products and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), MIBK (aka methyl isobutyl ketone) is reported to function in cosmetics as a denaturant, fragrance ingredient, and solvent.[1] This ingredient was first reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) in a safety assessment that was published in 2004.[2] At that time, the Panel issued a final report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products, based on the available animal and clinical data in the report.

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment was issued. In March 2023, the Panel determined that this safety assessment should be re-opened to include new carcinogenicity and toxicological data that were included in a National Toxicology Program (NTP) report; these studies were in progress at the time of the original report.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted in October 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Excerpts from the summary of the previous report on MIBK are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the summary section.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, MIBK (CAS No. 108-10-1) is the aliphatic ketone that conforms to the structure in Figure 1.[1] *MIBK has been described as a branched chain hydrocarbon that is photochemically reactive.*[2]

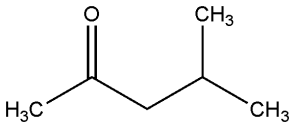

Figure 1. MIBK

Chemical Properties

MIBK occurs as a colorless liquid with a faint, ketonic, camphor odor.[2] *It has a molecular weight of 100.16 Da. Its solubility in water is 17 g/l (20°C); solubility has also been described as 2.04% by weight (28°C).*

Method of Manufacture

MIBK is manufactured by acetone condensation, followed by catalytic hydrogenation.[2] *Acetone is dimerized to diacetone alcohol at 0 to 20°C. Diacetone alcohol is then dehydrated at 100 to 120°C to 4-methyl-3-penten-2-one (aka mesityl oxide) in the presence of a weak acid. Finally, mesityl oxide is hydrogenated over nickel or copper at temperatures from 120 to 165°C.*

Impurities

MIBK is 99% pure (by mass) and may contain the following impurities < 0.3% dimethyl heptane, < 0.1% water, < 0.06% methyl isobutyl carbinol, < 0.03% mesityl oxide, < 0.002% acetic acid, and < 0.0002% non-volatiles.[2] *Another source indicates that MIBK is > 98% pure and contains 0.9% methyl n-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane. A 3% concentration of the contaminant, methyl n-butyl ketone, in commercial MIBK has been noted. However, in 1999, MIBK producers indicated that methyl n-butyl ketone was either no longer found in MIBK or was found in trace amounts (typically 0.01 to 0.06% and always less than 0.1%). Other impurities in MIBK include methyl amyl alcohol, acetone, and 3-methyl-2-butanone.*

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration

Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP data, MIBK is reported to be used in 2 formulations, specifically an "other" manicuring preparation and an aftershave lotion;[3] however, no concentrations of use were reported in response to the survey conducted by the Council in 2022[4] (Table 1). The results of the concentration of use survey conducted in 2003 indicated MIBK was used at up to 21% in other manicuring preparations, specifically, in a nail correction pen; in the use of nail correction pens there may be dermal contact with the skin adjacent to the nail.[2]

Although products containing MIBK may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In the European Union (EU), MIBK is categorized in Annex II, the list of substances prohibited in cosmetic products, due to carcinogenic potential.[5,6]

Non-Cosmetic

MIBK is approved for direct addition to food for human consumption as a component of synthetic flavoring substances and adjuvants (21CFR172.515). It is also an approved indirect food additive when used as a component of adhesives that are present in articles intended for use in packaging, transporting, or holding food (21CFR175.105) and as a solvent of polysulfide polymer-polyepoxy resins that form the food-contact surface of articles intended for packaging, transporting, or holding dry food (21CFR177.1650). MIBK has been approved as a denaturant in denatured alcohol and rum, with specifications for its acidity, color, distillation range, odor, and specific gravity (27CFR21.117). (It should be noted that the original safety assessment, the CFR citation code for this specifications was 27 CFR 21.161; the update was effective January 19, 2001.[7]) According to specifications established by the Alcohol and Tobacco Tax and Trade Bureau, the maximum concentration of MIBK that is listed for use as a denaturant of alcohol is 4.0%. MIBK is also listed in the National Formulary as an alcohol denaturant that is used as an excipient for drugs.

MIBK is used primarily in industrial coating solvents, lubricant oil dewaxing, and in rare metal refining.[2] It is also used in public health environmental studies for determining the presence of heavy metals in air and in biological materials.

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

In vitro partition coefficients of 70 to 90 between blood and air have been reported for MIBK.[2] The following partition coefficients were also reported: 90 (MIBK into blood), 79 (MIBK into water), and 926 (MIBK into oil).

Animal

Dermal

The percutaneous absorption of MIBK (1 ml) was determined using 8 outbred female guinea pigs.[2] A maximum percutaneous uptake rate of 1.1 µmol/min/cm^2 was observed at 10 to 45 min after the initiation of exposure.

Absorption, Distribution, Metabolism, and Excretion

Using a mass-spectrometric method, the presence of MIBK in human maternal blood samples collected immediately after delivery was confirmed.[2] Findings indicated that MIBK has the potential to enter the umbilical cord and cross the placenta.

Animal

Oral

MIBK was administered by gavage to Sprague Dawley rats.[2] The metabolite 4-methyl-2-pentanol (4-MPOL) was not detected in the plasma, liver, or lung. The authors concluded that metabolite concentrations were influenced by the route of MIBK administration.

Plasma levels of MIBK were determined up to 12 h after a single oral dose of MIBK to male rats.[8] Twenty-six male Sprague-Dawley rats were orally administered a single dose of 5 mmol/kg in corn oil, by gavage, according to Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 417. Two or three blood samples (1 ml) were taken by orbital bleeding from each rat at each of the following times after dosing: 0.125, 0.25, 0.5, 0.75, 1, 1.5, 3, 4.5, 6, 9 and 12 h.

MIBK in plasma was determined using gas chromatography/mass spectroscopy (GC/MS). MIBK was rapidly absorbed into the systemic circulation following oral exposure, with a mean maximum plasma concentration of 0.644 mmol/l occurring at 0.25 h.

Inhalation

MIBK metabolite 4-MPOL increased in a dose-related manner in plasma following inhalation by Sprague-Dawley rats.[2] Following inhalation exposure, 4-MPOL was detected in the plasma, liver, and lung.

Parenteral

Male guinea pigs were administered a single intraperitoneal dose of 450 mg/kg MIBK in corn oil.[2] Blood samples were collected at 1, 2, 4, 6, 8, 12, and 16 h post-dosing. The serum half-life and total clearance times for parent MIBK were 66 min and 6 h, respectively.

The metabolic fate of MIBK using groups of 8 male Charles River CD-1 mice was assessed. The animals received a single intraperitoneal injection of 5 mmol/kg MIBK dissolved in corn oil, and the injection volume was 10 ml/kg. The principal metabolites were 4-MPOL (reduction product) and 4-hydroxy-4 methyl-2- pentanone. The concentration of the reduction product in the brain was twice that seen in the blood at 15- and 30-min time intervals.

Human

Inhalation

Eight male volunteers were exposed to MIBK in a 12 m^3 exposure chamber (concentrations of 2.4 ppm [10 mg/m^3], 24.4 ppm [100 mg/m^3], and 48.8 ppm [200 mg/m^3]) for 2 h during light physical exercise.[2] The relative pulmonary uptake of MIBK was ~60%, and total pulmonary uptake increased linearly with increasing exposure concentrations. Average values for uptake were 0.2 mmol at 10 mg/m^3, 1.7 mmol at 100 mg/m^3, and 3.2 mmol at 200 mg/m^3. At the end of exposure, blood concentrations of MIBK increased linearly with increasing uptake. The blood clearance was 1.6 l/h/kg at all exposure concentrations. The concentration of MIBK in the urine was higher than that noted in arterial blood both at 0.5 and 3 h after exposure. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 h post-exposure. When human volunteers were exposed to 100 ppm (410 mg/m^3) of MIBK for 4 h in an environmental chamber, blood and breath samples collected at 90 min post-exposure indicated that most of the absorbed MIBK had been eliminated from the body.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

In an acute dermal toxicity study, undiluted MIBK was applied to the skin of 2 rabbits for 10 h either by flooding the test site or via a cotton pad saturated with the test substance.[2] Signs of systemic effects were not noted, and no treatment-related pathologic changes were observed at microscopic examination of internal organs.

The acute dermal toxicity of MIBK was assessed in Crl: CD BR rats in accordance with OECD TG 402.[9] A semi-occlusive patch with 2000 mg/kg bw undiluted MIBK was applied to 5 male and 5 female rats for 24 h. Dermal reactions were recorded twice daily on days 2, 3, and 4, and once daily from the 5th to 14th day. Rats were weighed before dosing and on days 1, 8, and 15. At study termination, necropsy was performed, organ weights were recorded, and tissues were examined microscopically. No animals died during the test or observation period and no clinical signs of toxicity were noted. Additionally, there were no irritation reactions or other dermal changes at the sites of application of the test article. Body weight gains were not affected, and there were no macroscopic changes observed at necropsy. The acute median lethal dose (LD_{50}) was determined to be greater than the test dose of 2000 mg/kg bw.

In another study performed in accordance with OECD TG 402, rabbits were administered 20 ml/kg of MIBK dermally for 4 h.[9] An LD_{50} of > 20 ml/kg bw was reported. (No other details, including number of animals not stated or whether the test site was occluded, were available.)

Oral

In mice, oral LD_{50}s of 1.5 ml/kg (10 - 40% emulsion in a 1% aqueous emulsion of a sodium sulfate derivative of 3,9-diethyl tridecanol-6) and 1900 mg/kg were reported for MIBK.[2] In another study, the average lethal dose for MIBK in mice dosed orally (stomach tube) was 2805 mg/kg. In rats, acute oral LD_{50} values of 2080 mg/kg, 4600 mg/kg, and 5.7 ml/kg have been reported. In a study in which 6 rats were given a single dose of 1 ml/kg MIBK, all rats died instantly; in most of the animals, 25% of the lung tissue was hemorrhagic. The researchers stated that MIBK may have been aspirated into the lungs when swallowed. In a similar study, 0.2 ml MIBK was placed in the oral cavity of 5 male rats, and the animals were held with the nostrils closed to promote entry of the test material into the trachea. Some of the animals (number not stated) died within 24 h; all deaths were due to respiratory arrest, cardiac failure, or both, rather than pulmonary edema. It was concluded that MIBK presents a potential aspiration hazard. In guinea pigs, the acute oral LD_{50} was between 1.6 - 3.2 g/ kg.

Inhalation

During 5 min of exposure to MIBK, a concentration-dependent decrease in respiratory rate was noted in male Swiss OF_1 mice; a 50% decrease in the respiratory rate (RD_{50}) was noted after exposure to MIBK at a concentration of 3195 ppm.[2] Mice

were exposed to a single exposure of MIBK (saturated air-vapor mixture) at concentrations ranging from 43 - 100 mg/l of air (20°C) for 0.25 - 22.6 h. Within 10 h post-exposure, 18 of 33 animals exposed to 82 mg/l for 0.5 h, 21 of 22 animals exposed to 86 mg/l for 1 h, and 5 of 10 animals exposed to 82 mg/l for 1.25 h died. In a study in which mice were exposed to MIBK (15 mg/l of air) for 2 h, narcosis was induced in all animals. An LC_{50} of 74.2 mg/l was reported in CF-1 male mice exposed to various concentrations of MIBK (1.0% v/v [41 mg/l] to 3.0% v/v [123 mg/l]) in a 10-l glass chamber. In another mouse study, exposure to 19,500 ppm MIBK induced anesthesia within 30 min, with recovery noted 5 min after exposure was discontinued; however, at concentrations >20,000 ppm, anesthesia followed by death occurred in most of the mice. In a 4-h inhalation study in rats (n = 6/ group), no animals exposed to 2000 ppm, but all animals exposed to 4000 ppm, died. In another 4-h inhalation study in rats, the threshold concentration for inhalation intoxication was 0.2 mg/l. Rats (number not stated) exposed to 21,000 ppm MIBK for 55 min died, and rats exposed to 4000 ppm MIBK for 6 h experienced loss of coordination and prostration. In guinea pigs, the acute inhalation toxicity of MIBK was evaluated by exposing groups of 10 animals to 0.1, 0.3, 1.0, 1.68, or 2.8 volume % (saturation) MIBK in an inhalation chamber. Death occurred within 4 h at a concentration of 1.0 volume %, and at progressively shorter periods at higher concentrations. In a study in which female guinea pigs were exposed to MIBK at concentrations of 1000 ppm (4100 mg/ m^3), 16,800 ppm (69,000 mg/m^3), or 28,000 ppm (115,000 mg/m^3) for 24 h, a decrease in the respiratory rate (narcotic effect during first 6 h) and minimal ocular or nasal irritation were noted during exposure to 1000 ppm MIBK. Ocular and nasal irritation, salivation, lacrimation, ataxia, progressive narcosis, and death were observed at higher concentrations.

Short-Term Toxicity Studies

Dermal

Seven applications of undiluted MIBK (3 ml/kg each) were applied for 5 – 12 h to the shaved skin of 2 rabbits (100 cm^2 area) over a period of 15 - 21 d.[2] (Whether or not the applications were occluded was not specified.) Local skin changes consisted of polymorphonuclear infiltration in the upper dermis. No systemic effects were noted.

Oral

Administration of increasing oral doses of an MIBK emulsion in 2% starch solution resulted in the death of 9 of 10 mice by day 24 of dosing; the first animal deaths were noted on day 8 (total dose of MIBK=3.82 g/kg), and the total average lethal dose was 9.35 g/kg.[2] No evidence of gross pathologic effects was observed in female Wistar rats (3 rats/group) given 0.5 or 1.0% MIBK in drinking water for 7 d.

Inhalation

B6C3F_1 mice and F344 rats (6 males and 6 females/group) were exposed to MIBK at concentrations of 101 ppm (44 mg/m^3), 501 ppm (2050 mg/m^3), or 1996 ppm (8180 mg/m^3) for 6 h/d for 5 d, followed by a 2-wk non-treatment period, and then an additional 4 d of dosing.[2] In high-dose female mice and male and female rats, relative liver weights and absolute and relative kidney weights were increased; a decrease in relative kidney weight was reported for high-dose male mice. Compared to untreated controls, no statistically significant histologic lesions were observed in mice at any of the concentrations tested. Hyaline droplet formation was observed in the kidneys of mid- and high-dose male rats. In a study in which male and female B6C3F_1mice and F344 rats were exposed to 100, 500, or 2000 ppm MIBK for 6 h/d, 5 d/wk, for 2 wk, the only microscopic changes reported were increases in regenerative tubular epithelium and hyaline droplets in the kidneys of male rats exposed to 500 or 2000 ppm MIBK. In another study in which 10 mice were exposed daily to 20,000 ppm MIBK for 15 d (20 min/d); 6 animals died. No signs of nasal irritation were observed during an inhalation study in which albino rats exposed to MIBK at 4.53 mg/l air for 6 h/d, 5 d/wk, for 4 wk. In another short-term test, 4 monkeys, 8 dogs, 40 mice, and 50 rats were exposed continuously (inhalation) to 100 or 200 ppm MIBK over a period of 2 wk. Increased kidney weights and microscopic evidence of toxic nephrosis of the proximal tubules were reported only for rats, and this finding was noted at both concentrations of exposure. Increased liver weight (rats) was also noted after exposure to 200 ppm.

Subchronic Toxicity Studies

Dermal

In a subchronic dermal toxicity study, MIBK (in sunflower oil) was applied to white rats (lower 2/3 of tail) daily at doses of 300 or 600 mg/kg for 4 mo.[2] Skin changes included reduced mitotic activity in hair follicles and increased thickness of horny and granular cell layers of the epidermis. Changes in the spleen included a decrease in the number of reactive centers in follicles and an increase in the number of iron-containing pigments in the area of the red pulp. A reduction in the lipid content of the cortical layer was noted in the adrenal glands.

Oral

Nephrotoxicity and increased liver and kidney weights, but no evidence of hepatic lesions, were observed in male and female Sprague-Dawley rats dosed orally with up to 1000 mg/kg MIBK daily for 13 wk.[2] The 50-mg/kg dose (lowest dose) was considered the no-observed-effect level (NOEL). No significant gross lesions or renal tubule cell hyperplasia were reported in a study involving rats that received 1.3% MIBK in drinking water daily (1.04 g/kg/d) for 120 d. MIBK was administered to 3 groups of Sprague-Dawley rats (30 males, 30 females) at doses of 50, 250, and 1000 mg/kg, respectively, daily for 13 wk. All animals that survived were killed at the end of the dosing period. Ten animals (5 males, 5 females) from each treatment group were subjected to gross and microscopic examination. In the highest dose group (1000 mg/kg), nephrotoxicity and increased liver and kidney weights were observed in males and females. Hepatic lesions were not observed at microscopic examination. These effects were

significantly less pronounced in females and males of the 250 mg/kg dose group and were not observed in the 50 mg/kg dose group.

Inhalation

In an inhalation study, B6C3F$_1$ mice and F344 rats were exposed to 50 ppm (205 mg/m^3), 250 ppm (1025 mg/m^3), or 1000 ppm (4100 mg/m^3) MIBK for 6 h/d, 5 d/wk, for 90 d.[2] No hepatic lesions at gross necropsy or microscopic examination were observed in mice or rats, and urinalysis and serum chemistry values were normal. An increase in the number of hyaline droplets in the proximal tubular cells of the kidney was noted in male rats of the 250 and 1000 ppm groups. In a study in which rats were exposed via inhalation to 86 to 127 mg/MIBK for 4 h/d, 5 d/wk, for 4.5 mo, some functional changes were noted. Groups of rats, dogs, and monkeys were exposed to 410 mg/m^3 MIBK vapor (100 mmol/25m^3) for 90 d in an altitude chamber. No biologically significant changes were reported for clinical chemistry and hematology parameters in dogs or monkeys. Microscopic examination of kidneys of rats revealed hyaline droplet degeneration of the proximal tubules (with occasional foci of tubular necrosis) in all animals exposed to MIBK, including those removed after 15, 22, 28, 71, or 85 d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

MIBK was applied to the skin (lower 2/3 of tail) of an unspecified number of male white rats daily (4 h/d) at doses of 300 or 600 mg/kg for 4 mo.[2] Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa. In an inhalation study, MIBK did not induce any treatment-related increases in embryotoxicity or fetal malformations in pregnant Fischer 344 rats or CD-1 mice (3 groups, 25 females in each group per species) that inhaled MIBK for 6 h/d at concentrations of 0, 300, 1000, or 3000 ppm on gestation days 6 - 15. There was evidence of treatment-related maternal toxicity only at the highest concentration tested.

Inhalation

To investigate the potential impact of MIBK on reproductive performance, a two-generation reproduction study was conducted in Sprague-Dawley rats.[10] Four groups of 30 F_0 males and 30 F_0 females were randomly bred to produce an F_1 generation, and a replicate breeding procedure (avoiding sibling mating) was conducted to produce an F_2 generation. The F_0 and F_1 generations were approximately 7 wk and 4 wk old at their initiation of exposures, respectively. The rats were subjected to whole-body inhalation exposure of MIBK for 6 h/d, 7 d/wk, at concentrations of 0, 500, 1000, or 2000 ppm. F_0 and F_1 males were exposed for 70 d prior to mating and throughout mating until 1 d prior to euthanasia. F_0 and F_1 females were exposed for 70 d prior to mating and throughout mating, gestation, and lactation until 1 d prior to euthanasia. Exposure of the F_0 and F_1 dams was suspended for 5 d following parturition (lactation/postnatal days (PND) 0 - 4), and resumed on PND 5. The offspring of the F_0 and F_1 generations (F_1 and F_2 pups, respectively) were potentially exposed to MIBK both in utero and through nursing via maternal milk during PND 0 - 21. Exposures for all groups of F_1 weanlings were suspended between PND 22 and PND 27 because of the death of one male pup in the 2000 ppm group; this pup had clinical signs of central nervous system (CNS) depression indicative of a sedative effect (e.g., rocking, lurching, or swaying while ambulating). Exposures were reinitiated for all surviving animals on PND 28.

Detailed physical examinations were conducted weekly for parental animals (F_0 and F_1). All animals were observed twice daily for appearance and behavior and were examined for pharmacotoxic signs within 1 h after completion of exposure. Each male pup was examined for balanopreputial separation beginning on PND 35, and each female for vaginal perforation beginning on PND 25. The left testis and epididymis from all F_0 and F_1 males in all dose groups were evaluated for homogenization-resistant spermatid counts and sperm. Microscopic evaluations were performed on diverse tissues such as adrenal glands, prostate, brain, seminal vesicles, cervix, coagulating gland, uterus, ovaries, etc. Quantitative histopathologic evaluation of 10 sections of the inner third of the ovary (including enumeration of primordial follicles) was conducted on 10 F_1 females from the control and high-dose groups. Furthermore, a qualitative assessment was performed to identify the presence or absence of growing follicles, astral follicles, and corpora lutea.

No MIBK-related mortalities of adult rats occurred during the study, and no adverse effects on male and female reproductive function or indicators of sexual maturation were observed. The authors concluded that MIBK, at all exposure levels, did not affect any reproductive parameters or offspring growth and development. During the initial 2-wk of exposure at 2000 ppm, a reduction in body weight gains and a slight decrease in food consumption were observed in both generations. Additionally, in the 2000 ppm group, there was an increase in liver weights associated with centrilobular hypertrophy for both the F_0 and F_1 generations. Male rats exhibited increased kidney weights with hyaline droplets across all exposure concentrations, indicating male rat-specific nephropathy. For reproductive endpoints, the highest concentration tested, 2000 ppm, was considered the no-observed-adverse-effect level (NOAEL). Apart from acute sedative effects, the NOAEL for systemic effects in parental animals (excluding male rat kidney effects) was determined to be 1000 ppm, based on the temporary decrease in body weights and food consumption. Regarding neonatal toxicity, the NOAEL was determined to be 1000 ppm based on acute CNS depressive effects and the one death on PND 22.

GENOTOXICITY STUDIES

MIBK was not genotoxic in numerous assays, including several Ames tests (up to 8000 µg/ml, with and without metabolic activation), an unscheduled DNA synthesis assay in rat hepatocytes (up to 100 µl/ml), a chromosomal damage assay using rat

liver RL$_4$ cells (up to 8000 µl/ml), a mitotic gene conversion assay in Saccharomyces cerevisiae strain JD1 (up to 5 mg/ml, with and without metabolic activation), a mitotic chromosome loss assay in Saccharomyces cerevisiae strain D61.M (up to 7.3 mg/ml), and an in vivo mouse micronucleus test (10 ml/kg; intraperitoneal administration).[2] However, in a mouse lymphoma assay performed using L5178Y/TK$^{+/-}$ mouse lymphoma cells (0.32 - 4.2 µl/ml MIBK, with and without metabolic activation), results were negative with metabolic activation but equivocal without metabolic activation. In cell transformation assays with BALB/3T3 mouse embryo cells (up to 7 µl/ml without and 5 µl/ml with metabolic activation), no transforming activity was observed with metabolic activation, but positive results were reported without metabolic activation for 4.8 µl/ml MIBK.

CARCINOGENICITY STUDIES

Details on the inhalation carcinogenicity studies summarized below can be found in Table 2.

B6C3F$_1$ mice and F344/N rats (50/sex/group) were exposed to MIBK (greater than 99% pure) by inhalation (0, 450, 900, or 1800 ppm; whole-body, 6 h/d, 5 d/wk) for 2 yr.[11] Male and female mice exposed to MIBK had increased liver tumors, and the incidences of eosinophilic foci were significantly increased in female mice exposed to 450 and 1800 ppm MIBK. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in male and female mice exposed to 1800 ppm. Male rats exposed to MIBK had tumors of the kidney, increased rates of hyperplasia of the kidney and adrenal gland, and mononuclear cell leukemia. The incidences of renal tubule hyperplasia were significantly increased in male rats exposed to 450 and 1800 ppm. Chronic nephropathy occurred in all male rats exposed to 1800 ppm and in 70 to 88% of exposed female rats. The incidences and severities of chronic nephropathy and mineralization in the renal papilla increased with increasing exposure concentration. Under the conditions of the 2-yr studies, there was *some evidence of carcinogenic activity* in male and female mice and male rats, and there was *equivocal evidence of carcinogenicity* in female rats. The liver was the primary site of MIBK-related toxicity in mice, and the kidney was the primary site of MIBK-related toxicity in rats (Table 3).

Mode of Action

Details of studies investigating the mode of action (MOA) underlying MIBK-induced tumors that are summarized below can be found in Table 4.

Inhalation

The MOA for the initiation of MIBK-induced liver tumors was investigated using male and female B6C3F$_1$, C57BL/6, and constitutive androstane receptor (CAR)/pregnane X receptor (PXR) knockout mice (16 sex/group).[12] These mice were exposed to either 0 or 1800 ppm MIBK via whole-body inhalation for 6 h/d, 5 d/wk, for a total of 10 d. The study concluded MIBK-induced hepatic effects are consistent with a phenobarbital-like MOA where the initiating events are activation of the CAR and PXR nuclear receptors and resultant hepatocellular proliferation, leading to rodent liver tumors. Overall, the MOA for rat and mouse liver tumor formation by phenobarbital and sodium phenobarbital and other CAR activators is considered qualitatively not plausible for humans.[12,13]Human hepatocytes are refractory to the mitogenic effects of CAR activator l (phenobarbital and sodium phenobarbital) and other CAR activators. These and other compounds do not stimulate replicative DNA synthesis (RDS) in cultured human hepatocytes and in in vivo studies performed in chimeric mice with humanized livers.[13]

Oral

To investigate whether MIBK operates through a non-genotoxic MOA to induce the male rat-specific renal tumor response following chronic exposure, 4 male and 4 female F344 rats were dosed by gavage with 0 or 1000 mg/kg MIBK in corn oil for 10 d.[14] In the positive control group, 4 male rats were dosed with 300 mg/kg D-limonene, a known inducer of α2u-globulin (α2u) nephropathy (α2u-N). The kidneys were removed and analyzed approximately 24 h after the final dose. MIBK caused an increase in protein droplets, accumulation of α2u, and renal cell proliferation in males, but not in females. The histological alterations caused by MIBK in male rat kidneys were similar to those induced by D-limonene, but they were of a milder degree. The investigators concluded that MIBK exerts renal effects through an α2u-N-mediated MOA.

In Vitro Cell Transformation

Two separate in vitro cell transformation studies were performed, one using cultured primary male C57BL/6 mouse hepatocytes and the other using cultured primary human hepatocytes.[9] Both types of hepatocytes were exposed to MIBK at 10 - 300 µM for 96 h. Certain nuclear receptors that link with carcinogenesis have been investigated, including CAR, PXR, aryl hydrocarbon receptor (AhR), and peroxisome proliferator-activated receptor-α (PPAR-α). Specifically, CAR, PXR, AhR, and PPAR-α were assessed by measurement of target genes, associated enzyme activities and cell proliferation. Phenobarbital and epidermal growth factor (EGF) were used as positive controls in the measurements of CAR activation and cell proliferation, respectively.

In the mouse hepatocyte study, cell viability was reduced at 10 µM (78% of controls) and at 300 µM (61% of controls). However, the researchers stated the finding at 10 µM was considered to be spurious in the absence of a concentration-response relationship. mRNA analysis revealed that cytochrome P450 (CYP) 2b10 mRNA expression was induced at all concentrations of MIBK, with a maximum of ~1.5 times at 300 µM. CYP1a2 mRNA expression showed marginal induction (~1.3 times) at 100 and 300 µM, without a clear concentration-response relationship. In contrast, MIBK treatment did not affect the mRNA expression of CYP3a11, CYP1a1, and CYP4a10. Furthermore, cell enzyme activities, including ethoxyresorufin-*O*-deethylase (EROD), pentoxyresorufin-*O*-dealkylase (PROD), benzyloxyresorufin-*O*-dealkylase (BROD), and benzoquinone reductase, were assessed.

PROD activity was increased (148% of controls) by exposure to 10 µM MIBK; however, this finding was considered questionable in the absence of any effects at higher concentrations. On the other hand, exposure to MIBK did not increase BROD, EROD, and benzoquinone reductase activities. Cells were also assessed for RDS immunohistochemically by bromodeoxyuridine (BrdU) incorporation. At any dose tested, MIBK did not induce RDS.

In the human hepatocyte study, cell viability was not reduced at any concentration. mRNA analysis revealed that CYP1A1 mRNA expression showed marginal induction (~1.3 times controls) at 300 µM MIBK in hepatocytes from one donor. CYP2B6 mRNA expression showed marginal induction (~1.6 times controls) at 300 µM MIBK in hepatocytes from one donor. In contrast, MIBK treatment did not affect the mRNA expression of CYP3A4 mRNA and CYP4A11 in hepatocytes from all three donors. Cell enzyme activities were also assessed. PROD, BROD, EROD, and benzoquinone reductase activity was not increased by exposure to MIBK in hepatocytes from any of the three donors. Exposure to MIBK at 300 µM increased RDS slightly (~1.7 times) in hepatocytes from one donor.

OTHER RELEVANT STUDIES

Neurotoxicity

MIBK (1.04 g/kg/d), administered to 5 female Wistar rats at a concentration of 1.3% in drinking water, did not induce any significant neurologic alterations.[2] The maximum motor-fiber conduction velocity in the tail nerve of male rats (number and strain not stated) was unaffected by treatment with MIBK (601 mg/kg, 5 times/wk for 55 wk).

The neurotoxicity of MIBK was evaluated using 3 groups of 12 Sprague-Dawley albino rats. The 3 groups were injected intraperitoneally with MIBK (10% in corn oil) at doses of 10, 30, and 100 mg/kg for 2 wk. At the end of the 2-wk period the doses were doubled, and the new doses of 20, 60, and 200 mg/kg were injected intraperitoneally 5 d/wk for 33 wk. The following non-neural lesions were observed in test animals: chronic respiratory disease, peritonitis, bone marrow hyperplasia, and increased splenic hematopoiesis. It was concluded that MIBK did not induce peripheral neuropathy when injected intraperitoneally at doses up to 200 mg/kg.

The influence of MIBK on the vestibulo-oculomotor reflex (VOR) of female Sprague-Dawley rats (number not stated) was studied. The test substance was administered by continuous intravenous infusion for 60 min. Test concentrations varied between 0.1 and 10%. MIBK had a depressive effect on the VOR.

Four cats were injected subcutaneously with 150 mg/kg bw undiluted MIBK twice daily, 5 times/wk, for up to 8.5 mo. A group of 4 control cats received subcutaneous doses of saline (0.2 ml/kg) 5 d/wk for up to 5 mo. No detectable damage to nerve tissues was observed. Four male Beagle dogs were injected subcutaneously with 300 mg/kg MIBK daily for 11 mo. No evidence of neurotoxicity was noted. In a similar study with 4 dogs, MIBK (>98% pure, with 0.9% methyl n-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane) was administered subcutaneously at a dose of 150 mg/kg twice daily for a year. No evidence of systemic toxicity or neurotoxicity was observed in any of the animals tested.

The neurotoxicity of MIBK in 6 young adult rats was studied. The animals were exposed to 1500 ppm MIBK for up to 5 mo. No signs of neurological dysfunction were noted at the end of the exposure period.

The effect of inhaled MIBK on the lever-pressing behavior of Holtzmann, Sprague-Dawley male rats on a match-to sample discrimination task were evaluated. A 2-min variable-interval schedule of reinforcement was used. The effect of 25 ppm MIBK on the variable response rate of one rat after the third hour of the experimental session was evaluated. The average response rate was 45 per min, which represented a 58% increase over the preexposure control rate of 26.5%. The response rate had not returned to control levels by day 7 post-exposure.

The neurobehavioral effects of MIBK were studied using 80 male Swiss OF1 mice (40 controls, 40 test animals). Four test groups (10 mice/group) were exposed to test concentrations of 662, 757, 807, and 892 ppm for 4 h in a 'behavioral despair' swimming test. A decrease in the duration of immobility in the swimming test was reported after exposure to MIBK; the duration of immobility (ID_{50}) was 803 ppm. The ID_{50} value was defined as the median active concentration that resulted in a 50% decrease in immobility.

The neurotoxicity of MIBK in rats was evaluated in a 13-wk (64 d of exposure) study using male Sprague-Dawley rats. Rats (CRL:CD (SD)BR/VAF Plus strain animals; 20/group) were exposed to MIBK at concentrations of 250, 750, or 1500 ppm for 6 h/d, 5 d/wk, for 13 wk. Untreated animals served as controls. The results of this study indicate that repeated MIBK exposure did not induce changes in schedule controlled operant behavior. An exposure concentration of 1500 ppm MIBK was considered the NOEL for subchronic neurotoxicity.

The effect of inhaled MIBK (25 - 75 ppm) on the behavior of young baboons (number and ages not stated) was determined in a match-to-sample discrimination task. Test animals were exposed to MIBK over a 7-d period, whereas the controls were exposed to clean air. MIBK did not impair a baboon's ability to discriminate or remember stimuli. Similarly, in a delayed match-to-sample discrimination task using 4 baboons (~2 yr old), the animals were exposed to 50 ppm MIBK for 7 d and accuracy of performance was affected minimally.

The neurotoxicity of MIBK using a clonal line of neuroblastoma cells (Neuro 2aE) produced no discernible cytopathological changes in cells exposed to 0.1% MIBK for 10 d. At a concentration of 0.2%, MIBK induced a depression of growth rates; MIBK (0.5%) caused widespread cell death.

Nephropathy

As noted in the 'Carcinogenicity; Mode of Action' section of the report, MIBK was evaluated to assess its ability to induce specific measures of α2u-N in the kidneys of male and female rats compared to D-limonene, a known inducer of α2u-N.[14] In the study in which 4 male and 4 female F344 rats were administered corn oil (control) or MIBK (1000 mg/kg; 5 ml/kg) and another group of 4 male rats were administered D-limonene (300 mg/kg; 5 ml/kg) for 10 consecutive days by gavage, rats were euthanized approximately 24 h following the final dose, and the kidneys and a small section of duodenum were analyzed. Kidneys from the male rats exhibited similar rate of histological changes as seen in the kidneys from the D-limonene-treated male rats, including basophilic proximal convoluted tubule, increased hyaline droplet accumulation, and a minimal number of cell debris-containing pars recta tubules at the junction of the outer stripe of outer medulla and inner stripe of outer medulla. Also noted was a minimal increase in mitotic activity and nuclear variability in the cortex. There were no changes noted in the female rats.

The ability of MIBK to induce measures of α2u-N, including renal cell proliferation, was evaluated in 84 male and 84 female F344 rats following exposure to 0, 450, 900, or 1800 ppm.[15] Rats were exposed 6 h/d for 1 or 4 wk, and the kidneys were excised approximately 18-h post-exposure to evaluate hyaline droplet accumulation, α2u staining of hyaline droplets, renal cell proliferation, and quantitative renal α2u concentration. Hyaline droplet accumulation associated with MIBK was observed in the proximal convoluted tubules of all MIBK-exposed male, but not female, rats. Increasing MIBK concentration showed increasing hyaline droplets in terms of size and pattern disruption. Hyaline droplet accumulation was also prominent in the D-limonene positive control group. Males exposed to 1800 ppm MIBK for 4 wk had solitary tubules at the junction of the outer and inner stripes of the outer medulla containing eosinophilic granular debris, which were consistent with precursors of granular casts. There was an exposure-related increase in concentration of α2u in the male rats at both 1 and 4 wk of exposure. Total protein was not changed in the male rats exposed to MIBK, but an increase was observed following D-limonene administration. Counts of mitotic figures in the cortical proximal tubule cells were 10 times higher in male rats exposed to 1800 ppm MIBK compared to controls. Further in vitro analysis estimated the dissociation constant (to describe MIBK binding to α2u) to be 1.27×10^{-5} M, within range of other chemicals known to bind to α2u and cause nephropathy.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

Immersion of the ear of a rabbit and the tails of mice in pure MIBK for 2 h resulted in pronounced inflammation and necrosis of the tissue.[2] Undiluted MIBK was applied to the skin of 2 rabbits for 10 h; immediate (moderate erythema) and delayed (erythema persisting for 24 h) reactions were observed. In another study in which a single 10-h occlusive patch of MIBK was applied to the shaved skin of rabbits (number of animals not specified), erythema was observed for up to 24 h post-application. MIBK (500 mg) induced moderate irritation of rabbit skin after a contact period of 24 h. In a 24-h occlusive patch test using 12 albino rabbits (6 with intact and 6 with abraded sites), 24 h after dosing with 0.5 ml MIBK, very slight erythema was observed at 3 intact skin sites and all 6 animals with abraded sites had slight or well-defined erythema, with 2 animals having very slight edema. At 72 h post-application, very slight erythema was observed in 2 animals with abraded sites, and no signs of irritation were observed in the remaining animals (intact or abraded sites). The primary irritation score = 0.75. Drying and flaking of the skin surface were observed after 10 ml/d MIBK was applied to the skin of rabbits for 7 d. Seven applications (3 ml/kg each, 5 - 12 h) of undiluted MIBK were applied to a 100 cm^2 area of shaved skin on 2 rabbits over a period of 15 - 21 d; drying of the skin and exfoliation were observed.

In guinea pigs, slight skin irritation was observed after undiluted MIBK (5 and 10 ml) was applied (under occlusive wrap) to depilated skin for 24 h. Application of 2 ml MIBK to the backs of guinea pigs daily for 31 d caused desquamation, but no clinical or histologic evidence of toxic neuropathy. Guinea pigs subjected to brief exposures of MIBK over a period of 3 mo had no noticeable skin changes.

Sensitization

Animal

The skin sensitization potential of MIBK was assessed in female albino guinea pigs according to OECD TG 406; the test group comprised 20 animals and the control group comprised 10 animals.[9] Intradermal induction was carried out with 0.1 ml of 5% MIBK in vehicle (corn oil), and epicutaneous induction was performed with undiluted MIBK. Challenge exposure was conducted with 30% MIBK under occlusive conditions. Skin reactions were evaluated at 24 and 48 h. Test and control animals displayed normal body weight gain throughout the investigation. Local reactions (reddening and swelling) were observed in all treated animals. Some irritation reactions were also observed in the control animals. During the study there were insufficient details available to determine if the whole test area was abraded or only partially abraded. Therefore, results of skin irritation were deemed unreliable. Under the experimental conditions, MIBK produced no sensitizing reaction.

In another study a guinea pig maximization test was performed on 20 test animals (10 control) in accord with OECD TG 406.[9] Intradermal induction was carried out with 0.1 ml of 5% MIBK in vehicle (corn oil), and epicutaneous induction was

performed with semi-occlusive patches with 0.1 ml of 5% MIBK in vehicle (corn oil) applied neat to the skin on filter paper. Challenge was performed under occlusion (up to 48 h) with 30% MIBK in corn oil. No indication of skin sensitization was observed.

OCULAR IRRITATION STUDIES

Animal

The ocular irritation potential of undiluted MIBK was evaluated using 1 rabbit.[2] Reactions, scored according to the Draize scale (0 - 110), were 8, 3, and 1 at 1, 24, and 72 h post-installation, respectively; the test substance induced conjunctivitis, with some edema and corneal injury. Ocular irritation was observed within 10 min after instillation of undiluted MIBK (0.1 ml) into the eye of a rabbit, with inflammation and conjunctival swelling noted within 8 h, and inflammation, swelling, and exudate evident at 24 h. All reactions had cleared by 60 h. In another study, 6 albino rabbits were administered undiluted MIBK (0.1 ml) into the left conjunctival sac; untreated eyes served as controls; MIBK induced slight, transient ocular irritation. One-tenth ml of MIBK was instilled into the conjunctival sac of New Zealand albino rabbits (4 to 6 animals); untreated eyes served as controls. Effects on the cornea, iris, and conjunctiva were scored at 1 – 21 d post-instillation, and it was concluded that MIBK induced mild ocular irritation in rabbits. In another Draize test using 4 to 6 rabbits, undiluted MIBK (0.1 ml) was instilled into the conjunctival sac of one eye of each animal; a Draize score of 5/110 was reported.

A single-exposure ocular irritation study on MIBK was performed using 3 New Zealand White rabbits in accordance with OECD TG 405.[9] Undiluted MIBK (0.1 ml) was instilled into the conjunctival sac. Ocular changes were assessed at 30 min, and at 1, 4, 24, 48, and 72 h. MIBK caused changes of the conjunctivae (slight chemosis, ocular discharge) that resolved within 24 h. One rabbit had a minor disturbance of the corneal epithelium that resolved within 48 h. Under the conditions of the study, MIBK was considered to be slightly irritating to rabbit eyes.

A study was performed in accordance with OECD TG 405 in which 0.1ml of MIBK was instilled into one eye of each of 4 rabbits, and observations were made on days 1, 2, 3, and 7.[9] The overall mean scores were 0.08/4 for cornea opacity, 0/2 for iris lesion, 0.8/3 for redness of conjunctivae, and 0.17/4 for chemosis. MIBK was slightly irritating.

CLINICAL STUDIES

Twelve volunteers of both sexes were exposed to various concentrations of MIBK for 15 min.[2] The sensory response limit was 100 ppm (410 mg/m^3), and the odor was found to be objectionable by most of the subjects at a concentration of 200 ppm (820 mg/m^3). In another study, the threshold for MIBK-induced irritation of the lungs was 0.03 to 0.1 mg/l after 1 min of respiration (number of subjects not stated.)

Symptoms of either nausea or respiratory irritation were reported in workers (number not stated) exposed to 100 ppm MIBK (410 mg/m^3). Tolerance to this level of exposure was acquired during the work week but was lost over the weekend. Complaints were largely eliminated when the level of exposure was reduced to 20 ppm (82 mg/m^3).

Six subjects inhaled MIBK (six, 20-min exposures) through face masks connected to ports on a 125-l aerosol chamber. Test concentrations for the series of 6 exposures ranged from 0.402 to 2.827 mg/l. The incidence of nasal, ocular, or throat irritation experienced by the subjects during one of the exposure sessions (results for exposure series 1 to 6 combined) was: nasal irritation (1 - 4 subjects), ocular irritation (1 - 3 subjects), and throat irritation (1 - 4 subjects). The results for throat irritation are based on the testing of only 4 subjects (test concentration range = 1.363 to 2.827 mg/l).

MIBK vapors have been reported to cause irritation of both the conjunctival and nasal mucosa at concentrations near 200 ppm. Exposure to higher concentrations caused lacrimation (indicative of marked irritation).

Eight male volunteers were exposed to MIBK at concentrations of 2.4 ppm [10 mg/m^3], 24.4 ppm [100 mg/m^3], and 48.8 ppm [200 mg/m^3] for 2 h during light physical exercise on three different occasions. Based on a questionnaire, nose and throat irritation were the most common symptoms. Neither symptom was experienced by more than 3 subjects at any of the 3 exposure concentrations. There were no significant, exposure-related effects on the performance of a simple reaction time task or a test of mental arithmetic.

The neurobehavioral effects of MIBK resulting from short-term inhalation exposure was evaluated in 10 male and 13 female subjects (18- to 32-yr-old). The 3-day test session began with a 2-h practice session on day 1, followed by 8 h of exposure to 100 ppm MIBK on day 2, and concluded with a 2-h post exposure session on day 3. The results of statistical analyses did not indicate any significant differences between male and female blood and breath concentrations of MIBK. Study results indicated that 4-h exposures to 100 ppm MIBK did not cause any significant neurobehavioral effects. The principal exposure-related effects were limited headache, nausea, throat irritation, and tearing.

The potential narcotic impact of MIBK on CNS function was studied. Heart rate, performance tests, and effects on local irritation, CNS symptoms, and mood were determined in 6 female and 6 male employees. The 12 employees were exposed to 10 and 200 mg/m^3 concentrations of MIBK in a 12-m^3 exposure chamber. The subjects were exposed individually for 2 h, and exposure sessions were separated by a 1-wk interval. The researchers concluded that 2 h of exposure to MIBK caused increased discomfort in the subjects tested, as measured by symptom ratings.

The occurrence of symptoms of irritation and CNS symptoms was evaluated using a questionnaire. Symptoms of local irritation to the eyes and airways were not significantly different when the two exposure concentrations were compared; however, a clear trend toward a significant increase was noted. The occurrence and/or intensity of CNS symptoms increased with exposure.

The effects of MIBK on olfactory function in 4 volunteers were reported. Subjects were exposed to 20 and 40 ppm of MIBK in an 18.1-m^3 chamber for 7 h on each of 3 consecutive days. After a 25-d non exposure period, a second identical exposure was performed. Olfactory adaptation and an MIBK-induced transient, olfactory perception threshold shift were reported at both exposure concentrations. Symptoms of eye, nose, or throat irritation and headache were present in some of the subjects. The authors concluded that individuals exposed professionally or environmentally to certain organic solvents may suffer temporary loss of the sense of smell, which hinders odor detection.

The potential narcotic impact of MIBK on CNS function was evaluated using two groups of 6 subjects exposed to 10 mg/m^3 (control) and 200 mg/m^3 MIBK for 2 h.[2] No consistent exposure-related effect on heart rate was identified, and the results of the simple reaction time performance test indicated no exposure-related differences in performance.

Case Report

In a case report, a 40-yr-old chemical factory worker with contact dermatitis had a negative patch test reaction to undiluted MIBK.[2] Findings in another case report indicated persistent cognitive deficits in a 44-yr-old employee of a poorly ventilated, indoor solvent extraction facility who had been exposed to ambient concentrations of MIBK in excess of 100 ppm (8 h/d) for 6 yr. The level of exposure to MIBK was twice the threshold limit value (TLV), short-term exposure limit of 50 ppm. The deficits noted included slowed information processing and impaired attention. Cognitive dysfunction was also noted in a coworker with the same history of exposure to MIBK.

Occupational Exposure

At the time of the original report, occupational limits from the American Conference of Governmental Industrial Hygienists (ACGIH) recommended a TLV– time-weighted average (TWA) of 50 ppm and a TLV–short-term exposure limit (STEL) of 75 ppm for atmospheric exposure to MIBK.[2] The National Institute of Occupational Safety and Health (NIOSH) proposed a TWA limit of 50 ppm MIBK (205 mg/m^3) in 1978. The Code of Federal Regulations (29CFR 1910.1000) included the Occupational Safety and Health Administration (OSHA) standard of 100 ppm MIBK (410 mg/m^3) established in 1983.

The short-term inhalation toxicity of MIBK in an occupational exposure was reported. Nineteen workers inhaled MIBK at concentrations up to 500 ppm (2050 mg/m^3) for 20 to 30 min/d, and 80 ppm (328 mg/m^3) for the remainder of the workday. Half of the workers had symptoms of weakness, loss of appetite, headache, ocular irritation, stomachache, nausea, vomiting, and sore throat. Insomnia, somnolence, heartburn, and intestinal pain were also reported by some of the workers (number not specified). Slightly enlarged livers and nonspecific colitis were reported for 4 and 6 workers, respectively. In another study, symptoms of either nausea or respiratory irritation were reported by workers exposed to 100 ppm MIBK. Complaints were reduced substantially when the level of exposure was reduced to 20 ppm. Exposure to 100 ppm MIBK for 4 h did not induce neurobehavioral effects in either of the 23 human subjects tested.

MIBK was detected in the brain, liver, lung, vitreous fluid, kidney, and blood in workers who died after exposure to several volatile organic solvents during spray painting. Workers (number of subjects not stated) exposed to 500 ppm MIBK for 30 min daily experienced weakness, loss of appetite, headache, burning eyes, stomachache, nausea, vomiting, and sore throat. An enlarged liver and colitis were also observed in some of the workers. In another case, workers exposed to 100 ppm MIBK experienced nausea, headache, and respiratory irritation.

The most recent occupational limits from the ACGIH recommend a TLV-TWA of 20 ppm (82 mg/m^3) and a TLV–STEL of 75 ppm (307 mg/m^3) for exposure to MIBK.[16] NIOSH lists a TWA limit of 50 ppm MIBK (205 mg/m^3), and also includes the OSHA standard of 100 ppm MIBK (410 mg/m^3).[17]

A field study on 20 workers exposed to mixed solvents (toluene, ethyl benzene, xylene) containing MIBK and one worker who was exposed to pure MIBK was performed.[18] The workers who were exposed to mixed solvents containing MIBK for 8 h had a TWA concentration of 21.9 ± 15 ppm MIBK. Their urinary concentration of 4-methyl-1-2 pentanol (urinary metabolite of MIBK) at 50 ppm corresponding TLV of MIBK, was 2.61 mg/g creatinine. In the subject exposed to pure MIBK, the TWA of MIBK in the air over 6 h was 42.3 ppm, and the corresponding concentration of 4-methyl-1-2-pentanol was 0.42 mg/g creatinine in urine.

In another study, unmetabolized MIBK in urine was examined to determine its usefulness as a low level marker in determining occupational exposure.[19] Twenty-seven furniture-making workers (19 men and 8 women) and 11 non-exposed controls were studied. In the morning, workers were equipped with a carbon cloth diffusive sampler for lipophilic solvents and a water-based diffusive sampler for hydrophilic solvents. At the end of the shift, workers were then invited to solvent vapor-free areas, the diffusive samplers were removed, and urine samples were taken for analysis. The arithmetic mean of MIBK exposure was 1.8 ppm while the geometric mean of MIBK exposure was 0.7 ppm. The maximum exposure concentration reached was 15.1 ppm. This is below the occupational exposure limit of 50 ppm set by the ACGIH as well as the Deutsch Forschungsgemeinschaft and the Japan Society for Occupational Health.

SUMMARY

MIBK is reported to function in cosmetics as a denaturant, fragrance ingredient and solvent. MIBK was previously reviewed by the Panel in a safety assessment that was published in 2004. At that time, the Panel issued a final report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products. In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment was issued. In March 2023, the Panel determined that this safety assessment should be re-opened due to new carcinogenicity data available from the NTP; these studies were in progress at the time of the original report.

According to 2023 VCRP survey data, MIBK is reported to be used in 2 formulations, (other manicuring preparations and aftershave lotions). In response to a concentration of use survey conducted by the Council in 2022, no uses were reported. MIBK is categorized in Annex II of the EU, the list of substances prohibited in cosmetic products, due to carcinogenic potential.

In an absorption and metabolism study in which male Sprague-Dawley rats were orally administered a single dose of 5 mmol/kg bw of MIBK in corn oil, by gavage, MIBK was rapidly absorbed following oral exposure. The mean maximum plasma concentration was 0.644 mmol/l occurring at 0.25 h.

An acute dermal toxicity study of MIBK was performed in Crl:CD BR rats. Five male and 5 female rats were treated with 2000 mg/kg bw of undiluted MIBK under a semi-occlusive patch for 24 h; the LD_{50} was determined to be greater than the test dose of 2000 mg/kg. Rabbits (number of animals not stated) were administered 20 ml/kg of MIBK dermally for 4 h. An LD_{50} of > 20 ml/kg bw was reported.

A two-generation reproduction study was conducted to evaluate the effects of MIBK on reproductive performance. MIBK was administered to 30 Sprague-Dawley rats via whole-body inhalation at concentrations of 0, 500, 1000, or 2000 ppm, 6 h daily, for 70 d prior to mating. The authors concluded that MIBK, at all exposure levels, did not affect any reproductive parameters nor offspring growth or development. For reproductive endpoints, the highest concentration tested, 2000 ppm, was considered the NOAEL. Apart from acute sedative effects, the NOAEL for systemic effects in parental animals (excluding male rat kidney effects) was determined to be 1000 ppm. Regarding neonatal toxicity, the NOAEL was determined to be 1000 ppm.

Male and female $B6C3F_1$ mice and F344/N rats (50/sex/group) were exposed via inhalation (whole-body) to 0, 450, 900, or 1800 ppm MIBK for 6 h/d, 5 d/wk, for 2 yr. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in male and female mice exposed to 1800 ppm. Male rats exposed to MIBK had tumors of the kidney, increased rates of hyperplasia of the kidney and adrenal gland, and mononuclear cell leukemia. Under the conditions of the 2-yr studies, there was *some evidence of carcinogenic activity* in male and female mice and male rats, and there was *equivocal evidence of carcinogenicity* in female rats. The liver was the primary site of MIBK-related toxicity in mice.

The MOA for the initiation of MIBK-induced liver tumors in mice was investigated in male and female $B6C3F_1$, C57BL/6, and CAR/PXR knockout mice. Mice were exposed to either 0 or 1800 ppm MIBK via whole-body inhalation for 6 h/d, 5 d/wk, for a total of 10 d. The study concluded MIBK-induced hepatic effects are consistent with a phenobarbital-like MOA; this MOA for rat and mouse liver tumor formation is considered not plausible for humans. The kidney was the primary site of MIBK-related toxicity in rats. MIBK was evaluated to assess its ability to induce specific measures of α2u-N in the kidneys of male and female rats compared to D-limonene, a known inducer of α2u-N. Kidneys from the male rats exhibited a similar rate of histological changes.

Cultured primary male C57BL/6 mouse hepatocytes and primary male human hepatocytes were exposed to MIBK (concentrations 10 - 300 µM) for 96 h. In the mouse study, cell viability was reduced at 10 µM but the results were considered spurious. CYP210 mRNA expression was induced at all concentrations of MIBK. CYP3a11, CYP1a, and CYP4a10 mRNA expression were unaffected. CYP1a2 expression was marginally induced. BROD, EROD, and benzoquinone reductase enzyme activity was not increased by exposure and exposure to MIBK did not induce RDS. In the human hepatocyte study, CYP1A1 and CYP2B6 mRNA expression was marginally induced at 300 µM in hepatocytes from one donor. CYP3A4 and CYP4A11 mRNA expression was unaffected by treatment with MIBK from all three donors. PROD, BROD, EROD and benzoquinone reductase activity was not increased by exposure to MIBK in hepatocytes from any of the three donors. However, exposure to MIBK at 300 µM increased RDS slightly (~1.7 times) in hepatocytes from one donor.

In the study in which MIBK was evaluated to assess its ability to induce specific measures of α2u-N in the kidneys of male and female rats as compared to D-limonene, 4 male and 4 female F344 rats were administered corn oil (control) or MIBK (1000 mg/kg; 5 ml/kg) and another group of 4 male rats were administered D-limonene, (300 mg/kg; 5 ml/kg) for 10 consecutive days by gavage. Kidneys from the male rats exhibited a similar rate of histological changes as seen in the kidneys from the D-limonene treated male rats. There were no changes noted in the female rats. The ability of MIBK to induce measures of α2u-N, including renal cell proliferation, was evaluated in 84 male and 84 female F344 rats following exposure to 0, 450, 900, or 1800 ppm MIBK for 6 h/d for 1 or 4 wk. Increased measures of α2u-N, renal cell proliferation and reversible binding of MIBK to α2u were observed.

The skin sensitization potential of MIBK was assessed in female albino guinea pigs; 20 animals comprised the test group and 10 animals comprised the control group. Intradermal induction was carried out with 0.1 ml of 5% MIBK in corn oil, epicutaneous induction was performed with undiluted MIBK, and challenge was conducted with 30% MIBK under occlusive conditions. MIBK produced no sensitizing reaction. In another study, a guinea pig maximization test was performed on 20 test animals (10 control).

Intradermal induction was carried out with 0.1 ml of 5% MIBK in corn oil, and semi-occlusive patches with 0.1 ml of 5% MIBK in corn oil were used for epidermal induction. No indication of skin sensitization was observed.

A single-exposure ocular irritation study on MIBK was performed using 3 New Zealand White rabbits. Undiluted MIBK is considered to be slightly irritating to rabbit eyes. MIBK was also slightly irritating to the eyes in another study using 4 rabbits.

Occupational limits from the ACGIH recommend a TLV-TWA of 20 ppm (82 mg/m^3) and a TLV–STEL of 75 ppm (307 mg/m^3) for exposure to MIBK. NIOSH lists a TWA limit of 50 ppm MIBK (205 mg/m^3), and also includes the OSHA standard of 100 ppm MIBK (410 mg/m^3). A field study on 20 workers exposed to mixed solvents (toluene, ethyl benzene, xylene) containing MIBK and one worker who was exposed to pure MIBK was performed. The TWA concentration of the urine of the workers exposed to the mixed solvents showed, after 8 h, 21.9 ± 15 ppm MIBK. In the subject exposed to pure MIBK, the 6-h TWA of MIBK was 42.3 ppm. In another study, urine of 27 furniture making workers and 11 non-exposed controls was examined to determine unmetabolized MIBK; the maximum exposure concentration reached was 15.1 ppm.

DISCUSSION

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years. In 2004, the Panel published a final report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products, based on the available animal and clinical data in that report. In March 2023, the Panel determined that this safety assessment should be re-opened to include new carcinogenicity and toxicological data that were included in an NTP report; these studies were in progress at the time of the original report.

This amended assessment reviews the safety of MIBK as used in cosmetic formulations. The Panel concluded that MIBK is safe as used in nail care products and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel noted that one reported use of MIBK is in an aftershave lotion. In accordance with the conclusion reached by the Panel, use in this product type is safe if the function is as an alcohol denaturant. Additionally, no concentrations of use of MIBK have were reported in response to the survey conducted by the Council in 2022. To determine safety, the Panel referred to the use concentration data that were included in the original safety assessment that was published in 2004, and it is that maximum concentration of use that was deemed safe.

Regarding the possible use of MIBK as a denaturant in cosmetics, the Panel noted that MIBK has been approved for use as a denaturant for alcohol. In keeping with the specification determined by the Alcohol and Tobacco Tax and Trade Bureau, the Panel agreed that MIBK could be considered safe for use as a denaturant in cosmetics at concentrations up to the maximum concentration of MIBK (4%) that is listed for use as a denaturant of alcohol (that can be consumed). It is important to note that because of the established regulations, the Panel maintains that cosmetic product formulators use MIBK as a denaturant at concentrations that do not exceed 4.0%.

The new studies that have been included from the NTP report did not raise concerns for the Panel. The MOA studies concluded that MIBK-induced hepatic effects are consistent with a phenobarbital-like MOA, where the initiating events are activation of the CAR and PXR nuclear receptors which results hepatocellular proliferation leading to rodent liver tumors. The Panel noted that concern for this effect was mitigated because the MOA for rat and mouse liver tumor formation initiated by phenobarbital and sodium phenobarbital and other CAR activators is considered not plausible for humans.

The Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that MIBK is safe as used in nail care products and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment.*

**Current concentrations of use are not reported; the expectation is that this ingredient would be used at concentrations comparable to that reported in the 2004 safety assessment.*

TABLES

Table 1. Frequency (2023; 1998) and concentration (2022; 2000) of use by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[3]	**1998[2]**	**2022[4]**	**2000[2]**
Totals	**2**	**2**	**NR**	**21**
Manicuring Preparations (Nail)				
Nail Polish and Enamel Remover	NR	2	NR	NR
Other Manicuring Preparations	1	NR	NR	21*
Shaving Preparations				
Aftershave Lotion	1	NR	NR	NR

NR – not reported

* MIBK was reported to be used at a concentration of 21%, specifically in a nail correction pen (volume = 3 ml); accordingly, some dermal contact would be expected.

Table 2. Inhalation carcinogenicity studies of MIBK

Animals/Group	Concentration/Dose	Procedure	Results	Reference
$B6C3F_1$ mice; 50/sex/group	0, 450, 900, or 1800 ppm	Mice were exposed whole body for 6 h/d, 5 d/wk, for 104 wk. Mice were housed in stainless steel chambers. Exposure valves in the chambers automatically opened and allowed vapors to flow through individual delivery lines to each exposure chamber. The vapor was then mixed and diluted with conditioned chamber air to achieve the desired exposure concentration. The total active mixing volume of each chamber was 1.7 m^3. MIBK concentrations were monitored by an on-line gas chromatograph. Samples were drawn every 28 min. Buildup and decay rates for chamber vapor concentrations were determined with animals present in the chambers. A T_{90} value of 12 min was selected for the studies. Chamber uniformity was monitored throughout the study. Animals were observed twice daily. Complete necropsies and microscopic examinations were performed on all mice. Complete histopathology was also performed.	Males - s*ome evidence of carcinogenic activity* Increased incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma at 1800 ppm Females - s*ome evidence of carcinogenic activity* Increased incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma at 1800 ppm. Increased incidence of eosinophilic foci in the liver at 450 and 1800 ppm. Female mice exposed to highest test concentration had decreased body weight.	[11]
F344/N rats 50/sex/group	0, 450, 900, or 1800 ppm	As above but performed with rats.	Males - s*ome evidence of carcinogenic activity* Increased incidences of renal tubule adenoma, adenoma/carcinoma in males exposed to 900 or 1800 ppm. Increased incidence of renal tubule carcinoma in males exposed to 1800 ppm. Increased incidence of renal tubule hyperplasia in males at 450 and 1800 ppm. Chronic nephropathy in all males at 1800 ppm. Transitional epithelial hyperplasia of renal pelvis in males exposed to 900 or 1800 ppm. Increased incidence of mineralization of renal papilla at all concentrations. Positive trend in incidences of mononuclear cell leukemia in males. Increased incidence in adrenal medulla hyperplasia in 1800 ppm Females - e*quivocal evidence of carcinogenic activity* Chronic nephropathy in 70 – 88% females at all concentrations. Two female rats exposed to 1800 ppm had renal mesenchymal tumors.	[11]

Table 3. Incidence of neoplastic and non-neoplastic lesions of the liver in mice and kidneys in rats [11]

Animals/Group	Neoplastic/ Non-Neoplastic	Effect	Chamber Control	450 ppm	900 ppm	1800 ppm
male $B6C3F_1$ mice; 50/sex/group	non neoplastic	eosinophilic focus	3/50	4/50	5/50	8/50
	neoplastic	hepatocellular adenoma	17/50	25/50	23/50	34/50
		hepatocellular carcinoma	12/50	12/50	10/50	9/50
		hepatocellular adenoma or carcinoma	27/50	34/50	28/50	37/50
female $B6C3F_1$ mice; 50/sex/group	non neoplastic	eosinophilic focus	4/50	11/50	10/50	14/50
	neoplastic	hepatocellular adenoma	13/50	15/50	20/50	23/50
		hepatocellular carcinoma	6/50	5/50	6/50	11/50
		hepatocellular adenoma or carcinoma	17/50	17/50	22/50	27/50
male F344/N rats; 50/sex/group	non neoplastic	renal tubule hyperplasia (standard eval)	1/50	11/50	3/50	18/50
		renal tubule hyperplasia (standard + extended eval combined)	1/50	14/50	7/50	21/50
		nephropathy	42/50	45/50	47/50	50/50
		pelvic transitional epithelium hyperplasia	1/50	5/50	6/50	19/50
		papilla mineralization	1/50	6/50	22/50	29/50
		adrenal medulla hyperplasia	13/50	18/48	18/50	24/50
	neoplastic	renal tubule adenoma (standard eval)	0/50	0/50	2/50	3/50
		renal tubule adenoma (standard + extended eval)	2/50	3/50	3/50	10/50
		renal tubular carcinoma (standard)	0/50	1/50	0/50	2/50
		renal tubular adenoma or carcinoma (standard and extended eval)	2/50	4/50	3/50	11/50
		mononuclear cell leukemia	25/50	26/50	32/50	35/50
female F344/N rats; 50/sex/group	non neoplastic	nephropathy	19/50	35/50	38/50	44/50
	neoplastic	malignant mesenchymal tumor	0/50	0/50	0/50	2/50

Table 4. Mode of action studies on MIBK-induced tumors

Animals/Group	Concentration/Dose	Procedure	Results	Reference
		Inhalation		
$B6C3F_1$ mice; 16/sex/group	0 or 1800 ppm	Mice were exposed whole body for 6 h/d, 5 d/wk, for a total of 10 d. Mice were implanted with an osmotic pump with 20 mg/ml of BrdU after day 1 of initial exposure.	Male and female $B6C3F_1$ mice showed an increase in liver weights that corresponded with hepatocellular hypertrophy and increased mitotic figures. Data shows induction of S-phase DNA synthesis. Gene expression showed maximally induced CAR-associated CYP2b10 and slightly increased PXR-associated CYP3a11 Compounds initiating liver tumors in rodents through the CAR MOA are not expected to be relevant in humans.	12
C57BL/6 mice; 16/sex/group	0 or 1800 ppm	As above.	Female C57BL/6 mice showed an increase in liver weights that showed hepatocellular hypertrophy and increased mitotic figures. Data shows induction of S-phase DNA synthesis Gene expression showed maximally induced CAR-associated CYP2b10 and slightly increased PXR-associated CYP3a11.	12
CAR/PXR KO mice; 16/sex/group	0 or 1800 ppm	As above.	No increase in induction of S-phase DNA synthesis. Mice exposed to 1800 ppm MIBK showed no evidence of activation of AhR, CAR, PXR or PPAR-α nuclear receptors via their associated transcripts.	12
		Oral		
F344 rats; 4/sex/group	0 or 1000 mg/kg in corn oil; 5 ml/kg positive controls: 300 mg/kg D-limonene males only)	To investigate whether MIBK operates through a non-genotoxic MOA to induce the male rat-specific renal tumor response following chronic exposure, rats were dosed by gavage for 10 consecutive days. The kidneys were removed approximately 24 h after the final dose. The left kidney was analyzed for histological alterations, which included the accumulation of protein (hyaline) droplets, staining for α2u, and the presence of proliferating cell nuclear antigen to determine renal cell growth rates. The right kidney was processed to measure total protein and α2u using ELISA. D-Limonene was used as the positive control in that it is an acknowledged inducer of α2u-N.	MIBK caused an increase in protein droplets, accumulation of α2u, and renal cell proliferation in males, but not in females. The histological alterations caused by MIBK in male rat kidneys were similar to those induced by D-limonene, but they were of a milder degree. The investigators concluded that MIBK exerts renal effects through an α2u-N-mediated MOA.	14

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Safety Assessment of MIBK (Methyl Isobutyl Ketone)[1]

MIBK (Methyl Isobutyl Ketone) is an aliphatic ketone that functions as both a denaturant and solvent in cosmetic products. Current use in cosmetic products is very limited, but MIBK is reported to be used in one nail correction pen (volume = 3 ml) at a concentration of 21%. The maximum percutaneous absorption rate in guinea pigs is 1.1 μmol/min/cm^2 at 10 to 45 min. Metabolites include 4-hydroxy-4-methyl-2-pentanone (oxidation product) and 4-methyl-2-pentanol (4-MPOL) (reduction product). Values for the serum half-life and total clearance time of MIBK in animals were 66 min and 6 h, respectively. In clinical tests, most of the absorbed MIBK had been eliminated from the body 90 min post exposure. MIBK was not toxic via the oral or dermal route of exposure in acute, short-term, or subchronic animal studies, except that nephrotoxicity was observed in rats dosed with 1 g/kg in a short-term study. MIBK was an ocular and skin irritant in animal tests. Ocular irritation was noted in 12 volunteers exposed to 200 ppm MIBK for 15 min in a clinical test. A depression of the vestibulo-oculomotor reflex was seen with intravenous infusion of MIBK (in an emulsion) at 30 μM/kg/min in female rats. The no-observed-effect level in rats exposed orally to MIBK was 50 mg/kg. Both gross and microscopic evidence of lung damage were reported in acute inhalation toxicity studies in animals. Short-term and subchronic inhalation exposures (as low as 100 ppm) produced effects in the kidney and liver that were species and sex dependent. Dermal doses of 300 or 600 mg/kg for 4 months in rats produced reduced mitotic activity in hair follicles, increased thickness of horny and granular cell layers of the epidermis, a decrease in the number of reactive centers in follicles (spleen), an increase in the number of iron-containing pigments in the area of the red pulp (spleen), and a reduction in the lipid content of the cortical layer of the adrenal glands. Neuropathological changes in the most distal portions of the tibial and ulnar nerves were observed in young adult rats which inhaled 1500 ppm MIBK for up to 5 months. No adverse effects were seen in any other neurological end point by any route of exposure in other studies using rats or other animal species. Clinical tests demonstrated a threshold for MIBK-induced irritation of the lungs at 0.03 to 0.1 mg/L after 1 min of respiration. MIBK was not mutagenic in the Ames test or in a mitotic gene-conversion assay in bacteria. Mammalian mutagenicity test results were also negative in the following assays: mouse lymphoma, unscheduled DNA synthesis, micronucleus, cell transformation, and chromosome damage. MIBK did not induce any treatment-related increases in embryotoxicity or fetal malformations in pregnant Fischer 344 rats or CD-1 mice that inhaled MIBK at concentrations of 300, 1000, or 3000 ppm. There was evidence of treatment-related maternal toxicity only at the highest concentration tested. MIBK applied to the tail of rats daily at doses of 300 or 600 mg/kg for 4 months produced changes in the testes, including a reduction in the number of spermatocytes, spermatids, and spermatozoa. An ongoing carcinogenicity study of MIBK being conducted by the National Toxicology Program will be considered when the results are available. On the basis of the information that is currently available, MIBK is considered safe as used in nail polish removers and as an alcohol denaturant in cosmetic products.

INTRODUCTION

This safety assessment focuses on the use of MIBK (Methyl Isobutyl Ketone) in cosmetic products. MIBK functions as both an alcohol denaturant and solvent, but most current cosmetic uses are solvent uses.

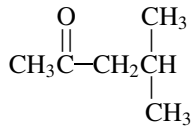
The European Chemical Industry Ecology and Toxicology Centre (ECETOC) prepared an earlier review of the toxicity of MIBK (ECETOC 1987). ECETOC concluded that the relatively high volatility of MIBK, its rapid atmospheric phototransformation, ready biodegradability, and low mammalian and aquatic toxicity indicate that the environmental hazards of MIBK are negligible.

In a more recent review, the World Health Organization (WHO) reached a similar conclusion (WHO 1990). The WHO concluded that the relatively high volatility, rapid atmospheric phototransformation, ready biodegradability, and low mammalian and aquatic toxicity of MIBK indicate that adverse environmental effects of this substance are only likely to occur after accidental spills or from uncontrolled industrial effluents.

CHEMISTRY

Chemical and Physical Properties

MIBK (CAS no. 108-10-1) is the aliphatic ketone that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



MIBK has also been described as a branched chain hydrocarbon that is photochemically reactive (Billmaier et al. 1974). Other names for this chemical are as follows: Methyl Isobutyl Ketone; Isopropylacetone; 4-Methyl-2-Pentanone; and 2-Pentanone, 4-Methyl- (Wenninger, Canterbery, and McEwen

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TABLE 1
Chemical and physical properties of MIBK

Property	Value	References
Octanol/water partition coefficient (Log P)	1.966	Barrat 1997
	1.38	WHO 1990
	1.31	Tanii, Tsuji, and Hashimoto 1986
Molecular weight	100.16	WHO 1990
Physical form	Liquid	Verschueren 1983
Color	Colorless	Verschueren 1983
Odor	Faint, ketonic, and camphor	Budavari 1989
Taste	Sweet	Verschueren 1983
Solubility in water	17 g/L (20°C)	Verschueren 1983
	2.04% by weight (28°C)	EPA 1979
Boiling point	116.2°C (116°C to 119°C) at 101 kPa	EPA 1979
Freezing point	−80.26°C (range: −80°C to −85°C)	EPA 1979
Melting point	−84.7°C	EPA 1979
Flashpoint	14°C (closed cup)	Verschueren 1983
Autoignition temperature	460°C	Verschueren 1983
Explosion limits in air	1.4 to 7.5% vol at 101 kPa	Verschueren 1983
Specific gravity	0.8017 at 20°C/4°C	Verschueren 1983
	Not more than 0.799	Committee of Revision of the United States Pharmacopeial Convention 2000
Refractive index (n_D^{20})	1.395 to 1.397	Verschueren 1983
Viscosity	0.58 to 0.61 mPa at 20°C	Verschueren 1983
Vapor density (air = 1)	3.45	Verschueren 1983
Vapor pressure	1.99 kPa (20°C)	Verschueren 1983
	15 mm Hg (20°C)	EPA 1979
Concentration in saturated air	27 g/m^3 at 20°C and 101 kPa	Verschueren 1983

2000), and MIK; 2-Methyl-4-Pentanone; Hexanone; Hexone; Isopropyl-Acetone; 4-Methyl Pentan-2-One; 4-methyl-2-Oxopentane; 2-Methyl Propyl Methyl Ketone; and Isobutyl-methyl ketone (WHO 1990).

The chemical and physical properties of MIBK are summarized in Table 1.

Methods of Production

The commercial production of MIBK involves acetone condensation, followed by catalytic hydrogenation (Environmental Protection Agency [EPA] 1976; Chemical Manufacturers Association [CMA] 1999b).

According to Zakhari et al. (1977), acetone is dimerized to diacetone alcohol by a liquid phase reaction at 0°C to 20°C over a fixed-bed, alkaline catalyst. Diacetone alcohol is then dehydrated at 100°C to 120°C to 4-methyl-3-penten-2-one (aka mesityl oxide) in the presence of a weak acid. Finally, mesityl oxide is hydrogenated over nickel or copper at temperatures from 120°C to 165°C.

The CMA (1981) confirmed this process, noting that MIBK is typically manufactured via the aldol condensation of acetone to form diacetone alcohol. This is described as an enclosed, continuous process. Diacetone alcohol is then dehydrated to form mesityl oxide, site-limited intermediate, which is hydrogenated to MIBK. The crude MIBK is purified by continuous distillation.

Impurities

MIBK is 99% pure (by mass) and may contain the following impurities: <0.3% dimethyl heptane, <0.1% water, <0.06% methyl isobutyl carbinol, <0.03% mesityloxide, <0.002% acetic acid, and <0.002% nonvolatiles (WHO 1990). Another source indicates that MIBK is >98% pure and contains 0.9% methyl *n*-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane (Eastman Kodak Company 1992).

Spencer et al. (1975) reported a 3% concentration of the contaminant, methyl *n*-butyl ketone (3.0%) in commercial MIBK. In 1999, however, MIBK producers indicated that methyl *n*-butyl ketone was either no longer found in MIBK or was found in trace amounts (typically 0.01% to 0.06% and always less than 0.1%) (CMA 1999a). Other impurities in MIBK include: methyl amyl alcohol, acetone, and 3-methyl-2-butanone (CMA 1999b).

Reactivity

MIBK does not undergo hydrolysis. However, because it is a branched-chain ketone, it may be photochemically active. The

half-life for the evaporation of MIBK is 33 h (Mackay and Wolkoff 1973).

MIBK has been described as dangerous when exposed to heat, flame (moderate explosion hazard), or oxidizers (Lewis 2000). Some of the oxidizing agents that MIBK may react violently with include peroxides, nitrates, and perchlorates. Additionally, when heated, MIBK may form peroxides by auto-oxidation; the peroxides may explode spontaneously (WHO 1990). MIBK ignites on contact with potassium *tert*-butoxide, and can react vigorously with reducing materials (Lewis 2000).

Analytical Methods

MIBK has been analyzed by gas chromatography (DiVincenzo, Kaplan, and Dedinas 1976; Raccio and Widomski 1981; Fernandes 1985; Cobb and Braman 1991), gas chromatography with flame ionization detection (EPA 1973; Moshlakova and Indina 1986), gas chromatography with mass spectroscopy (Zlatkis and Liebich 1971; Bellanca et al. 1982; Weller and Wolf 1989), high-resolution capillary gas chromatography (Clair, Tua, and Simian 1991), and infrared spectroscopy (Committee of Revision of the United States Pharmacopeial Convention 2000).

USE

Purpose in Cosmetics

MIBK functions as a denaturant and solvent in cosmetic products (Wenninger, Canterbery, and McEwen 2000). Frequency of use data provided by the Food and Drug Administration (FDA) in 1998 indicated that MIBK is used in 2 out of a total of 34 products in the nail polish and enamel remover category (FDA 1998). Data submitted to CTFA in 2000 indicate that MIBK is used at a concentration of 21%, specifically in a nail correction pen (volume = 3 ml) (CTFA 2000).

Cosmetic products containing MIBK are applied to the nail and may come in contact with skin adjacent to the nail or the ocular and nasal mucosae. These products could be used on a weekly basis, and could be applied frequently over a period of several years.

MIBK is included in the CTFA *List of Japanese Cosmetic Ingredients* (Santucci 1999). It has precedent for use without restrictions in nail makeup preparations. In Japan, MIBK is not used in cleansing preparations, hair care preparations, treatment preparations, make-up preparations, fragrant preparations, suntan/sunscreen preparations, eyeliner preparations, lip preparations, oral preparations, and bath preparations.

MIBK is not included among the substances listed as prohibited from use in cosmetic products marketed in the European Union (European Economic Community 1999).

Noncosmetic Use

MIBK is used primarily in industrial coating solvents, lube oil dewaxing, and in rare metal refining (EPA 1979). It is also used in public health environmental studies for determining the presence of heavy metals in air and in biological materials. For example, lead in air and biological materials can be extracted with MIBK and then analyzed by atomic absorption spectrophotometry (Zakhari et al. 1977).

MIBK has been approved as denaturant in denatured alcohol and rum, with specifications for its acidity, color, distillation range, odor, and specific gravity (27 CFR 21.161). Specifications for the composition of completely denatured alcohol formulas (27 CFR 21.21; 21.22; 21.23; 21.24) and specially denatured spirit formulas (27 CFR 21.31; 21.32; 21.49) containing MIBK are available. According to these specifications, established by the Bureau of Alcohol, Tobacco, and Firearms, the maximum concentration of MIBK that is listed for use as a denaturant of alcohol is 4.0%.

MIBK is also listed in the *National Formulary* as an alcohol denaturant that is used as an excipient for drugs (Committee of Revision of the United States Pharmacopeial Convention 2000).

MIBK has been approved for use as a component of synthetic flavoring substances and adjuvants (21 CFR 172.515) and as a component of adhesives that are present in articles intended for use in packaging, transporting, or holding food (21 CFR 175.105), and as an optional component (solvent-use only) of polysulfide polymer-polyepoxy resins that form the food-contact surface of articles intended for packaging, transporting, or holding dry food (21 CFR 177.1650).

BIOLOGICAL PROPERTIES

Fate of Inhaled MIBK

Hjelm et al. (1990) exposed eight male volunteers (18 to 35 years old; weights = 68–90 kg) to MIBK (concentrations of 2.4 ppm [10 mg/m^3], 24.4 ppm [100 mg/m^3], and 48.8 ppm [200 mg/m^3]) for 2 h during light physical exercise on three different occasions. Exposures took place in a 12-m^3 exposure chamber. Pulmonary retention of MIBK was described as fairly constant throughout the exposure period. The relative pulmonary uptake of MIBK was ≈60%, and total pulmonary uptake increased linearly with increasing exposure concentrations. Average values for uptake were 0.2 mmol at 10 mg/m^3, 1.7 mmol at 100 mg/m^3, and 3.2 mmol at 200 mg/m^3. At the end of exposure, blood concentrations of MIBK increased linearly with increasing uptake. No tendency toward saturation kinetics was observed over the range of doses tested. The apparent blood clearance was 1.6 L/h/kg at all exposure concentrations. The concentration of MIBK in the urine was higher than that noted in arterial blood both at 0.5 h and 3 h after exposure. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 h post exposure. Results concerning the irritation potential (nose and throat) of MIBK and effects on the central nervous system that were recorded during the study are included in the section on Short-Term Inhalation Toxicity later in this report.

Fate of MIBK Applied to the Skin

Hjelm et al. (1991) evaluated the percutaneous absorption of MIBK using eight outbred female guinea pigs. Initially, to determine blood clearance values, MIBK was infused into each animal at a rate of 0.478 μmol MIBK per minute, corresponding to 0.680 to 0.928 μmol/minute/kg body weight, for 30 min. The average blood clearance of MIBK was 201 ml/min/kg body weight.

After a 2.5-h nontreatment period, the percutaneous absorption part of the study was begun. Hair on the back of each animal was clipped and epicutaneous exposure (150 min) was carried out by filling a glass cylinder, secured to the application site, with 1 ml of MIBK. Arterial blood was analyzed for MIBK using gas chromatography. A maximum percutaneous uptake rate of 1.1 μmol/min/cm^2 was reached at 10 to 45 min after the initiation of exposure. A decrease in the uptake rate to 0.56 μmol/min/cm^2 was noted during the latter part of exposure (75 to 135 min after the initiation of exposure).

Distribution

Using a mass-spectrometric method, Dowty, Laseter, and Storer (1976) demonstrated MIBK in human maternal blood samples collected immediately after delivery. The authors interpreted this finding as indicating the potential for MIBK to enter the umbilical cord and cross the placenta.

In vitro partition coefficients of 70 to 90 between blood and air have been reported (Sato and Nakajima 1979; Hjelm et al. 1990). Sato and Nakajima (1987) reported the following partition coefficients for MIBK: 90 (MIBK into blood), 79 (MIBK into water), and 926 (MIBK into oil).

Bellanca et al. (1982) reported that MIBK was detected in the brain, liver, lung, vitreous fluid, kidney, and blood (at concentrations ranging from 0.14 to 0.52 and 0.04 to 0.22 mg/100 g, respectively) in workers who died after exposure to several volatile organic solvents during spray painting.

Regardless of the route of administration, Duguay and Plaa (1995) reported that the amount of MIBK detected in the plasma and liver of Sprague-Dawley rats was proportional to the initial MIBK administered dose. Based on a linear-regression analysis for plasma and liver concentrations versus dose, the correlations were statistically significant. A dose-related increase in MIBK concentration in the lungs was also noted.

Metabolism

DiVincenzo, Kaplan, and Dedinas (1976) evaluated the metabolism of MIBK using male guinea pigs (weights = 250–450 g). A single dose of the test substance (450 mg/kg in corn oil) was administered intraperitoneally to each animal and blood samples were collected at 1, 2, 4, 6, 8, 12, and 16 h post dosing. After centrifugation of the samples, sera were assayed within 48 h. 4-Hydroxy-4-methyl-2-pentanone (HMP) and 4-methyl-2-pentanol (4-MPOL) were MIBK metabolites identified in the serum by gas chromatography and confirmed using gas chromatography–mass spectrometry. The authors stated that HMP and 4-MPOL result from the oxidation and reduction of MIBK, respectively. The serum half-life and total clearance time for parent MIBK were 66 min and 6 h, respectively. The total clearance time for HMP was 16 h. It was also stated that the hydroxylation products of MIBK, such as 4-MPOL, are expected either to be conjugated with sulfate or glucuronic acid and excreted in the urine or to enter intermediary metabolism to be converted to carbon dioxide.

Lande et al. (1976) reported that enzymatic ketonic reduction of MIBK to the alcohol 4-MPOL occurs in the liver, and that conjugation with glucuronic acid can occur prior to elimination in the urine.

DiVincenzo et al. (1980) demonstrated that 16 h was required for the elimination of both HMP and 4-MPOL metabolites.

According to Hjelm et al. (1990), inhaled MIBK accumulates in adipose tissue, because it is easily soluble in blood and has a high affinity for fat.

Proteins, chiefly hemoglobin, are the major carriers of MIBK in the blood (Lam et al. 1990).

According to the WHO, the structure of MIBK precludes the metabolic production of 2,5-hexanedione, a neurotoxic agent formed from methyl *n*-butyl ketone and hexane (WHO 1990).

Granvil, Sharkawi, and Plaa (1994) studied the metabolic fate of MIBK using groups of eight male Charles River CD-1 mice. The animals received a single intraperitoneal (IP) injection of 5 mmol/kg MIBK. MIBK was dissolved in corn oil, and the injection volume was 10 ml/kg. The animals were killed by decapitation and blood and brain samples were collected at 15, 30, 60, and 90 min post injection. The principal metabolites were 4-MPOL (reduction product) and 4-hydroxy-4 methyl-2-pentanone (oxidation product). The concentration of the reduction product in the brain was twice that seen in the blood at 15- and 30-min time intervals.

Duguay and Plaa (1995) reported that the MIBK metabolite 4-MPOL increased in a dose-related manner in the plasma, following oral or inhalation exposure using Sprague-Dawley rats. When MIBK was administered by gavage, 4-MPOL was not detected in the plasma, liver, or lung. However, following inhalation exposure, 4-MPOL was detected in all of the tissues. The authors concluded that metabolite concentrations were influenced by the route of MIBK administration.

Excretion

Zlatkis and Liebich (1971) reported that MIBK can also be eliminated unchanged in the urine. As indicated above, the metabolism of MIBK is an oxidative-reductive metabolic conversion.

Human volunteers were exposed to 100 ppm (410 mg/m^3) MIBK for 4 h in an environmental chamber. This group represented one of four groups exposed to MIBK, methyl ethyl ketone, or mixtures of the two. Ninety-eight male and female subjects were randomly assigned to the four exposure groups. Steady-state blood concentrations of MIBK were attained after

2 h of exposure. Blood and breath samples collected at 90 min post exposure indicated that most of the absorbed MIBK had been eliminated from the body (Dick et al. 1990).

Effect on Enzyme Activity

Lapin et al. (1982) stated that MIBK (50 and 200 mM) inhibited the enzyme activity of creatine kinase from rat muscle and adenylate cyclase from rat brain in vitro. Cunningham, Sharkawi, and Plaa (1989) reported that MIBK reduced the activity of mouse (CD-1 mice) liver alcohol dehydrogenase in vitro.

According to Raymond and Plaa (1995a), the oral administration to male rats of 1362 mg/kg MIBK in 5% polyoxyethylated castor oil produced increased renal cytochrome P-450 and aniline hydroxylase activity and increased liver and renal aminopyrine *N*-demethylase activity. No histopathology was noted. The authors were uncertain about the toxicological significance of these findings.

Effect on Cholestatic Activity

Plaa and Ayotte (1985) evaluated the effect of MIBK on the acute cholestatic response (change in bile flow) induced by sodium taurolithocholate using Sprague-Dawley rats (weights = 250–300 g). Animals were pretreated with MIBK daily with oral doses of 3.75 or 7.5 mmol/kg at a dose volume of 10 ml/kg for 3 or 7 days. Sodium taurolithocholate in a vehicle consisting of albumin, dextrose, and NaCl was then injected intravenously (5 to 25 mg/kg). Control animals were pretreated with corn oil. MIBK potentiated the decrease in bile flow that was induced by sodium taurolithocholate (TLC). Pretreatment with MIBK (7.5 mmol/kg oral doses) for three days, followed by intravenous (IV) dosing with sodium taurolithocholate (15 mg/kg) resulted in a 79% decrease in bile flow, compared to the 55% decrease in bile flow that was induced by similar pretreatment with corn oil followed by dosing with sodium taurolithocholate. Decreased bile flow was not noted in rats dosed only with MIBK.

The effect of MIBK on the cholestatic activity of manganese, with or without bilirubin, was evaluated by this same laboratory (Vézina, Ayotte, and Plaa 1985; Vézina and Plaa 1987, 1988) using male Sprague-Dawley rats. MIBK was administered by gavage at doses ranging from 188 to 1502 mg/kg once daily for 1, 3, or 7 days. MIBK was not cholestatic over the range of doses tested. However, it potentiated the cholestasis induced by a manganese-bilirubin combination, administered 18 h after dosing with MIBK. MIBK dosing for 3 or 7 days caused dose-related enhancement of cholestasis that had been induced by the manganese-bilirubin combination. Potentiation of the cholestasis induced by manganese alone was noted after dosing with 750 mg MIBK/kg for 3 days. In other experiments, two metabolites of MIBK (HMP and 4-MPOL) also potentiated the cholestatic effect of manganese or the manganese-bilirubin combination in male Sprague-Dawley rats.

Dahlstrom-King et al. (1990) demonstrated the potentiation of taurolithocholic acid–induced reduction in bile flow after oral dosing with MIBK (same procedure) in rats. Study results also indicated that this effect is not caused by alteration of the kinetics of taurolithocholic acid.

Joseph et al. (1992) evaluated the effect of MIBK on bile flow in male Sprague-Dawley rats. During oral exposure, the rats received MIBK in corn oil at the following doses for 3 days: 1.5 mmol/kg (0.5 MED [minimal effective dose]), 3 mmol/kg (1 MED), and 6 mmol/kg (2 MED). The MED was defined as the smallest dose of MIBK that potentiated the cholestatic response. Inhalation exposure consisted of exposure to 200, 400, or 600 ppm MIBK for 4 h.

In this study, cholestasis induced by taurolithocholic acid or manganese-bilirubin combinations was enhanced following oral or inhalation exposure to MIBK. When compared to control rats, the decrease in bile flow was more pronounced and lasted longer in rats preexposed to MIBK. Additionally, the oral administration of 1.5, 3.0, or 6 mmol/kg or 4 h of inhalation exposure to 200, 400, or 600 ppm resulted in equivalent MIBK plasma concentrations. No observable diminution in bile flow was noted in rats that received MIBK (alone) either orally or by inhalation for 3 days (Duguay and Plaa 1993). It has been suggested that MIBK potentiates lithocholate-induced cholestasis by reducing the bile salt pool and interfering with the rate of hepatic secretion of bile salts (Joseph et al. 1992).

In a study by Duguay and Plaa (1997) using male Sprague-Dawley rats, MIBK inhalation potentiated taurolithocholic acid (30 μmol/kg) and manganese-bilirubin (4.5 mg/kg Mn and 25 mg/kg bilirubin) induced cholestasis in a dose-related manner. The rats were exposed to MIBK for 3 days (4 h per day) at concentrations equivalent to 0.5, 1.0, 1.5, or 2.0 times the MEC (minimal effective concentration). The MEC was estimated to be 400 ppm for 3 days of exposure (4 h/day) to MIBK.

Antimicrobial Activity

The threshold concentration of MIBK for the inhibition of bacterial (*Pseudomonas putida*) growth was 275 mg/L in a 16 h study (WHO 1990).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Mice

McOmie and Anderson (1949) evaluated the acute oral toxicity of MIBK in five fasted mice (weights not stated). MIBK was administered intragastrically as a 10% to 40% emulsion in 1% aqueous Tergitol (dose volume = 0.2 ml/10 g). The Tergitol used in the emulsion is defined as the sodium sulfate derivative of 3,9-diethyl tridecanol-6. MIBK caused excitement and generalized involuntary movements; light anesthesia was also induced. At necropsy of mice that died, hyperemia of the stomach wall and duodenum was a common finding. The congested area usually extended throughout the length of the gut and along the mesenteric blood vessels. At microscopic examination, albuminous degeneration of the liver was the most significant finding. An LD_{50} of 1.5 ml/kg was reported.

Batyrova (1973) stated that the average lethal dose for MIBK in mice dosed orally (stomach tube) was 2.85 (2.638–3.078) g/kg. The number, strain, and weights of the animals tested were not stated.

Zakhari et al. (1977) evaluated the acute oral toxicity of MIBK using six groups of eight CF-1 male mice (weights = 23–34 g). Doses ranging from 0.9 to 3.5 g/kg per group were administered. An LD_{50} of 1.90 ± 0.68 g/kg was reported. All animals in the highest dose group died, whereas the mortality rate was 13% in the 0.9-g/kg dose group.

Rats

Acute oral LD_{50} values of 2.08 (1.91–2.27) g/kg (Smyth, Carpenter, and Weil 1951); 4.6 (3.932–5.382) g/kg (Batyrova 1973); and 5.7 ml/kg (CMA 1981) have been reported in studies involving rats.

Panson and Winek (1980) evaluated the acute oral toxicity of MIBK using six rats (three males, three females; body weights = 200–226 g). Each animal was given a single dose of 1 ml/kg and then observed over a 24-h period. Necropsy was then performed. All of the rats died instantly. Lung weights ranged from 1.42 to 2.51 g (mean = 1.84) and the lung weight/body weight ratio ranged from 0.70 to 1.23 (mean = 0.88). In most of the animals, 25% of the lung tissue (all right lobes and caudal lobe included) was hemorrhagic. In one animal, 50% of the lung tissue was hemorrhagic. A blood clot at the base of the heart was also noted in one animal. Thus, MIBK may be aspirated into the lungs when swallowed.

The Exxon Chemical Company (1982) evaluated the aspiration hazard and toxicity of MIBK using five male albino rats (weights = 179–267 g). The animals were anesthetized with diethyl ether vapor to the point of apnea, and 0.2 ml of the test substance was placed in the oral cavity of each. Next, the animals were held in a vertical position with mouths held open and nostrils closed at end of expiration phase of breathing cycle. The nostrils were closed to promote entry of the test material into the trachea. Negative controls were dosed with tap water. At 24 h post dosing, the lungs were removed from animals that died and surviving animals that were killed under ether anesthesia by exsanguination from the abdominal aorta. Some of the animals (number not stated) died; all deaths were due to respiratory arrest, cardiac failure, or both, rather than pulmonary edema. None of the negative-control animals died. It was concluded that MIBK presents a potential aspiration hazard.

Guinea Pigs

An acute oral LD_{50} in the range between 1600 and 3200 mg/kg has been reported for guinea pigs (CMA 1981).

Acute Intraperitoneal Toxicity

Mice

Zakhari et al. (1977) evaluated the acute intraperitoneal toxicity of MIBK using six groups of 8 to 10 CF-1 male mice (weights = 20–23 g). The doses injected per group ranged from 0.25 to 1.25 g/kg. An acute IP LD_{50} of 0.59 ± 0.23 g/kg was reported at 24 h post injection. All animals in the 1.25-g/kg dose group died, whereas no deaths occurred in the 0.25-g/kg dose group. These authors also reported that the IP administration of MIBK to cats caused pulmonary vascular effects. The threshold dose for these effects was 8 mg/kg. However, bronchoconstriction was not noted after IP administration of MIBK at doses ranging from 4 to 32 mg/kg in the cats.

Guinea Pigs

Divincenzo and Krasavage (1974) evaluated the hepatotoxicity of MIBK was evaluated using mature guinea pigs. The test substance (in corn oil) was injected intraperitoneally at doses of 500 and 1000 mg/kg (four animals per dose). Blood was drawn at 24 h post injection and the animals were then killed. Serum ornithine carbamyl transferase (OCT) activity in the blood was measured using a spectrophotometric procedure. OCT is an enzyme that is found predominantly in the liver, and is released into the blood stream whenever liver cells are ruptured. MIBK induced a slight, but insignificant increase in serum OCT activity. One of the animals dosed with 1000 mg/kg MIBK died. Serum OCT activity in this animal was comparable to that observed at the 500-mg/kg dose. Neither histologic evidence of liver damage nor lipid deposition was observed in any of the guinea pigs tested.

Multiple Species

The Eastman Kodak Company (1982a) evaluated the acute intraperitoneal toxicity of MIBK using four groups of six male rats (Carworth Farms; weights = between 128 and 210 g) and four groups of six male guinea pigs (strain not stated; weights = 210–770 g). Both groups of six animals received MIBK in doses of 0.5, 1.0, 2.0, and 4.0 ml/kg body weight, respectively. The following signs were observed in rats after dosing: weakness, ataxia, prostration, dyspnea, and vasodilation. Signs indicative of demyelination or other nervous system damage were not observed. Deaths (rats) occurred anywhere from less than one day to six days after dosing, and the time to death was inversely related to the dose administered. The mortality data were as follows: 6/6 (4.0 ml/kg), 6/6 (2.0 ml/kg), 1/6 (1.0 ml/kg), and 0/6 (0.5 ml/kg). An acute IP LD_{50} (rats) of 1.14 ml/kg was reported. For guinea pigs, the authors reported that the signs of intoxication and time to death were similar to the data reported for rats above. The mortality data were as follows: 6/6 (4.0 ml/kg), 4/6 (2.0 ml/kg), 3/6 (1.0 ml/kg), and 2/6 (0.5 ml/kg). An acute IP LD_{50} of 0.919 ml/kg was reported.

Acute Intravenous Toxicity

Zakhari et al. (1977) injected MIBK intravenously into nine male cats to determine whether the pulmonary effects noted following inhalation (study described earlier) were limited to this route of exposure. Geometrically increasing doses of MIBK ranging from 4 to 128 mg/L were injected (single injection per dose). Dosing with 4 mg/kg MIBK resulted in no response.

MIBK (8 mg/kg) induced a 17% increase in mean pulmonary arterial pressure and a 34% decrease in mean pulmonary arterial flow. The authors stated that these results were indicative of an intense increase in pulmonary vascular resistance (84%), and that, most likely, this increase in resistance was caused by pulmonary vasoconstriction.

In another experiment, MIBK was injected intravenously into eight male cats to determine whether bronchoconstriction could be produced by this route of exposure. The IV injection of MIBK at doses ranging from 4 to 32 mg/kg did not result in an increase in pulmonary resistance or transpulmonary pressure. The authors concluded that bronchoconstriction, previously reported to be induced by MIBK inhalation, was not observed after intravenous dosing. Both a precipitous hypotension and apneic response (unexpected results) were observed simultaneously after the injection of 64 mg/kg MIBK (Zakhari et al. 1977).

Acute Dermal Toxicity

The acute dermal toxicity of MIBK was evaluated using two rabbits. Undiluted MIBK was applied (10 h of exposure) either by flooding the test site or placement of a cotton pad impregnated with the test substance. Signs of systemic effects were not noted during the 10-day observation period. At microscopic examination, there were no pathologic changes in the internal organs that resulted from exposure to MIBK. Irritation reactions are reported in the section on Skin Irritation later in this report (McOmie and Anderson 1949).

Acute Inhalation Toxicity

Mice

A concentration-dependent decrease in respiratory rate during 5 min of exposure to MIBK was noted in male Swiss OF_1 mice. A 50% decrease in the respiratory rate (RD_{50}) was noted after exposure to MIBK at a concentration of 3195 ppm. In this study, the reflex decrease in the respiratory rate of mice was measured as an index of sensory irritation (De Ceaurriz et al. 1981). In an earlier study, the decreased respiratory rate induced by MIBK was said to have been due to a narcotic effect (Specht et al. 1940).

McOmie and Anderson (1949) exposed six groups of mice (6 to 33 mice/group) to MIBK (saturated air-vapor mixture) at concentrations ranging from 43 to 100 mg/L of air (20°C). Each group received a single exposure and the duration ranged from 0.25 to 22.6 h.

Mortality data were provided for three of six groups. In the group exposed to 82 mg/L for 0.5 h, 18 of 33 animals died. In the group exposed to 86 mg/L for 1 h, 21 of 22 animals died. And in the group exposed to 82 mg/L for 1.25 h, 5 of 10 died. Deaths occurred by 10 h post exposure. Signs of irritation (e.g., closed eye, pawing at nose) were reported for all animals tested. Intense excitement and rapid, shallow respiration was noted after 3 min of exposure. The behavior of some mice ranged from convulsive movements to depression, with some animals lying prone. Furthermore, in the group of 33 mice, 30 animals had a loss of righting reflex in 30 min. At microscopic examination of animals that died, damage to the lung was the most common finding. Congestion, and, in some instances, hemorrhage and pneumonia were noted. Congestion noted in the liver and kidney was not as severe (McOmie and Anderson 1949).

Batyrova (1973) reported that narcosis was induced in all mice (number, weights, and strain not stated) exposed to MIBK (15 mg/L of air) for 2 h. An exposure concentration of 23.3 (18.49–29.36) mg/L was classified as moderately fatal.

Zakhari et al. (1977) evaluated the acute inhalation toxicity of MIBK using five groups of 10 CF-1 male mice (weights = 20–23 g). The animals were exposed to various concentrations of the test substance (1.0% *v/v* [41 mg/L] to 3.0% *v/v* [123 mg/L]) in a 10-L glass chamber. The mortality rate was determined after 45 min of exposure. Exposure to 1.0% *v/v* MIBK and to a saturated concentration of 3% MIBK resulted in no deaths and a mortality rate of 80%, respectively. The LC_{50} (95% fiducial limit) was 74.2 ± 25.8 mg/L.

The CMA (1981) reported that the acute inhalation toxicity of MIBK was evaluated using 10 mice. Exposure to 19,500 ppm MIBK induced anesthesia within 30 min. Recovery from this effect was noted within 5 min after exposure was discontinued. MIBK (concentrations above 20,000 ppm) also induced anesthesia within 30 min, which was followed by death of most of the animals. Congestion of the lungs was observed at gross necropsy.

Cometto-Muñiz and Cain (1991) stated that the RD_{50} (mice) for MIBK is 3195 ppm. The RD_{50} is defined as the concentration of an irritant that is expected to cause a 50% decrease in respiratory rate. Alarie (1966) had noted that the measurement of a decrease in respiratory rate of experimental animals (specifically mice) exposed to airborne irritants serves as an index of sensory irritation.

Rats

Smyth, Carpenter, and Weil (1951) evaluated the acute inhalation toxicity of MIBK (4-Methyl-2-Pentanone) using two groups of six rats (weights and strain not stated). The two groups were exposed to test concentrations of 2000 and 4000 ppm, respectively, for 4 h. None of the animals exposed to 2000 ppm died, whereas, 4000 ppm resulted in death of all six animals.

Batyrova (1973) exposed rats (number, weights, and strain not stated) to MIBK at a concentration of 0.2 mg/L for 4 h. The threshold concentration for inhalation intoxication (change in conditioned reflex activity, using the maze procedure) was 0.2 mg/L.

The CMA (1981) stated that all rats (number and strain not stated) exposed to 21,000 ppm MIBK for 55 minutes died. Rats exposed to 4,000 ppm MIBK for 6 h experienced loss of coordination and prostration.

Guinea Pigs

Specht (1938) evaluated the acute inhalation toxicity of MIBK was evaluated using 10 female guinea pigs (weights ≈300 g).

The animals were exposed to the following concentrations of MIBK in a 1-cm^3 inhalation chamber: 2.8 volume % (saturation), 1.68 volume %, 1.0 volume %, 0.3 volume %, and 0.1 volume %. Death occurred within 4 h at a concentration of 1.0 volume % and at progressively shorter periods at higher concentrations. At an exposure concentration of 1.68%, 9 of the 10 animals died by 142 min post exposure. Marked irritation, indicated by lacrimation and salivation, was observed in guinea pigs at higher concentrations. The animals that died during exposure were subjected to gross and microscopic evaluations. Gross changes were described as slight and consisted mainly of congestion, especially in the brain and lungs. At microscopic examination, a fine droplet fatty metamorphosis was present in many liver cells. However, most liver cells were normal and many sections of the liver had no pathology. No abnormalities in the kidneys or heart were observed. However, congestion and hypertrophy of the spleen was evident. Both gross and microscopic pathology was described as slight, resembling that of most acute reactions to solvent exposures. Gross findings in survivors of the study were not different from those noted in controls.

This same laboratory (Specht et al. 1940) exposed female guinea pigs to MIBK at concentrations of 1000 ppm (4100 mg/m^3), 16,800 ppm (69,000 mg/m^3), and 28,000 ppm (115,000 mg/m^3) for 24 h. A decrease in the respiratory rate (narcotic effect during first 6 h) and minimal ocular or nasal irritation were noted during exposure to 1000 ppm MIBK. The following signs were noted at higher concentrations: ocular and nasal irritation, salivation, lacrimation, ataxia, progressive narcosis, and death. Half of the animals exposed to the highest test concentration (28,000 ppm) died within 45 min of exposure. The following observations were made in some of the animals that were subjected to necropsy/microscopic examination: fatty livers and congestion of the brain, lungs, and spleen. No damage to the heart and kidneys was noted.

Cats

Batyrova (1973) determined the threshold for MIBK-induced irritation of the lungs using cats (number, weights, and strain not stated) with fistulae of the parotid gland. Salivation served as the index for respiratory irritation. After 15 min of exposure, the irritation threshold was between 0.25 and 0.50 mg/L.

Zakhari et al. (1977) evaluated the pulmonary and systemic vascular response following inhalation exposure to MIBK using nine male cats (weights = 2.9–3.4 kg). MIBK was volatilized by injection of a measured volume into a stream of air entering a breathing bag. The bag was connected to the inlet of a respirator and a cat was exposed to MIBK vapor (5 min/concentration) according to a sequence of increasing concentrations (*v*/*v*): 0.01% (0.41 mg/L), 0.05% (2.05 mg/L), 0.10% (4.1 mg/L), 0.25% (10.25 mg/L), 0.5% (20.5 mg/L), and 1.0% (41.0 mg/L).

Compared to controls, an MIBK concentration–dependent increase in mean pulmonary arterial pressure and pulmonary vascular resistance was observed.

Pulmonary vasoconstriction was noted at all concentrations of MIBK. The lowest test concentration (0.01% MIBK) caused a small, but significant, increase (2%, $p < .05$) in mean pulmonary arterial pressure. The greatest increase in mean pulmonary arterial pressure (9% increase) and pulmonary vascular resistance (18% increase) were noted during exposure to 1% MIBK. The preceding changes were accompanied by a steady recovery of mean pulmonary arterial flow to control levels. Concerning systemic effects, changes in mean arterial pressure were variable (i.e., no overall pattern observed). Mean left atrial pressure remained unchanged. Nonsignificant increases (3% to 4%) in mean arterial pressure and systemic vascular resistance were noted during the inhalation of MIBK concentrations ranging from 0.01% to 0.25%. MIBK (1%) induced a nonsignificant 4% to 5% decrease in mean arterial pressure and systemic vascular resistance.

In this same report, the effect of MIBK inhalation on respiratory responses was evaluated using eight male cats (weights = 2.7–3.3 kg). The animals (free-breathing, close-chest) were exposed to the following vapor concentrations of MIBK (*v*/*v*): 0.01%, 0.05%, 0.10%, 0.25%, 0.5%, and 1.0% (5 min exposure/test concentration). Like the preceding experiment, these vapor concentrations were prepared in breathing bags. A constant stream of vapor was provided by a pump that was placed between the vapor bag and the inlet port (connected to the tracheal cannula).

Compared to controls, MIBK induced significant changes in airway resistance and transpulmonary pressure. The first statistically significant increases in transpulmonary pressure and airway resistance were observed during ventilation with 0.10% and 0.05% MIBK, respectively. These two parameters reached a maximum during the inhalation of 0.5% MIBK. Decreased dynamic compliance, beginning with the inhalation of 0.05% MIBK and reaching a maximum during 0.25% MIBK inhalation, was also noted. The magnitude of these bronchopulmonary responses was classified as somewhat attenuated during the inhalation of 1.0% MIBK. Bronchoconstriction was the primary finding in these inhalation experiments. This effect was characterized by a small but statistically significant increase in pulmonary resistance and no increase in tracheal air flow (Zakhari et al. 1977).

Dogs

Zakhari et al. (1977) administered the following concentrations of MIBK to dogs of either sex (weights = between 18 and 24 kg) via the inlet of a respirator: 0.01%, 0.05%, 0.10%, 0.25%, 0.50%, and 1.0%. The duration of exposure to each concentration was 5 min. The chest of each animal was opened and various hemodynamic parameters were studied.

At a concentration as low as 0.05%, MIBK induced an increase in mean pulmonary arterial pressure (5.1% increase), effective mean pulmonary arterial pressure (5.8% increase), and pulmonary vascular resistance (6.9% increase). The increase in each parameter was intensified at higher concentrations.

Exposure concentrations up to 0.5% induced either no effect or a nonsignificant decrease in myocardial contractility. However, exposure to 1% MIBK induced a significant decrease (15.9%) in myocardial contractility. Significant decreases in left ventricular pressure and systemic vascular resistance were also noted. The observed increases in heart rate were described as consistent and concentration dependent, but statistically nonsignificant. Decreases in mean pulmonary arterial flow, stroke volume, and stroke work were observed at high concentrations of exposure (0.5% and 1.0% MIBK) (Zakhari et al. 1977).

Short-Term Oral Toxicity

Batyrova (1973) reported that the administration of increasing oral doses of MIBK (emulsion in 2% starch solution) resulted in the death of 9 of 10 mice by day 24 of dosing. The first animal deaths were noted on day 8 (total dose of MIBK = 3.82 g/kg). In most of the animals, severe clonic-tonic spasms occurred prior to death. The total average lethal dose was 9.35 g/kg.

The Carnegie Mellon Institute of Research (1983) administered MIBK at concentrations of 0.5% and 1.0% in drinking water to two groups of three Wistar female rats (4 weeks old), respectively, for 7 days. Two groups of three rats served as untreated controls. Pale kidneys were noted in two of three rats dosed with 1% MIBK, and all three rats dosed with 0.5% MIBK had pale, mottled kidneys. Similar findings were reported for both untreated control groups. The authors concluded that no evidence of gross pathologic effects was observed in animals dosed with MIBK. The results of the subchronic study are included in the section on Subchronic Oral Toxicity later in this report.

Short-Term Dermal Toxicity

McOmie and Anderson (1949) made seven applications (3 ml/kg each, 5 to 12 h) of undiluted MIBK to a 100-cm^2 area of shaved skin on each of two rabbits over a period of 15 to 21 days. At microscopic examination, there were no pathologic changes in the internal organs that resulted from exposure to MIBK. Local changes in the skin consisted of polymorphonuclear infiltration in the upper dermis. Hair follicles appeared normal, and there was no evidence of sloughing of keratin. No systemic effects were noted. Irritation reactions are included in the section on Skin Irritation later in this report.

Short-Term Inhalation Toxicity

Mice

McOmie and Anderson (1949) subjected 10 mice to 15 20-min exposures to a saturated air–MIBK vapor mixture (74 to 98 mg/L of air). Deaths (six animals) occurred on days 1, 6, and 9 post inhalation.

CMA (1981) stated that, following daily exposures to 20,000 ppm MIBK for 15 days (20 min/day), 6/10 exposed mice died.

The Bushy Run Research Center (1982) exposed three groups of $B6C3F_1$ mice (six males, six females) to MIBK at concentrations of 101 ppm (44 mg/m^3), 501 ppm (2050 mg/m^3), and 1996 ppm (8180 mg/m^3), respectively, for 9 days (6 h/day). A fourth group served as the untreated control. The first 5 days and the remaining 4 days of exposure were separated by a 2-week nontreatment period. A fourth group served as the control.

At the highest exposure concentration (1996 ppm MIBK), an increase in liver weight (as a % of body weight) was observed in female mice, but not in male mice. A significant increase in both absolute and relative kidney weights (females) and a decrease in relative kidney weight (males) were also noted in the 1996 ppm exposure group. No ophthalmological lesions or alterations in body weight resulted from exposure to 1996 ppm MIBK. No statistically significant effects on liver weight, kidney weight, or other organ weights were observed in mice exposed to 501 ppm MIBK.

At a concentration of 101 ppm, a statistically significant decrease in liver weight (as a % of body weight) was observed in male mice but not in female mice. No significant changes in kidney weight or other organ weights were noted in male or female mice exposed to 101 ppm MIBK. Compared to controls, no statistically significant histologic lesions were observed at any of the concentrations tested (Bushy Run Research Center 1982).

Rats

The Bushy Run Research Center (1982) exposed three groups of F-344 rats (six males, six females) to MIBK at concentrations of 101 ppm (44 mg/m^3), 501 ppm (2050 mg/m^3), and 1996 ppm (8180 mg/m^3), respectively, for 9 days (6 h/day). A fourth group served as the untreated control. The first 5 days and the remaining 4 days of exposure were separated by a 2-day nontreatment period.

In the highest dose group (1996 ppm), an increase in liver weight (as % of body weight) and a significant increase in both absolute and relative kidney weights were noted in male and female rats. Epithelial regeneration of the proximal convoluted tubules was also noted at 1996 ppm. No ophthalmological lesions or alterations in body weight resulted from exposure to 1996 ppm MIBK. In the 501-ppm exposure group, a nonsignificant increase in kidney weight and a statistically significant increase in liver weight were observed in male rats, but not in female rats. In both 501- and 1996-ppm exposure groups, hyaline droplet formation was observed in the kidneys of male rats. No microscopic abnormalities were noted in rats exposed to 101 ppm MIBK (Bushy Run Research Center 1982).

Phillips et al. (1987) conducted a 2-week probe study on MIBK using male and female Fischer-344 rats and B6C3F1 mice; six males, six females per group per species. Groups within each species were exposed to MIBK at concentrations of 100, 500, and 2,000 ppm, respectively, 6 h per day, 5 days/week for 2 weeks. A fourth group per species served as the untreated control. None of the animals died. A slight increase in both

absolute and relative liver weight was noted in male rats exposed to 2000 ppm MIBK. The only microscopic changes reported were increases in regenerative tubular epithelium and hyaline droplets in the kidneys of male rats exposed to 500 or 2000 ppm MIBK.

Hazleton Labs, Inc. (1992) evaluated the short-term inhalation toxicity of MIBK using young adult albino rats (Charles River Caesarian-derived strain; 10 males, 10 females). The animals were exposed to MIBK at a concentration of 4.53 mg/L of air 5 days per week (6 h/day) for 4 weeks. The control group was exposed to filtered room air. No signs of irritation (i.e., nasal bleeding) were observed in any of the test animals throughout the entire exposure series, although slight nasal bleeding was noted in the control group. Compared to the control group, exposed females had a significantly higher adrenal/body weight ratio. Additionally, exposed females had a 1.4% increase in lymphocytes and a 1.0% decrease in segmented neutrophils.

Multiple Species

In a range-finding study, a laboratory at the Wright-Patterson Air Force Base Aerospace Medical Research Laboratory (1971) continuously exposed 4 monkeys, 8 dogs, 40 mice, and 50 rats to a mean concentration of 100 ppm MIBK for 2 weeks. Control groups consisted of 3 monkeys, 4 dogs, 20 mice, and 25 albino rats.

A comparison of the results for test and control groups revealed no signs of toxicity during exposure, no differences in cortical activity (based on electroencephalogram [EEG]), no differences in hematologic or clinical chemistry measurements between dogs or monkeys, and, no differences at gross examination of tissues. However, compared to controls, a significant increase in kidney weight and in the kidney-to-body weight ratio ($p < .01$) was noted in rats exposed to MIBK. Growth was also slightly depressed in rats.

When this experiment was repeated at a higher level of exposure (200 ppm MIBK), the following statistically significant effects were reported: increased kidney weight and kidney-to-body weight ratio ($p < .01$), increased liver weight and liver-to-body weight ratio ($p < .01$), and increased heart-to-body weight ratio ($p < .05$). In both experiments (rats), the kidneys were primarily affected. At microscopic examination, toxic nephrosis of the proximal tubules was observed in tissues from rats exposed to 100 and 200 ppm MIBK (Wright-Patterson Air Force Base Aerospace Medical Research Laboratory 1971).

Subchronic Inhalation Toxicity

Mice

In a Union Carbide Corporation (1983) study, subsequently reported by Phillips et al. (1987), three groups of $B6C3F_1$ mice (14 males, 14 females) were also exposed to the same concentrations of MIBK in the rat study described above. A fourth group served as the control.

Growth retardation was not observed in any of the animals tested. A slight increase in liver weight (~11%) and in the liver weight per body weight ratio was noted in male mice exposed to 1000 ppm MIBK. Liver weight was also slightly increased in male mice exposed to 250 ppm MIBK. Exposure to 1000 ppm MIBK resulted in no hepatic lesions at gross necropsy or microscopic examination and urinalysis and serum chemistry values were normal.

Rats

Batyrova (1973) exposed a group of 70 rats to MIBK at concentrations ranging from 86 to 127 mg/m^3 (average concentration = 115 ± 14 mg/m^3) 5 days per week (4 h/day) for 4.5 months.

An increase in the time required for traversing a maze was observed in rats with a previously developed and reinforced food reflex. The conditioned reflex was less clearly developed and easily slowed during the action of random external stimuli. Disruption of the speed of extinction of the elementary defensive reflex (in Lyubimov chamber) and the ability to practice the reflex at the end of exposure was also noted. Other findings included narcosis, disruption of the detoxifying function of the liver, and decreased eosinophil count. Compared to controls, the number of eosinophils in the blood of test animals was noticeably less. It was also noted that the adrenaline load and painful irritation yielded no differences in the eosinopenic reaction in test and control rats.

The author also reported decreased weight of the liver and adrenal glands in animals exposed to MIBK compared to controls. Weight coefficients of internal organs were compared at 2 months after initiation of exposure, at the end of exposure, and at 1 to 2 months after the end of exposure. Disruption of blood circulation and dystrophic changes in the parenchymatous elements (up to necrobiosis) were detected in the central nervous system and in the most important internal organs.

In another experiment by this author, subchronic exposure to MIBK at a concentration of 30 mg/m^3 induced insignificant changes in the function of the central nervous system, which is said to be most sensitive to the effects of MIBK (Batyrova 1973).

In a Union Carbide Corporation (1983) study, subsequently reported by Phillips et al. (1987), three groups of F-344 rats (14 males and 14 females in each group) were exposed to 50 ppm (205 mg/m^3), 250 ppm (1025 mg/m^3), and 1000 ppm (4100 mg/m^3) MIBK 5 days per week (6 h/day) for 90 days. A fourth group served as the untreated control. Growth retardation was not observed in any of the animals tested. A slight increase in liver weight (~11%) and in the liver weight per body weight ratio was noted in male rats exposed to 1000 ppm MIBK. It is important to note that exposure to 1000 ppm MIBK resulted in no hepatic lesions at gross necropsy or microscopic examination and that urinalysis and serum chemistry values were normal. However, an increase in the number of hyaline droplets in the proximal tubular cells of the kidney was noted in male rats of the 250- and 1000-ppm exposure groups. No other gross or microscopic changes in the kidney were observed. The authors stated

that the significance of an increase, compared to controls, in the occurrence of hyaline droplets in male rats was not known. Additionally, it was noted that the presence of hyaline droplets did not appear to be associated with major alterations in kidney function.

According to Alden et al. (1984), increased hyaline droplet formation is thought to be related to a rat-specific protein, α-2u-globulin, which is found predominantly in male rats. Alden (1986) indicated that the hyaline droplet, renal effects observed in male rats exposed to MIBK may be specific to the male rat, and, therefore, these effects (in male rats) do not constitute an appropriate model for man.

In a commentary on the Union Carbide study above, EPA (1991) noted that evidence of increased renal α-2u-globulin levels (indicative of alpha 2u-globulin nephropathy) was not reported.

Multiple Species

The Wright-Patterson Air Force Base Aerospace Medical Research Laboratory (1971) conducted a subchronic inhalation toxicity study of MIBK in three species: rats, dogs, and monkeys. Male Wistar albino rats (100), male Beagle dogs (8), and male *Macaca mulatta* monkeys (2) were exposed to 410 mg/m^3 MIBK vapor (100 millimoles/25 m^3) for 90 days in an altitude chamber. The control group (no MIBK exposure) was maintained in a separate altitude chamber. Liver function tests (dogs only) involved the intravenous injection of bromsulphalein, followed by determination of the dye concentration 15 min later. Tissue sections from the following organs (test and control animals) were subjected to gross and microscopic examination: heart, lung, brain, liver, spleen, kidney, adrenal glands, and pituitary gland.

The results of clinical chemistry and hematology tests on dogs and monkeys revealed no biologically significant differences between test and control animals. There were also no significant differences in liver function test results between test and control dogs. Gross examination also revealed no differences in the tissues examined (heart, lung, brain, liver, spleen, kidney, adrenal glands, and pituitary gland) between test and control animals.

Microscopic examination of kidney sections revealed hyaline droplets in one test and one control dog, fat in a few tubules at the corticomedullary junction in dogs (classified as common finding in untreated dogs), and focal chronic inflammation of the kidney in one monkey. Statistically significant increases in liver and kidney weights and organ-to-body weight ratios for these tissues were noted in rats exposed to MIBK. This increase in liver weight was not associated with any pathological changes. However, microscopic examination of kidneys revealed hyaline droplet degeneration of the proximal tubules (with occasional foci of tubular necrosis) in each of the 100 rats exposed to MIBK, including those that were removed from the inhalation chamber after 15, 22, 28, 71, and 85 days. It is important to note that a trend toward a linear progression of hyaline droplet degeneration during exposure was observed, but that this pattern was not associated with all animals. Additionally, the hyaline droplets appeared larger with time. This observation was thought to have resulted from the coalescence of smaller droplets.

Microscopic examination of rat kidneys, removed after 15 days of exposure, indicated a gradual reversion of tubular damage with time. Kidney damage was completely reversed in rats observed up to 60 days post exposure. Recovery from MIBK-induced kidney lesions was also noted in rats that were serially killed for reversibility studies after 90 days of exposure. However, recovery was not as rapid as that noted in animals exposed for shorter periods. Growth rate (rats) was unaffected by continuous exposure to MIBK (Wright-Patterson Air Force Base Aerospace Medical Research Laboratory 1971).

Subchronic Oral Toxicity

In a study reported by the Carnegie Mellon Institute of Research (1983), the subchronic oral toxicity of MIBK was evaluated using five Wistar female rats (4 weeks old). MIBK was administered at a concentration of 1.3% in drinking water daily for 120 days (MIBK dose = 1.04 g/kg/day). Two groups of five rats that received tap water served as untreated controls. Neurological evaluations for any treatment-related effects during the study included observations of any changes in balance, strength, coordination, or behavior. The animals were killed by CO_2 narcosis and subjected to gross and microscopic examination. Body weight gain in animals dosed with MIBK was not significantly different from that noted in controls. MIBK induced a statistically significant increase in relative and absolute kidney weight. No significant gross lesions were noted in any of the tissues examined. Tubular cell hyperplasia was noted in the kidney of one of the five rats. Results concerning the neurotoxicity of MIBK are included in the section on Neurotoxicity later in the report text.

WHO (1990) described a subchronic oral toxicity study of MIBK using three groups of Sprague-Dawley rats (30 males, 30 females). The test substance was administered to the three groups in doses of 50, 250, and 1000 mg/kg, respectively, daily for 13 weeks. All animals that survived were killed at the end of the dosing period. Ten animals (five males, five females) from each treatment group were subjected to gross and microscopic examination. In the highest dose group (1000 mg/kg), nephrotoxicity and increased liver and kidney weights were observed in males and females. Hepatic lesions were not observed at microscopic examination. These effects were significantly less pronounced in females and males of the 250-mg/kg dose group, and were not observed in the 50-mg/kg dose group. Thus, the 50-mg/kg dose of MIBK was considered the no-observed-effect level.

Subchronic Dermal Toxicity

Malyscheva (1988) evaluated the dermal toxicity of MIBK (in sunflower oil) using white rats (males, number not stated). The test substance was applied to the tail (lower 2/3) of each animal daily in doses of 300 mg/kg. Intermittent application involved 600-mg/kg doses. The duration of the study was 4 months.

Control animals were dosed with sunflower oil according to the same test procedures.

The intermittent application of MIBK resulted in an undulatory increase in the activity of copper-containing oxidase. At 1 month of daily application, there was a sharp increase (82.4%) in enzyme activity. This was not a consistent finding following this type of exposure because a 7% to 20% increase in enzyme activity was noted in other groups dosed according to the same procedure. At 2 months of daily application, a 160% increase in enzyme activity was noted.

In the control group and the group subjected to intermittent administration for 2 months, the increase in enzyme activity was 60% to 70%. Following 3 months of administration, the increases in enzyme activity were as follows: intermittent administration (39.67%), daily administration (101.93%), and control group (32.6%).

An increase in the number of binuclear hepatocytes, reduced mitotic activity of these cells, and an increase in the number of hepatocytes with pathology were observed in the liver. Morphological changes in the internal organs (liver, adrenal glands, spleen, and testes) and skin of white rats were noted after monotonic administration of MIBK. The following changes were observed in the liver: increase in number of binuclear hepatocytes, decrease in mitotic activity of the cells, and an increase in the number of hepatocytes with pathology. A reduction in the lipid content of the cortical layer was observed in the adrenal glands and changes in the spleen included a reduction in the number of reactive centers in follicles and an increase in the number of iron-containing pigments in the area of the red pulp. Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa. The daily application of MIBK caused changes in the skin. Mitotic activity in the basal layer and the sprout layer of the hair follicles was reduced, and the thickness of the horny layer of the epidermis and the granular cell layer was increased. Following intermittent exposure, similar, but smaller, changes in the skin and liver were observed (Malyscheva 1988).

Chronic Intraperitoneal Toxicity

MIBK was injected intraperitoneally into rats (strain and number not specified) five times per week for 35 weeks. During the first 2 weeks the animals were injected with doses of 10, 30, or 100 mg/kg body weight. For the remainder of the study, the doses were doubled. After 3 to 4 weeks of dosing, body weight gain suppression was noted. Transient narcosis was observed during the first 4 weeks of treatment with the highest dose (100 mg/kg) (CMA 1981).

Effect of MIBK on the Hepatotoxicity of Other Agents

1,2-Dichlorobenzene

Brondeau et al. (1989) investigated the effect of MIBK on the hepatotoxicity of inhaled 1,2-dichlorobenzene (DCB) using male Sprague-Dawley rats and OF1 mice. The aim of this study was to compare the interactive liver responses of MIBK with DCB and to explain them in terms of metabolic changes. Thus, in rats, the mitochondrial enzyme glutamate dehydrogenase (GLDH) was assessed in serum as a quantitative sign of hepatic necrosis, and liver cytochrome P-450 content and glutathione-*S*-transferase (GST) activity as indicators of phase I and phase II metabolic effects, respectively. Three groups of rats (five per group) were exposed to MIBK at concentrations of 595, 1280, and 3020 ppm, respectively, for 4 h in 200-L inhalation chambers. After an 18-h nontreatment period, the rats were exposed to 377 ppm DCB for 4 h. Three groups of eight mice were exposed to MIBK at concentrations of 664, 1477, and 3260 ppm, respectively, followed by exposure to 263 ppm DCB, according to the same procedure. Control mice and rats inhaled air only. At 24 h post exposure to DCB, the rats were exsanguinated from the abdominal aorta, and serum GLDH activity was measured. The livers were removed, homogenized, centrifuged, and the microsomal pellet that resulted from ultracentrifugation was assayed for cytochrome P-450 activity. The mice were killed at 48 h post exposure to DCB. Liver glucose-6-phosphatase (G-6-Pase) staining intensity was measured in the periportal, mediolobular and centrilobular areas.

Exposure of rats to MIBK alone did not cause any modifications in serum GLDH activity. However, exposure to DCB alone increased GLDH activity. Preexposure to 595, 1280, or 3020 ppm MIBK caused a dose-related increase in DCB-induced GLDH activity. Compared to controls, MIBK caused a dose-dependent elevation of rat liver cytochrome P-450 content. Rat liver GST activity was also increased following exposure to MIBK.

In the mice, neither MIBK nor DCB alone induced any constant effect on G-6-Pase staining intensity. However, successive exposure to MIBK and DCB induced a significant decrease in G-6-Pase staining intensity, varying from 29% to 49% when compared to the DCB-only exposed group.

The authors summarized their results as follows: MIBK increased liver cytochrome P-450 content and GST activity, but did not affect serum GLDH activity in rats. Preexposure to MIBK enhanced the DCB-induced increase in serum GLDH activity, whereas the increases in cytochrome P-450 content and GST activity were identical to those resulting from exposure to MIBK alone. In mice, MIBK interacted with DCB on centrolobular liver G-6-Pase (Brondeau et al. 1989).

Chloroform

According to Vézina, Ayotte, and Plaa (1985), a single oral dose of MIBK administered to male Sprague-Dawley rats enhanced the hepatotoxicity of a single IP dose of chloroform that was administered 24 h later. The no-observed-effect level of MIBK was 375 mg/kg, and 560 mg/kg was the minimal-effect level.

A more recent oral study by Vézina et al. (1990) exposed male Sprague-Dawley rats to MIBK and its two major metabolites, 4-MPOL and 4-hydroxymethyl isobutyl ketone. The authors

reported significant increases in liver damage induced by chloroform (0.5 ml/kg, dissolved in corn oil to yield 10 mL/kg). Three doses of each chemical were administered orally to three groups of six animals, respectively (total of 9 groups) 24 h before dosing with chloroform. The minimally effective dose of MIBK and each of the two metabolites that was needed for potentiation of chloroform-induced hepatotoxicity was approximately 5 mmol/kg. Liver damage was demonstrated by elevation of the plasma activity of two transferases, alanine aminotransferase and ornithine carbamoyl transferase, and by the severity of the morphological changes (necrosis and inflammation) observed.

In a second series of experiments, these same authors studied the enzyme inducing properties of MIBK. They assayed cytochrome P-450 liver content and the activity of aniline hydroxylase, 7-ethoxycoumarin *O*-deethylase, and aminopyrine *N*-demethylase. The liver content of cytochrome P-450 and the oxidation of aniline and 7-ethoxycoumarin were significantly increased following a single oral dose (7.5 mmol/kg or greater) or multiple doses (5.0 and 7.5 mmol/kg/day for 5 days) of MIBK. Repetitive administration of MIBK also caused an increase in the activity of aminopyrine demethylase. MIBK also caused a significant increase in the 52.1- and 54.1-kDa microsomal proteins, which probably corresponded to cytochrome P-450 isozymes (Vézina et al. 1990).

Other Agents

Krishnan et al. (1992) evaluated the effect of MIBK on hexachlorobenzene (HCB)-induced hepatic porphyria using groups of female Sprague Dawley rats (weights = 125–150 g). The first dosing schedule consisted of the simultaneous oral administration of HCB (50 mg/kg in 10 ml/kg corn oil daily, 5 days per week) and MIBK (7.5 mmol/kg in 10 ml/kg corn oil daily, 3 days per week) for 6 weeks. The second dosing schedule consisted of initial oral dosing with 25 or 50 mg HCB/kg daily for 12 consecutive days. Dosing with HCB was followed by oral dosing with 7.5 mmol MIBK every other day for 27 days. The simultaneous administration of HCB and MIBK resulted in a reduction in the severity of HCB-induced porphyria. The sequential administration procedure for both chemicals (MIBK dosing after initial dosing with HCB) resulted in enhancement of the porphyrinogenic response. The authors concluded that the effect of combined exposure to HCB and MIBK on hepatic porphyria depends on the sequence of administration of both chemicals. Furthermore, it was suggested that the mechanism involved in this interaction may invoke both the induction and inhibition of specific hepatic isoenzymes by MIBK.

Raymond and Plaa (1995b) dosed groups of 12 male Sprague-Dawley rats (weights = 175–200 g) orally with the hepatotoxicant, carbon tetrachloride (in corn oil) 18 h after oral dosing with MIBK in corn oil. MIBK was administered at potentiator dosages of 0.3, 1.5, 3.0, 12.0, or 20 mmol/kg, and carbon tetrachloride was administered at dosages of 0.005, 0.01, 0.05, 0.1, and 0.5 ml/kg. The extent of potentiation of carbon tetrachloride–induced hepatotoxicity in male rats was found to be dependent on MIBK and carbon tetrachloride concentrations. MIBK administration induced a dose-dependent potentiation of carbon tetrachloride toxicity. Hepatotoxicity was indicated by an increase in plasma alanine transaminase activity and the concentration of bilirubin.

The MED of MIBK decreased 10-fold when the dose of carbon tetrachloride was increased from 0.01 ml/kg to 0.1 ml/kg. The MED was defined as the smallest dose of a potentiator that was able to produce a statistically significant enhanced response to carbon tetrachloride–induced injury. The results of this study suggested that a given level of liver injury induced by a ketone-haloalkane combination could be evaluated on the basis of the potentiator × hepatotoxicant product (Pilon, Brodeur, and Plaa 1988).

Ocular Irritation

McOmie and Anderson (1949) evaluated the ocular irritation potential of undiluted MIBK using one rabbit. Reactions were scored according to the Draize scale (0 to 110). Draize irritation scores were 8, 3, and 1 at 1, 24, and 72 h post instillation, respectively. The test substance induced conjunctivitis, with some edema and corneal injury. Light accommodation was unaffected, and pupillary damage was not observed. The eye was described as grossly normal at day 7 post instillation

CMA (1981) stated that ocular irritation was observed within 10 min after instillation of undiluted MIBK (0.1 ml) into the rabbit eye. Inflammation and conjunctival swelling were noted within 8 h post instillation. Inflammation, swelling, and exudate were evident at 24 h; however, reactions had cleared by 60 h.

The Exxon Chemical Company (1982) evaluated the ocular irritation potential of MIBK using six albino rabbits. Undiluted MIBK (0.1 ml) was instilled into the left conjunctival sac of each animal. Untreated eyes served as controls. Reactions were scored at 1, 4, and 24 h and at 2, 3, 4, and 7 days post instillation according to the Draize scale (0 to 110). Additional readings at 10 and 14 days were taken, depending on the types of reactions that were observed. Blinking was observed in all six animals immediately after instillation. One animal had slight iritis at 1 and 4 h, which had cleared by 24 h. Slight to moderate conjunctivitis was noted in all rabbits from 1 h to day 2 post instillation. Reactions had cleared within 4 days post instillation. Corneal reactions were not observed throughout the experiment in any of the animals tested. MIBK induced slight, transient ocular irritation.

Kennah et al. (1989) studied the ocular irritation potential of MIBK using New Zealand albino rabbits (four to six animals). The test substance (0.1 ml) was instilled into the conjunctival sac of one eye of each animal. Untreated eyes served as controls. The cornea, iris, and conjunctiva were scored at days 1, 2, 3, 7, 10, 14, and 21 post instillation. A Draize score was computed at each observation period by averaging the total scores of all rabbits tested. Draize scores of 5 and 2 (110 max) were reported for 100% and 2% MIBK, respectively. It was concluded that MIBK induced mild ocular irritation in rabbits.

Gautheron et al. (1994) evaluated the ocular irritation potential of undiluted MIBK in the Draize test using four to six rabbits. The test substance (0.1 ml) was instilled into the conjunctival sac of one eye of each animal. A Draize score of 5 (maximum = 110) was reported.

Skin Irritation

McOmie and Anderson (1949) made seven applications (3 ml/kg each, 5 to 12 h) of undiluted MIBK to a 100-cm^2 area of shaved skin on each of two rabbits over a period of 15 to 21 days. Drying of the skin and some exfoliation were the only reactions observed.

These same authors applied undiluted MIBK (10 h of exposure) to the skin of two rabbits either by flooding the test site or placement of a cotton pad impregnated with the test substance. The reactions observed were classified as immediate (moderate erythema) and delayed (erythema persisting for 24 h). Additional study results are included in the earlier section on Acute Dermal Toxicity (McOmie and Anderson 1949).

Batyrova (1973) reported that the immersion of the ear of a rabbit and the tails of mice in pure MIBK for 2 h resulted in pronounced inflammation and necrosis of the tissues. In another experiment in the same study, no noticeable skin changes were observed in guinea pigs subjected to brief exposures to MIBK over a period of 3 months.

In a series of studies reported by CMA (1981), rabbits (shaved skin) were patch tested with MIBK in a single, 10-h occlusive patch test. Erythema was observed for up to 24 h post application. Drying and flaking of the skin surface were observed after MIBK was applied to the skin of rabbits daily (10 ml/day) for 7 days. Slight skin irritation was observed after undiluted MIBK (5 and 10 ml) was applied (under occlusive wrap) to depilated skin of guinea pigs for 24 h. Reportedly, there was no clinical evidence of absorption. MIBK (500 mg) induced moderate irritation of rabbit skin after a contact period of 24 h. The application of MIBK (2 ml) to the backs of guinea pigs daily for 31 days caused desquamation, but no clinical or histologic evidence of toxic neuropathy.

The Exxon Chemical Company (1982) evaluated the skin irritation potential of MIBK using 12 albino rabbits. The application sites of six animals were abraded. Four gauze patches (adhesive backing), each containing 0.5 ml MIBK, were applied to clipped abdominal skin of each animal. Patches were secured with dental damming and gauze binders for 24 h. Reactions were scored at 24 and 72 h post application according to the following scales: 0 (no erythema) to 4 (severe erythema [beet redness] to slight eschar formation [injuries in depth]) and 0 (no edema) to 4 (severe edema [raised more than 1.0 ml, extending beyond the area of exposure]). At 24 h post application, very slight erythema was observed at three intact skin sites (three animals respectively) and no signs of irritation were noted in the remaining three animals (intact skin sites). Slight or well-defined erythema at all abraded sites (six animals) was noted at 24 h; very slight edema was observed in two of the animals. At 72 h post application, very slight erythema was observed in two animals (abraded application sites); no signs of irritation were observed in the remaining animals (abraded or intact skin). MIBK induced slight, transient erythema (primary irritation score = 0.75).

NEUROTOXICITY

Oral Dosing

The Carnegie Mellon Institute of Research (1983) administered MIBK to each of five Wistar female rats (4 weeks old) at a concentration of 1.3% in drinking water daily for 120 days (MIBK dose = 1.04 g/kg/day). Two groups of five rats that received tap water served as untreated controls. Neurological evaluations for any treatment-related effects during the study included observations of any changes in balance, strength, coordination, or behavior. The animals were killed by CO_2 narcosis and subjected to gross and microscopic examination. MIBK did not induce any significant neurologic alterations. Additionally, no discernible neurotoxic gross effects were noted. Additional results for this subchronic study are included in the section on Subchronic Oral Toxicity earlier in this report.

Nagano et al. (1988) reported that the maximum motor-fiber conduction velocity in the tail nerve of male rats (number and strain not stated) was unaffected by treatment with MIBK (601 mg/kg, 5 times/week for 55 weeks). However, treatment with MIBK (201 mg/kg) facilitated the neurotoxic effect of methyl *n*-butyl ketone (401 mg/kg).

Intraperitoneal Injection

In a study by the Eastman Kodak Company (1977), the neurotoxicity of MIBK was evaluated using three groups of 12 Sprague-Dawley albino rats. The three groups were injected intraperitoneally with MIBK (10% in corn oil) at doses of 10, 30, and 100 mg/kg, respectively for 2 weeks. At the end of the 2-week period, the doses were increased to 20, 60, and 200 mg/kg, respectively. The new doses were injected intraperitoneally 5 days per week for 33 weeks. The test groups were referred to as low-, mid-, and high-dose groups. Control rats (group of 12) were injected with corn oil for 2 weeks and then distilled water for the remainder of the study. At the end of the study, some of the surviving animals in the highest dose group were killed and tissues (sciatic, tibial, peroneal, and sural nerves and interosseous muscles from the right hindlimb) subjected to microscopic examination. Tissues (spinal cord, medulla, and sciatic nerve) from some of the survivors of all dose groups were also examined microscopically.

The mortality rate per dose level was comparable to that noted in the control group. A significant decrease (>10%, compared to control group) in mean body weight gain was noted only in the high-dose group. This finding was first noted after 17.5 weeks and persisted to the end of the study. The following non-neural lesions were observed in test animals: chronic respiratory disease (2 rats—high dose; 1 rat—low dose), peritonitis

(4 rats—high dose), bone marrow hyperplasia (1 rat—high dose), and increased splenic hematopoiesis (1 rat—high dose). These pathologic changes were either spontaneous occurrences or were due to an irritative property of the test substance. Tissue lesions (neural and non-neural) were not observed in the control group. Except for a transient anesthetic effect in the high-dose group, observed initially after 1 month of dosing, no neurologic signs were observed in either of the test groups. At microscopic examination, senile changes in the nucleus gracilis of the medulla oblongata were observed in one mid-dose and one low-dose animal, but not in high-dose or control animals. It was concluded that MIBK did not induce peripheral neuropathy when injected intraperitoneally at doses up to 200 mg/kg (Eastman Kodak Company 1977).

Sharkawi et al. (1994) studied the effect of MIBK on the duration of ethanol-induced loss of righting reflex and on ethanol elimination using two groups of seven Charles River CD-1 mice (weights not stated). MIBK was dissolved in corn oil and injected intraperitoneally (2.5 or 5.0 mmol/kg) 30 min before ethanol (4 g/kg, intraperitoneally). MIBK significantly prolonged the duration of ethanol-induced loss of righting reflex when administered at a dose of 5 mmol/kg. The concentrations of ethanol in blood and in the brain upon return of the righting reflex were similar in MIBK-treated and control animals. MIBK did not induce ataxia or loss of righting reflex in any of the mice at doses of 2.5 or 5 mmol/kg (Cunningham et al. 1989). MIBK (5 mmol/kg) also prolonged the duration of ethanol-induced loss of righting reflex in a more recent study involving CD-1 mice.

Intravenous Injection

Tham et al. (1984) evaluated the influence of MIBK on the vestibulo-oculomotor reflex (VOR) of female Sprague-Dawley rats (number not stated; weights = 250–300 g). The effect of MIBK on VOR was studied by recording nystagmus that was induced by accelerated rotation. The VOR connects the labyrinth with the eye muscles via the brainstem, thereby eliciting ocular movements in response to acceleration or deceleration of the head. The test substance (in an emulsion of lipids) was administered by continuous intravenous infusion for 60 min. Test concentrations varied between 0.1% and 10%. MIBK had a depressive effect on the VOR. The threshold limit for this effect was 0.2 mM/L (20 ppm) at an infusion rate of 30 μM/kg/min. It was suggested that solvents cause depression or excitation of the VOR by interaction with central pathways in the reticular formation and the cerebellum.

Subcutaneous Injection

Spencer and Schaumburg (1976) reported a study in which 4 cats (weights = 2–3 kg) were injected subcutaneously with 150 mg undiluted MIBK/kg body weight twice daily, 5 times/week, for up to 8.5 months. Injection sites on the back were rotated. The composition of the test substance was as follows: MIBK (98.79%), methyl *n*-butyl ketone (0.94%), acetone (0.02%), other light impurities (0.14%), and heavy impurities (0.11%). A group of four control cats received subcutaneous doses of saline (0.2 ml/kg) 5 days per week for up to 5 months. None of the animals died. Biopsies were taken from the right and left hind feet after 45 and 135 days of dosing with MIBK. Biopsy results indicated no detectable damage to nerve tissues.

The Eastman Kodak Company (1982b) evaluated the neurotoxicity of MIBK using four purebred, male Beagle dogs (9 to 30 months old). Each dog was injected subcutaneously with a dose of 300 mg/kg daily for approximately 11 months and then subjected to electromyographic examination. No evidence of neurotoxicity was noted in either of the four dogs tested.

In another study using dogs, the neurotoxicity of MIBK was evaluated (four dogs; mean age = 13 months). MIBK was >98% pure and also consisted of 0.9% methyl *n*-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane. The test substance was administered subcutaneously at a dose of 150 mg/kg twice daily for a year. The animals were necropsied at the end of the study. No evidence of systemic toxicity or neurotoxicity was observed in any of the animals tested (Eastman Kodak Company 1992).

Inhalation Exposure

Rats

Spencer et al. (1975) studied the neurotoxicity of MIBK in six young adult rats (ages and strain not stated). The animals were exposed to 1500 ppm MIBK in an 18.5-L glass exposure chamber for up to 5 months. The animals were then killed and tissues (muscle, brain, and peripheral nerves) subjected to gross and microscopic evaluation. Weight gain was described as normal. Slight narcosis was observed during exposure. However, no signs of neurological dysfunction were noted at the end of the exposure period. Consistently, many axons containing large numbers of dilated, glycogen-filled mitochondrial remnants, adaxonal Schwann cell invaginations, and rare focal swellings were noted in the most distal portions of the tibial and ulnar nerves. Distal nerve fiber degeneration was not observed. Results of examination of sampled areas of the central nervous system and proximal parts of the peripheral nervous system were unremarkable. The authors stated that the neuropathological changes noted may have been related to the presence of 3% methyl *n*-butyl ketone in the commercial grade of MIBK that was used in this study.

In a study by Geller, Rowlands, and Kaplan (1978), the effect of inhaled MIBK on the lever-pressing behavior of Holtzmann, Sprague-Dawley male rats (~90 to 120 days old) on a match-to-sample discrimination task were evaluated. Rats were exposed to the test substance in chambers made of glass and steel. Animal weights were gradually reduced to 80% of the normal body weight and the animals were then trained to press a lever for a liquid food reward. A 2-min variable-interval schedule of reinforcement was used. The effect of 25 ppm MIBK on the variable response rate of one rat after the third hour of the experimental session was evaluated. The average response rate was 45 per

minute, which represented a 58% increase over the preexposure control rate of 26.5%. The response rate had not returned to control levels by day 7 post exposure.

De Ceaurriz et al. (1984) studied neurobehavioral effects of MIBK using 80 male Swiss, OF1 mice (40 controls, 40 test; weights = 20–25 g). Four test groups (10 mice/group) were exposed to test concentrations of 662, 757, 807, and 892 ppm, respectively, for 4 h in a 'behavioral despair' swimming test. At the end of each exposure period, mice were placed in a glass cylinder containing water. Neurobehavioral effects were determined by measuring the duration of immobility in this test. Transient periods of immobility were accompanied by periods of intensive swimming activity. The decrease in immobility time served as an indicator of MIBK-induced behavioral toxicity. Control groups were exposed concurrently to clean filtered air. A decrease in the duration of immobility (ID_{50} = 803 ppm) in the swimming test was reported after exposure to MIBK. The ID_{50} value was defined as the median active level that caused a 50% decrease in immobility.

In a study by Eastman Kodak Company (1996), published later by David et al. (1999), the neurotoxicity of MIBK in rats was evaluated in a 13-week (64 days of exposure) study using male Sprague-Dawley rats. Sixty five rats (CRL:CD (SD)BR/VAF Plus strain; 134 days old; weights = 338 ± 12 g) were restricted to 13 to 18 g of feed per day and used for schedule-controlled operant behavior (SCOB) testing. Systemic toxicity was evaluated using 64 rats of the same strain (68 days old; weights = 352 ± 12 g) that were fed ad libitum. Both sets of animals (20 per group) were exposed to MIBK at concentrations of 250, 750, or 1500 ppm 5 days per week (6 h/day) for 13 weeks. Untreated animals served as controls. Exposure was carried out in 420-L stainless steel and glass inhalation chambers. Each SCOB test session consisted of four fixed ratio (FR) sessions of 20 lever presses for each food pellet, followed by two fixed-interval (FI) sessions of 120 s for each food pellet. FR running rates, postreinforcement pause duration, FI response rates, and index of curvature values were presented as a mean for each animal, based on values determined on Tuesday through Friday of each week. The testing of SCOB animals continued post exposure for 2 weeks (i.e., through day 102). On day 107, 20 SCOB animals (5/group, selected at random) were perfused systemically for the collection of neurological tissues. The remaining SCOB animals were killed and necropsied on day 108. The animals in the systemic toxicity test were used for comparative purposes to determine whether feed restriction masked overt signs of systemic toxicity. These animals were killed and necropsied on day 88.

One death in a control animal was reported. Clinical signs observed during the study included minor piloerection and sialorrhea, and minimal to minor reduced activity (less movement, decreased alertness, and slower response to tapping on chamber wall). No statistically significant differences in FR running rate, FR pause duration, FI response rate, or index of curvature (each analyzed as % of baseline) were observed between test (all doses) and control groups. Thus, no differences in the performance of schedule-controlled operant behavior were noted.

Differences in body weight between animals fed ad libitum and controls were not statistically significant at either administered dose. However, for SCOB animals, only mean terminal body weights in groups exposed to 1500 ppm were significantly higher ($p \leq 0.05$) than those of controls. An increase in mean terminal body weights over those noted in controls was also reported for the 750-ppm exposure groups. Regarding organ weights of animals fed ad libitum, the mean absolute liver and kidney weights for all exposure groups and the relative (to body weight) liver and kidney weights for 750 and 1500 ppm exposure groups were statistically higher ($p \leq 0.05$) than control values. No other differences in organ weight were observed in animals fed ad libitum. In SCOB animals, the mean absolute liver weights for 750 and 1500 ppm exposure groups and the mean relative (to body weight) liver weights for 250 and 750 ppm exposure groups were statistically higher ($p \leq 0.05$) than control values. Mean absolute and relative (to body weight) kidney weights of all exposure groups were comparable to the control group. No other differences in organ weight were observed in SCOB animals.

At gross examination, no test substance–related changes were noted in SCOB animals or animals fed ad libitum. None of the tissues examined were examined microscopically. The results of this study indicate that repeated MIBK exposure did not induce changes in schedule controlled operant behavior. An exposure concentration of 1500 ppm MIBK was considered the no-observed-effect level (NOEL) for subchronic neurotoxicity (Eastman Kodak Company 1996; David et al. 1999).

Baboons

Geller et al. (1978) studied the effect of inhaled MIBK (25 to 75 ppm) on the behavior of young baboons (number and ages not stated) in a match-to-sample discrimination task. A match-to-sample task is an operant behavior procedure that measures perceptual acuity and discrimination performance. Two large stainless steel exposure chambers, for test and control animals respectively, were used. The test animals were exposed to MIBK over a 7-day period, whereas the controls were exposed to clean air. Each group was provided with an intelligence panel instrumented with a row of three round, translucent discs. Under the appropriate experimental conditions, pressing either of the two end discs would result in the release of a food pellet (reward). Each trial began with the illumination of one of the stimuli on the center key (probe stimulus). The following records were kept during the test procedure: number of probe stimuli presented during each 15-min segment, number of correct matching responses on the left and right keys and the number of incorrect responses on these keys, any extra responses, and the time required for a baboon to respond with a key press after a stimulus was activated (reaction time). Performance of the match-to-sample discrimination task was not impaired over the range of MIBK concentrations tested. However, it is important to note that one of the

baboons exposed to 50 ppm MIBK made extra responses on each day of testing. These changes were thought to reflect alterations in the animal's level of anxiety. No further increase in responses was noted when the test concentration was increased to 75 ppm for an additional 48 h. It was concluded that MIBK did not impair a baboon's ability to discriminate or remember stimuli.

In a subsequent study (Geller et al. 1979), the effect of inhaled MIBK on a delayed match-to-sample discrimination task was evaluated using four juvenile baboons (~2 years old). The animals were exposed to 50 ppm MIBK for 7 days. Two animals were exposed to the test substance and two animals served as controls (clean air exposure). Accuracy of performance was affected minimally. However, increased and decreased extra responses during the delay intervals were noted. Termination of the stimulus activated a timer for 2 min (defined as the delay interval). MIBK also caused a slowing of the response times for all four baboons during most or all of the exposure sessions. The authors stated that this effect could be an early manifestation of the incoordination and narcosis that is observed at much higher concentrations of MIBK.

In Vitro Study

Selkoe, Luckenbill-Edds, and Shelanski (1978) evaluated the neurotoxicity of MIBK using a clonal line of neuroblastoma (Neuro 2aE) derived from a spontaneously occurring murine tumor (C1300). Using light microscopy, it was determined that MIBK produced no discernible cytopathological changes in cells exposed to 0.1% MIBK for 10 days. At a concentration of 0.2%, MIBK induced a depression of growth rates; however, the cells appeared normal. MIBK (0.5%) caused widespread cell death. The cells that survived either appeared normal or a fine granular cytoplasm was observed.

GENOTOXICITY

The genotoxicity of MIBK has been evaluated in many assay systems. The results of those tests are presented in Table 2. In most assay systems, MIBK is not genotoxic. Equivocal results in a mouse lymphoma assay and a positive result in a cell transformation assay, however, were reported by O'Donoghue et al. (1988). The study and results are further described below.

O'Donoghue et al. (1988) present the results of several MIBK genotoxicity assays, including: Salmonella/microsome (Ames) assay, L5178Y/TK^{+}/$^{-}$ mouse lymphoma (ML) assay, BALB/3T3 cell transformation (cT) assay, unscheduled DNA synthesis (UDS) assay, and micronucleus (MN) assay.

The Ames test used the following *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation. MIBK was tested at concentrations of 0.04, 0.1, 0.4, 1.0, and 4.0 μl/plate. The positive controls were as follows: 2-aminoanthracene (1.0 μg/plate), 2-nitro-*O*-phenylenediamine (10 μg/plate), sodium azide (5.0 μg/plate), 2-aminoanthracene (4.0 μg/plate), and 9-aminoacridine (75 μg/plate). DMSO served as the negative control. MIBK was not mutagenic in any of the strains. The positive controls were mutagenic.

MIBK was tested in the L5178Y/TK^{+}/$^{-}$ mouse lymphoma assay. The forward mutation frequency at the thymidine kinase locus in mouse lymphoma cells was evaluated. MIBK was tested at concentrations ranging from 0.32 to 4.2 μl/ml both with and without metabolic activation. Ethyl methanesulfonate (0.5 and 1.0 μl/ml) served as the positive control for assays without metabolic activation. 7,12-Dimethylbenz[a]anthracene (5.0 and 7.5 μl/ml) served as the positive control for cultures with metabolic activation. DMSO served as the negative control.

Results were negative when MIBK was tested with metabolic activation, but were equivocal when MIBK was tested at high concentrations without metabolic activation. A significant increase in the mutation frequency (at least 2× that noted in controls) was noted at concentrations of 1.8, 3.2, and 4.2 μl/ml. Both positive controls were mutagenic. The results of a second mouse lymphoma assay were also equivocal (without metabolic activation) at the highest doses tested. In this assay, a significant increase in the mutation frequency was noted at doses of 2.1, 2.9, and 3.7 μg/ml.

The authors noted that the mutation frequency in test cultures was not dose related and that repeat testing with replicate cultures did not result in a consistent positive effect. Furthermore, the greatest response to MIBK was noted at doses that resulted in 96% to 99% lethality. The authors noted that doses that result in 90% to 100% lethality may not be relevant in determining mutagenicity. They noted that if the doses that resulted in >90% lethality are not considered, then the few remaining increases were not concentration-dependent and the results would be considered negative. Regarding results for DMSO control cultures, it was noted that more than a twofold difference in mutation frequencies was observed when control cultures for the eight mouse lymphoma assays were compared. However, the results for DMSO control cultures were within historical control ranges for the testing laboratory. The authors also stated that the absence of increases in the mutagenic frequency in test cultures over that observed in the DMSO control range provides additional evidence for a negative conclusion on the mutagenic potential of MIBK.

MIBK was evaluated in the unscheduled DNA synthesis assay (rat hepatocytes). MIBK was tested at concentrations ranging from 0.010 to 100 μl/ml. The positive control was 2-acetylaminofluorene (2-AAF) at 2 and 20 μg/ml and DMSO served as the negative control. The test substance was classified as positive if it induced a dose-related response and at least one dose produced a significant increase in the average net nuclear grains (compared to control), or if the test substance induced a significant increase in the mean net nuclear grain count in at least two successive doses. MIBK did not induce a positive response, meaning that there was no significant increase in the net nuclear grain counts at any of the doses tested. It is important to note that because of the high level of toxicity at doses of 10 and 100 μl/ml,

TABLE 2
MIBK genotoxicity

Test system	Protocol and dose	Results	Reference
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	Preincubation assay (modification of procedure by Haworth et al. 1983) with and without metabolic activation; 0.1 ml	Negative	Zeiger et al. 1992
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538; *Escherichia coli* strains WP_2 and WP_2 *uvr* A	Preincubation assay (Brooks and Dean 1981) with and without metabolic activation; up to 8000 μg/ml	Negative	Brooks, Meyer, and Hutson 1988
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538; *Escherichia coli* strains WP_2 and WP_2 *uvr* A	Ames test with and without metabolic activation; up to 8000 μg/ml	Negative	Brooks, Meyer, and Hutson 1988
Salmonella typhimurium strains TA98, TA100, and TA1535	Ames test with and without metabolic activation; up to 0.1–2000 μg/plate	Negative	Goodyear Tire & Rubber Company 1982
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without metabolic activation; 0.01–10 μl/plate	Negative	Litton Bionetics 1991
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538; *Saccharomyces cerevisiae strain* D4	Ames test with and without metabolic activation; up to 5 μl/plate	Negative	Litton Bionetics 1977
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without metabolic activation; 0.04–4 μl/plate	Negative	O'Donoghue et al. 1988
L5178Y/$TK^{+}/^{-}$ mouse lymphoma cells	Mouse lymphoma assay (Clive and Spector 1975; Clive et al. 1979) with and without metabolic activation; 0.32–4.2 μl/ml	Negative, with metabolic activation; equivocal, without metabolic activation	O'Donoghue et al. 1988

Rat hepatocytes	Unscheduled DNA synthesis assay (Williams 1977, 1979); 0.010-100 μl/ml	Negative	O'Donoghue et al. 1988
CD-1 mice	Micronucleus cytogenetic assay (in vivo)—mice dosed IP with 10 ml/kg; bone marrow samples obtained after animals killed	Negative	O'Donoghue et al. 1988
BALB/3T3 clone A31-1 mouse embryo cells	Cell transformation assay; with metabolic activation (1–4 μl/ml) and without metabolic activation (2–4.8 μl/ml)	Without metabolic activation, three type III foci in 15 dishes (statistically significant positive result) at highest dose. No transforming activity with metabolic activation	O'Donoghue et al. 1988
BALB/3T3 clone A31-1 mouse embryo cells	Cell transformation assay repeated with metabolic activation (2–5 μl/ml) and without metabolic activation (4–7 μl/ml)	Without metabolic activation, two type II foci in 15 dishes. No confirmation of preceding test results, because transformation frequency not significantly increased over that noted in negative control (phosphate-buffered saline) cultures. No transforming activity with metabolic activation	O'Donoghue et al. 1988
Saccharomyces cerevisiae strain JD1	Mitotic gene conversion assay with and without metabolic activation; up to 5 mg/ml	Negative	Brooks, Meyer, and Hutson 1988
Saccharomyces cerevisiae strain D61.M	Mitotic chromosome loss assay; 4.8–7.3 mg/ml	Negative	Zimmermann, Scheel, and Resnick 1989
Rat liver RL_4 cells	Chromosome damage assay; up to 8000 μl/ml	Negative	Brooks, Meyer, and Hutson 1988

it was not possible to determine the average net nuclear grains. Results for the positive control were classified as positive.

The mutagenicity of MIBK was evaluated in the micronucleus cytogenetic assay. A single dose of the test substance (in corn oil) was administered intraperitoneally (dose = 10 ml MIBK in corn oil/kg body weight) to groups of 10 CD-1 mice (5 males, 5 females per group). The animals were killed at 12, 24, or 48 h post dosing. The positive-control group was dosed with triethylene melamine (0.25 mg/kg) and examined at 24 h post dosing. Corn Oil served as the negative control. After the animals were killed, bone marrow samples were obtained and smears prepared. One thousand polychromatic erythrocytes were scored on coded slides for the presence of micronuclei. Micronucleated normocytes were also counted. MIBK was not mutagenic.

MIBK was also tested in the BALB/3T3 mouse embryo cell transformation assay. BALB/3T3 clone A31-1 cells were harvested during exponential growth. Based on the results of a preliminary cytotoxicity assay, the following concentrations were tested: 2, 4, 3.6, and 4.8 μl/ml (without metabolic activation) and 1.0, 2.0, and 4.0 μl/ml (with metabolic activation). The assay was repeated at concentrations of 4.0, 5.0, 6.0, and 7.0 μl/ml (without activation) and 2.0, 3.0, 4.0, and 5.0 μl/ml (with activation). At the end of the incubation period, transformation plates were fixed, stained, and scored for type II and type III foci. The transformation frequency for each treatment condition was expressed as the number of transformed foci per surviving cell. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) served as the positive control. Phosphate buffered saline served as the negative control. Test results were classified as ambiguous. In the first assay, the 4.8-μl/ml dose of MIBK induced three type III foci in 15 dishes. This number of type III foci, together with a reduced cloning efficiency, yielded a positive statistical analysis in the nonactivated system. In cultures with metabolic activation, no transforming activity was present. When the assay was repeated, MIBK (dose = 5 μl/ml) induced two type III foci in 15 plates with 100% cell survival. Because the resulting transformation frequency was not significantly increased over that reported for the negative control, it was not possible to confirm the results of the first BALB/3T3 assay (without metabolic activation). Like the first assay, results for MIBK were negative with metabolic activation.

Taking into consideration the marginal response to MIBK at the highest cytotoxic concentration in the mouse lymphoma assay and the lack of reproducibility in the BALB 3T3 transformation assay, and based on negative results for MIBK in the Ames, unscheduled DNA synthesis, and micronucleus assays, the authors concluded that it is unlikely that MIBK would be genotoxic in mammalian systems (O'Donoghue et al. 1988).

CARCINOGENICITY

No studies of MIBK carcinogenic potential were found. MIBK, however, is among the chemicals that have been approved by the National Toxicology Program (NTP) for testing in a toxicology and carcinogenesis study (NTP 1999).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Dermal Exposure

Malyscheva (1988) applied MIBK to the tails (lower 2/3) of an unspecified number of male white rats daily (4 h/day) in doses of 300 or 600 mg/kg for 4 months. Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa. The magnitude of each reduction was not stated, and no statistical analysis of the results was included.

Inhalation Exposure

Tyl et al. (1987) evaluated the reproductive and developmental toxicity of MIBK in 100-day-old virgin male and virgin female Fischer 344 rats (NIH:(F-344)/H1aBR (F141 + 3)) and 6-week-old virgin male and virgin female CD-1 mice (outbred albino Crl:CD-1-(ICR)BR). The animals were mated and then divided into four groups per species. Three groups, 25 females in each group, per species were exposed to MIBK vapor at concentrations of 300, 1000, and 3000 ppm (mean analytical values of 305, 1012, and 2997 ppm), respectively, on gestation days 6 through 15. Group 4 animals served as untreated controls. On gestation day 21, the animals were killed and live fetuses examined for external, visceral, and skeletal alterations.

Overall the authors concluded that the results indicated that MIBK did not induce any treatment-related increases in embryotoxicity, or fetal malformations in pregnant Fischer 344 rats or CD-1 mice that inhaled MIBK at concentrations of 300, 1000, or 3000 ppm . There was also no evidence of treatment-related maternal toxicity in mice or rats exposed to 300 or 1000 ppm MIBK (Tyl et al. 1987).

Study results are presented separately for the two species below.

Mice

Of the 25 pregnant mice exposed to 3000 ppm MIBK, three died after the first exposure. No treatment-related changes in body weight were noted. Maternal body weight gain was significantly elevated only after exposure to 3000 ppm MIBK. Clinical observations, associated only with dams in this exposure group, included irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, and lacrimation. Significant increases in absolute (117.8% of controls) and relative (104.5% of controls) liver weight in the 3000-ppm exposure group were the only treatment-related changes in maternal organ weights that were noted. Neither maternal body weights (absolute or corrected for gravid uterine weight), gravid uterine weight, nor absolute or relative maternal kidney weight differed between either of the three test groups. No treatment-related findings were observed at gross necropsy.

The pregnancy rate was equivalent for control and test groups of mice. Twenty-two litters were evaluated in each exposure group. No treatment-related effects in the following gestational parameters were noted: number of corpora lutea, total implantations, viable or total nonviable implants per litter, % preimplantation loss, % live fetuses, and sex ratio (% males). Following exposure to 3000 ppm MIBK, a significant increase in the number of dead fetuses (but not early or late resorptions) per litter, compared to controls, was noted. A significant reduction in total body weight per litter was also noted following exposure to 3000 ppm MIBK. Compared to controls, no statistically significant, treatment-related increases in the number of fetuses or litters (with one or more affected fetuses) with individual malformations, pooled external, visceral, skeletal malformations, or total malformations in any treatment group were noted. This finding was true for all exposure groups. Visceral variations included an increase in the incidence of dilated lateral ventricles of the cerebrum and dilated renal blood vessels. An increased incidence (compared to controls) of reduced ossification (indicative of toxicity) in the vertebrae, sternebrae, limbs, and skull plates was observed after exposure to 3000 ppm.

Rats

None of the female rats died during the study, delivered early, or had aborted fetuses. Evidence of maternal toxicity included significant reductions in body weight and significantly reduced weight gain. Food consumption (g/dam/day) was significantly reduced in the 3000-ppm exposure group, and only during the exposure period. Exposure-related clinical signs, observed only at 3000 ppm, were as follows: loss of coordination, negative tail and/or toe pinch, paresis (partial hindlimb paralysis), muscular weakness in hindlimbs, piloerection, lacrimation, and red perioral encrustation. A slight but statistically significant elevation in maternal relative kidney weight (104% of controls) was observed in the 3000-ppm exposure group. The following parameters were unaffected by treatment: absolute kidney weight, relative and absolute liver weight, gravid uterine weight, and absolute or corrected body weight. No exposure-related findings were noted at gross necropsy.

The pregnancy rate in rats was slightly reduced in the highest dose group (65.7% at 3000 ppm), but was not significantly different from the control group. For the other two dose groups and the control group, the pregnancy rates were considered equivalent (86.2% for control, 86.7% at 300 ppm, and 80.6% at 1000 ppm). The litters evaluated were as follows: 25 controls, 26 at 300 ppm, 25 at 1000 ppm, and 23 at 3000 ppm. No treatment-related effects on the following parameters were noted: number of corpora lutea, total implantations, viable or nonviable implantations (resorptions or dead fetuses) per litter, % preimplantation loss, % live fetuses, and sex ratio (% males). At an exposure concentration of 3000 ppm, fetal body weight per litter (males, females, or total) was significantly reduced (≈93% to 94% of control values; $p < .001$). Fetal body weight was slightly reduced at 300 ppm (≈97% of control values; $p < .05$), but not at 1000 ppm. No statistically significant, treatment-related increases in the incidence of external, visceral, skeletal, or total malformations in rat fetuses were noted. An increased incidence of five skeletal variations involving the vertebrae, sternebrae, and distal limbs was noted following exposure to 3000 ppm MIBK. This finding was considered indicative of toxicity.

CLINICAL ASSESSMENT OF SAFETY

Acute Inhalation Toxicity

Twelve volunteers of both sexes were exposed to various concentrations of MIBK for 15 min. This duration of exposure was chosen because, presumably, it permitted an accurate observation of olfactory fatigue and increasing or decreasing irritation of mucous membranes. The sensory response limit was 100 ppm (410 mg/m^3), and the odor was found to be objectionable by most of the subjects at a concentration of 200 ppm (820 mg/m^3). MIBK (200 ppm) was also found to be irritating to the eyes during inhalation exposure (Silverman, Schulte, and First 1946).

The threshold for MIBK-induced irritation of the lungs was 0.03 to 0.1 mg/l after 1 min of respiration. The number and weights of the subjects involved in this study were not stated (Batyrova 1973).

Short-Term Inhalation Toxicity

Elkins (1959) reported symptoms of either nausea or respiratory irritation in workers exposed to 100 ppm MIBK (410 mg/m^3). Tolerance to this level of exposure was acquired during the work week, but was lost over the weekend. Complaints were largely eliminated when the level of exposure was reduced to 20 ppm (82 mg/m^3).

National Institute of Occupational Safety and Health (NIOSH) (1978) reported that workers exposed to 500 ppm MIBK for 30 min daily experienced weakness, loss of appetite, headache, burning eyes, stomach ache, nausea, vomiting, and sore throat. An enlarged liver and colitis were also observed in some of the workers. In another case, workers exposed to 100 ppm MIBK experienced nausea, headache, and respiratory irritation.

Hazleton Labs, Inc. (1982) reported that six subjects (19 to 49 years old) inhaled MIBK (six, 20-min exposures = exposure session) through face masks connected to ports on a 125-L aerosol chamber. Test concentrations for the series of six exposures ranged from 0.402 to 2.827 mg/L. The incidence of nasal, ocular, or throat irritation experienced by the subjects during one of the exposure sessions (results for exposure series 1 to 6 combined) is indicated as follows: nasal irritation (one to four subjects), ocular irritation (one to three subjects), and throat irritation (one to four subjects). The results for throat irritation are based on the testing of only four subjects (test concentration range = 1.363 to 2.827 mg/L).

The Shell Chemical Corporation (1983) stated that MIBK vapor causes irritation of both the conjunctival and nasal mucosa at concentrations near 200 ppm. Exposure to higher concentrations causes lacrimation (indicative of marked irritation).

WHO (1990) reported on an occupational exposure in which 19 workers inhaled MIBK at concentrations up to 500 ppm (2050 mg/m^3) for 20 to 30 min/day, and 80 ppm (328 mg/m^3) for the remainder of the work day. Half of the workers had symptoms of weakness, loss of appetite, headache, ocular irritation, stomach ache, nausea, vomiting, and sore throat. Insomnia, somnolence, heartburn, and intestinal pain were also reported by a few workers. Slightly enlarged livers were observed in four workers, and six workers had nonspecific colitis. No abnormalities were noted at clinical chemistry examination. Reportedly, work practices at this facility had improved greatly 5 years after this study was conducted. The highest levels of exposure to MIBK ranged from 100 to 105 ppm (410 to 430 mg/m^3), and the general concentration of exposure was 50 ppm (205 mg/m^3). However, gastrointestinal and central nervous system effects were reported by a few workers. Slight liver enlargement persisted in two workers, but the workers did not complain of the initial symptoms.

Hjelm et al. (1990) presented the results of exposing eight male volunteers (18 to 35 years old; weights = 68 to 90 kg) to MIBK at concentrations of 2.4 ppm [10 mg/m^3], 24.4 ppm [100 mg/m^3], and 48.8 ppm [200 mg/m^3] for 2 h during light physical exercise on three different occasions. Based on a questionnaire, nose and throat irritation were the most common symptoms. Neither symptom was experienced by more than three subjects at either of the three exposure concentrations. There were no significant, exposure-related effects on the performance of a simple reaction time task or a test of mental arithmetic. Results concerning the basic human toxicokinetics of MIBK are included in the section on Distribution and Excretion.

Neurotoxicity

Dick et al. (1992) evaluated neurobehavioral effects resulting from short-term inhalation exposure to MIBK using 10 male and 13 female subjects (18 to 32 years old). The 3-day test session began with a 2-h practice session on day 1, followed by 8 h of exposure to 100 ppm MIBK on day 2, and concluded with a 2-h postexposure session on day 3. Inhalation exposure to 100 ppm MIBK on day 2 (in an environmental chamber) was according to the following procedure: (1) 2-h preexposure period; (2) two 2-h exposure periods, experiments 1 and 2, respectively; and (3) 2-h postexposure period. Neurobehavioral tests administered during each of the two 2-h test periods consisted of the following: five psychomotor tests (choice reaction time [CRT], simple reaction time [SRT], visual vigilance, dual task, and short-term memory scanning), one neurophysiological test (eye blink reflex), and one sensorimotor test (postural sway).

The results of statistical analyses did not indicate any significant differences between male and female blood and breath concentrations of MIBK. Study results indicated that 4-h exposures to 100 ppm MIBK did not cause any significant neurobehavioral effects. The principal exposure-related effects were limited headache, nausea, throat irritation, and tearing. These authors also stated that the primary health hazards from acute MIBK inhalation are mucous membrane irritation of the eyes, nose, and respiratory tract at concentrations <500 ppm and central nervous system depression at higher concentrations (Dick et al. 1992).

Iregren, Tesarz, and Wigaeus-Hjelm (1993) studied the potential narcotic impact of MIBK on central nervous system (CNS) function. Heart rate, performance tests, and rating scales for local irritation, CNS symptoms, and mood were determined in six female and six male employees (ages = 19 to 47 years; all healthy) at the National Institute of Occupational Health. The 12 employees were exposed to 10 and 200 mg/m^3 concentrations of MIBK in a 12-m^3 exposure chamber. The subjects were exposed individually for 2 h, and exposure sessions were separated by a 1-week interval. Exposure started with a 90-min period of light physical exercise on a bicycle ergometer. During the last 30 min of exposure, the subjects were relaxing on a bed. Average MIBK concentrations in the exposure chamber were 201 $\pm$ 3 mg/m^3 and 11.9 $\pm$ 1.44 mg/m^3 for the two exposure levels.

The SRT performance test measured reaction time to an easily discriminable but temporally uncertain stimulus during 6 min, using a signal density of 16 signals per minute. Performance was evaluated with respect to level and variability of latencies to 80 stimuli. The results of the SRT test indicated no differences in performance that were attributed to exposure. Compared to the 10 mg/m^3 level of exposure, a decrease in heart rate (seven subjects), an increase in heart rate (four subjects), and no change in heart rate (one subject) were noted after exposure to 200 mg/m^3 MIBK. Thus, no consistent exposure-related effect on heart rate was identified. Mood ratings of activity and stress varied during exposure sessions. However, differences in these parameters were not noted between low and high concentrations of exposure.

The occurrence of symptoms of irritation and CNS symptoms was evaluated using a questionnaire. For irritation and CNS symptoms, the symptoms index was expressed as differences from the preexposure measurement. Symptoms of local irritation to the eyes and airways were not significantly different when the two exposure concentrations were compared; however, a clear trend toward a significant increase was noted. The occurrence and/or intensity of CNS symptoms increased with exposure. The authors concluded that 2 h of exposure to MIBK caused increased discomfort in the subjects tested, as measured by symptom ratings (Iregren, Tesarz, and Wigaeus-Hjelm 1993).

Gagnon, Mergler, and Lapare (1994) reported on the effect of MIBK on olfactory function in four volunteers (two men, two women; ages = 27 to 57 years old). The subjects were exposed to 20 and 40 ppm MIBK, respectively, in an 18.1-m^3 chamber for 7 h on each of 3 consecutive days. After a 25-day nonexposure period, a second identical exposure was performed. Olfactory

adaptation and an MIBK-induced transient, olfactory perception threshold shift were reported at both exposure concentrations. Symptoms of eye, nose, or throat irritation and headache were present in some of the subjects. The authors concluded that individuals exposed professionally or environmentally to certain organic solvents may suffer temporary loss of the sense of smell, which hinders odor detection.

Case Reports

van Joost et al. (1984) diagnosed contact dermatitis in a 40-year-old man who had worked in a chemical factory for approximately 2 years. In the workplace, he was exposed to a variety of chemicals that were used in the manufacture of pesticides. Patch tests of various chemicals were performed using International Contact Dermatitis Research Group routine batteries. Reactions were scored at 48 and 72 h. Patch test results for undiluted MIBK were negative.

Grober and Schaumburg (2000) reported persistent cognitive deficits for a 44-year-old male employee of a poorly ventilated, indoor solvent extraction facility who had been exposed to ambient concentrations of MIBK in excess of 100 ppm (8 h/day) for 6 years. The level of exposure to MIBK was twice the threshold limit value, short-term exposure limit of 50 ppm. The deficits noted included slowed information processing and impaired attention. The pattern of cognitive deficits was said to have been best accounted for by an impairment in the limited-capacity working memory system that supports the performance of activities of everyday life that are not routine. The presence of impaired working memory in the worker correlated with the functional magnetic resonance imaging (MRI) finding of diminished cerebral blood volume and diminished mean transit time in both frontal lobes, relative to the remainder of the cerebrum. Cognitive dysfunction was also noted in a coworker with the same history of exposure to MIBK. Most likely, the persistent cognitive deficits resulted from chronic exposure to MIBK. It is important to note that no symptoms were reported for other employees in the work area (same exposure) who wore protective breathing devices.

Occupational Safety

NIOSH (1978) proposed a time-weighted average (TWA) limit of 50 ppm MIBK (205 mg/m^3) in 1978. The Code of Federal Regulations (29CFR 1910.1000) includes the OSHA standard of 100 ppm MIBK (410 mg/m^3) established in 1983.

The American Conference of Governmental Industrial Hygienists (ACGIH 2000) recommended a threshold limit value–time-weighted average (TLV-TWA) of 50 ppm and a threshold limit value–short-term exposure limit (TLV-STEL) of 75 ppm for occupational atmospheric exposure to MIBK. The TLV-TWA is defined as the time-weighted average concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. The TLV-STEL is defined as the concentration to which workers can be exposed continuously for a short period of time without suffering from (1) irritation, (2) chronic or irreversible tissue damage, or (3) narcosis of sufficient degree to increase the likelihood of accidental injury, impair self-rescue or materially reduce worker efficiency, provided that the daily TLV-TWA is not exceeded.

Gray (2000) presented information suggesting that MIBK, as an industrial degreasing agent, removes lipid from the skin, causing reddening, scaling, blistering, and peeling, and is irritating to the eyes and respiratory tract. The author noted that, in the chemical industry, the use of skin and eye protection is advised when handling MIBK.

SUMMARY

MIBK is an aliphatic ketone that functions as both a denaturant and solvent in cosmetic products. One method of production is acetone condensation, followed by catalytic hydrogenation. MIBK may contain the following impurities: dimethyl heptane, methyl isobutyl carbinol, mesityloxide, acetic acid, 4-methyl-2-hydroxypentane, and methyl n-butyl ketone. According to the Chemical Manufacturers Association, MIBK producers indicated in 1999 that MnBK (known neurotoxin) is either not found in MIBK or is found in trace amounts (typically 0.01 to 0.06% and always less than 0.1%).

Frequency of use data provided by FDA in 1998 indicate that MIBK is used in two cosmetic products. However, use concentration data provided by CTFA in 2000 indicate that MIBK is used in one nail correction pen (volume = 3 ml) at a concentration of 21%.

According to regulations established by the Bureau of Alcohol, Tobacco, and Firearms, the maximum concentration of MIBK that is listed for use as a denaturant of alcohol is 4.0%. MIBK is also listed in the *National Formulary* as an alcohol denaturant that is used as an excipient for drugs.

The metabolites, 4-hydroxy-4-methyl-2-pentanone (MIBK oxidation product) and 4-methyl-2-pentanol (4-MPOL) (MIBK reduction product) were detected in blood samples from guinea pigs injected intraperitoneally with MIBK. Values for the serum half-life and total clearance time for MIBK that have been determined are 66 min and 6 h, respectively. Hydroxylation products of MIBK, such as 4-MPOL, are expected either to be conjugated with sulfate or glucuronic acid and excreted in the urine or to enter intermediary metabolism to be converted to carbon dioxide.

In a study in which MIBK was administered orally or by inhalation exposure to groups of guinea pigs, the amount of MIBK detected in the plasma and liver was proportional to the administered dose. The metabolite, 4-hydroxy MIBK was detected in the plasma regardless of the route of exposure. 4-MPOL was detected in the plasma after inhalation exposure, but not after oral doses of MIBK were administered. 4-MPOL and 4-hydroxy-4-methyl-2-pentanone were the principal MIBK metabolites in mice dosed intraperitoneally with MIBK.

The percutaneous absorption of MIBK was demonstrated in a study involving guinea pigs. A maximum percutaneous uptake rate of 1.1 μmol/min/cm^2 was observed at 10 to 45 min after the initiation of exposure.

Both gross and microscopic evidence of lung damage have been reported in acute inhalation toxicity studies in which mice and guinea pigs were exposed to MIBK. Increased pulmonary arterial pressure was noted in acute inhalation toxicity studies in which cats or dogs were exposed to MIBK. Bronchoconstriction was also observed in cats that inhaled MIBK.

Acute oral LD_{50} values of 4.6 (3.932–5.382) g/kg and 2.08 (1.91–2.27) g/kg have been reported for MIBK in studies involving rats. An acute oral LD_{50} of 1.5 ml/kg (mice) for MIBK has also been reported.

In an acute dermal toxicity study, undiluted MIBK was applied to the skin of two rabbits for 10 h either by flooding the test site or placement of a cotton pad impregnated with the test substance. Signs of systemic effects were not noted and no treatment-related pathologic changes were observed at microscopic examination of internal organs.

The following acute intraperitoneal LD_{50} values for MIBK have been reported: 1.14 ml/kg (rats), 0.59 $\pm$ 0.23 g/kg (mice), and 0.919 ml/kg (guinea pigs). Intraperitoneal injection of MIBK was associated with pulmonary vascular effects in cats.

Increased pulmonary arterial pressure, but not bronchoconstriction, was induced in cats dosed intravenously with MIBK.

In short-term exposure experiments, male and female Fischer-344 rats inhaled MIBK (concentrations up to ~2000 ppm) 6 h per day over a period of 9 days to 2 weeks. Concentrations of approximately 500 or 2000 ppm induced hyaline droplet formation in the kidneys of male rats, and epithelial regeneration of the proximal convoluted tubules was also noted at the highest concentration. Increases in liver and kidney weight were also observed at these concentrations. B6C3F1 mice exposed to MIBK according to the same procedure had increased liver weight (~2000 ppm, females only). An increase (females) and decrease (males) in kidney weight was also noted at this concentration. No changes in kidney weight occurred after exposure to ~500 ppm.

In another short-term test, monkeys, dogs, mice, and rats were exposed continuously (inhalation) to 100 or 200 ppm MIBK over a period of 2 weeks. Increased kidney weight and microscopic evidence of toxic nephrosis of the proximal tubules were reported only for rats, and this finding was noted at both concentrations of exposure. Increased liver weight (rats) was also noted after exposure to 200 ppm. Other study results indicated increased adrenal weight only in female albino rats exposed to MIBK at a concentration of 4.53 mg/L of air 5 days per week (6 h/day) for 4 weeks. No "clear-cut" test substance-related abnormalities were noted at gross necropsy.

No evidence of gross pathologic effects was observed in Wistar female rats that ingested MIBK at concentrations of 0.5% and 1.0% MIBK in drinking water for 7 days.

In a short-term dermal toxicity study, seven applications of undiluted MIBK (3 ml/kg each, 5 to 12 h) were made to the shaved skin of two rabbits over a period of 15 to 21 days. Local skin changes consisted of polymorphonuclear infiltration in the upper dermis. No systemic effects were noted.

The subchronic inhalation toxicity of MIBK was evaluated using groups of male and female F-344 rats. The animals were exposed to concentrations ranging from 50 to 1000 ppm 5 days per week (6 h/day) for 90 days. Increased liver weight was noted following exposure to 1000 ppm; however, hepatic lesions were not observed at gross or microscopic examination. Groups exposed to 250 or 1000 ppm MIBK had increased numbers of hyaline droplets in proximal tubule cells, which may be specific to the male rat. Male $B6C3F_1$ mice exposed to MIBK had increased liver weight following exposure to 250 or 1000 ppm, but hepatic lesions were not observed at gross or microscopic examination.

Increased liver and kidney weight was observed in male Wistar albino rats exposed to 410 mg/m^3 MIBK for 90 days. At microscopic examination, hyaline droplet degeneration of the proximal tubules was observed in kidneys from each of the 100 rats. Kidney damage was completely reversed in rats observed up to 60 days post exposure. In the same study (same dose and exposure duration), gross examination revealed no differences in tissues examined between test dogs and monkeys and controls. Liver function test results (dogs only) also indicated no differences between test and control dogs.

Nephrotoxicity and increased liver and kidney weight, but no evidence of hepatic lesions, was observed in male and female Sprague-Dawley rats dosed orally with 1000 mg/kg MIBK daily for 13 weeks. The 50-mg/kg dose (lowest dose) was considered the NOEL. No significant gross lesions and renal tubule cell hyperplasia were reported in a study involving rats that received daily oral doses of 1.04 g/kg MIBK (in drinking water) for 120 days.

In a subchronic dermal toxicity study, MIBK (in sunflower oil) was applied to white rats (lower 2/3 of tail) daily at doses of 300 or 600 mg/kg for 4 months. Skin changes included reduced mitotic activity in hair follicles and increased thickness of horny and granular cell layers of the epidermis. Changes in the spleen included a decrease in the number of reactive centers in follicles and an increase in the number of iron-containing pigments in the area of the red pulp. A reduction in the lipid content of the cortical layer was noted in the adrenal glands.

Inhalation exposure of Sprague-Dawley rats to MIBK (up to 3020 ppm) did not cause any modifications in serum GLDH activity. However, preexposure of rats to MIBK caused a dose-related increase in DCB-induced GLDH activity. In this study, the mitochondrial enzyme, GLDH was assessed in serum as a quantitative sign of hepatic necrosis. In other studies (oral dosing), the hepatotoxicity of chloroform in Sprague-Dawley rats was enhanced after the administration of MIBK or its major metabolites, 4-MPOL and 4-hydroxymethyl isobutyl ketone (minimally effective dose of each = 5 mmol/kg), and the

hepatotoxicity of carbon tetrachloride was also enhanced after MIBK administration (dose response; doses up to 20 mmol/kg).

The dermal administration of MIBK in sunflower oil to white rats (lower 2/3 of tail) at daily doses of 300 or 600 mg/kg for four months caused an increase in the number of binuclear hepatocytes, reduced mitotic activity of these cells, and an increase in the number of hepatocytes with pathology. Neither histologic evidence of liver damage nor lipid deposition was observed in mature guinea pigs injected intraperitoneally with a single dose of 500 or 1000 mg/kg MIBK.

MIBK had an additive effect on the hepatotoxicity of 1,2-dichlorobenzene, chloroform, hexachlorobenzene, and carbon tetrachloride.

Neuropathological changes in the most distal portions of the tibial and ulnar nerves were observed in young adult rats exposed (inhalation) to 1500 ppm MIBK for up to 5 months. The neuropathological changes observed may have been related to the presence of 3% methyl *n*-butyl ketone in the commercial grade of MIBK that was tested. No differences in the performance of schedule-controlled operant behavior were noted between Sprague-Dawley rats exposed to MIBK (inhalation) at concentrations up to 1500 ppm for 13 weeks and control rats. At gross examination, no test substance-related changes were noted.

In a 7-day study in which the effect of inhaled MIBK (25 to 75 ppm) on the behavior of young baboons was evaluated, it was concluded that MIBK did not impair each animal's ability to discriminate or remember stimuli presented in a match-to-sample discrimination task.

The oral dosing of rats with 601 mg/kg MIBK five times per week for 55 weeks had no effect on the maximum motor-fiber conduction velocity in the tail nerve.

MIBK did not induce peripheral neuropathy in groups of Sprague-Dawley albino rats injected intraperitoneally with MIBK (10% in corn oil) at doses up to 100 mg/kg for 2 weeks.

The intravenous infusion of female Sprague-Dawley rats with MIBK (in an emulsion) resulted in depression of the vestibulo-oculomotor reflex. The threshold limit for this effect was 0.2 mM/L (20 ppm) at an infusion rate of 30 μM/kg/min.

Based on the results of an electromyographic examination, neurotoxicity was not observed in male Beagle dogs injected with MIBK (300 mg/kg) daily for 11 months. No evidence of systemic toxicity or neurotoxicity was observed in dogs injected subcutaneously with MIBK (150 mg/kg) twice daily for a year. Tissue samples from the brainstem, nerves, and muscles were examined at necropsy. The test substance (98% pure) contained 0.9% methyl *n*-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane. In another study, MIBK containing 0.9% methyl *n*-butyl ketone was injected subcutaneously (150 mg) into cats five times per week for up to 8.5 months. The analysis of biopsy specimens from the hind feet indicated no detectable damage to nerve tissues.

MIBK (0.5%) induced widespread cell death in a clonal line of neuroblastoma cells (Neuro 2aE) derived from a spontaneously occurring murine tumor (C1300). No discernible cytopathological changes and depressed growth rate were reported after exposure to concentrations of 0.1% and 0.2% MIBK, respectively.

The results of ocular irritation studies involving albino rabbits indicate that undiluted MIBK is an ocular irritant.

Single 24-h patch applications of undiluted MIBK induced reactions ranging from slight to moderate skin irritation in rabbits and slight skin irritation in guinea pigs. Repeated applications (seven) of undiluted MIBK over a 15- to 21-day period caused drying of the skin and exfoliation in rabbits. In another study, repeated applications of MIBK to guinea pigs (daily for 31 days) resulted in desquamation. The immersion of a rabbit's ear and tails from mice in pure MIBK for 2 h caused pronounced inflammation and necrosis.

The threshold concentration of MIBK for the inhibition of bacterial growth (*Pseudomonas putida*) was 275 mg/L in a 16-h study.

MIBK was not mutagenic in the Ames test (*Salmonella typhimurium* strains) or in the mitotic gene conversion assay (*Saccharomyces cerevisiae* strain) with or without metabolic activation. Mammalian mutagenicity test results (with or without metabolic activation) for MIBK in the following assays were also negative: mouse lymphoma, unscheduled DNA synthesis, micronucleus, cell transformation, and chromosome damage.

MIBK is among the chemicals that have been approved by the NTP for testing in a toxicology/carcinogenesis study.

MIBK did not induce any treatment-related increases in embryotoxicity or fetal malformations in pregnant Fischer 344 rats or CD-1 mice that inhaled MIBK at concentrations of 300, 1000, or 3000 ppm. There was evidence of treatment-related maternal toxicity only at the highest concentration tested. In another study, MIBK was applied to the skin (lower 2/3 of tail) of an unspecified number of male white rats daily (4 h/day) at doses of 300 or 600 mg/kg for four months. Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa.

Blood and breath samples were obtained from subjects who inhaled MIBK during six 20-min exposure sessions. Results at 90 min post exposure indicated that most of the absorbed MIBK had been eliminated from the body. In another group of subjects exposed to MIBK (inhalation) for 2 h during light physical exercise, the apparent blood clearance was 1.6 L/h/kg at all exposure concentrations. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 h post exposure. MIBK was detected in the following tissues of subjects who died following exposure to several volatile organic solvents during spray painting: brain, liver, lung, vitreous fluid, kidney, and blood.

The threshold for MIBK-induced irritation of the lungs of human subjects was 0.03 to 0.1 mg/L after 1 min of respiration. Ocular irritation was noted in 12 volunteers exposed to 200 ppm MIBK (inhalation) for 15 min. Nasal, ocular, and throat irritation were experienced by no more than four of six volunteers subjected to six 20-min exposures (inhalation) to MIBK

at concentrations ranging from 0.402 to 2.827 mg/L. In another inhalation study, irritation of the nose and throat were the most common symptoms reported by three of the eight volunteers exposed to MIBK at concentrations up to 48.8 ppm during light physical exercise for 2 h.

Ocular irritation, nausea, and sore throat were experienced by approximately half of the 19 workers exposed to MIBK daily at concentrations up to 500 ppm for 30 min and 80 ppm for the remainder of the day. Slightly enlarged livers and nonspecific colitis were reported for 4 and 6 workers, respectively. In another study, symptoms of either nausea or respiratory irritation were reported by workers exposed to 100 ppm MIBK. Complaints were reduced substantially when the level of exposure was reduced to 20 ppm.

Exposure to 100 ppm MIBK for 4 h did not induce neurobehavioral effects in either of the 23 human subjects tested. In another study, the potential narcotic impact of MIBK on CNS function was evaluated using two groups of six subjects exposed to 10 mg/m^3 (control) and 200 mg/m^3 MIBK, respectively, for 2 h. No consistent exposure-related effect on heart rate was identified, and the results of the simple reaction time performance test indicated no exposure-related differences in performance.

In a case report, a 40-year-old worker (with contact dermatitis) at a chemical factory had a negative patch test reaction to undiluted MIBK. Findings in another case report indicated persistent cognitive deficits in a 44-year-old employee at an indoor solvent extraction facility who did not wear a protective breathing device.

The most recent occupational limits from the ACGIH recommended a TLV-TWA of 50 ppm and a TLV-STEL of 75 ppm for atmospheric exposure to MIBK.

DISCUSSION

MIBK is used as a solvent and denaturant in cosmetic products. The Panel expressed concern over the neurotoxicity potential of this ingredient, based on published data indicating that MnBK (methyl *n*-butyl ketone, a known neurotoxin) is present as an impurity in MIBK at concentrations as high as 3.0%. According to the Chemical Manufacturers Association, MIBK producers indicated in 1999 that MnBK is either not found in MIBK or is found in trace amounts (typically 0.01% to 0.06% and always less than 0.1%). After considering the new impurities data, data indicating that the only reported use of MIBK in cosmetics is in a nail correction pen (total volume of pen = 3 ml; 21% MIBK), and the observation that significant dermal absorption of the nail correction fluid would not be likely under normal use conditions, the Panel agreed that MIBK could be used safely as a solvent in nail polish removers in a controlled application system. However, given the known neurotoxic effects of MnBK, the Panel stressed the importance of continued efforts to limit the concentration of this impurity in MIBK. Furthermore, the Panel stressed the importance of avoiding inhalation exposure to MIBK, based on evidence of lung, kidney, or liver damage in animal studies and respiratory irritation or liver effects reported in human occupational exposure studies on MIBK.

Though one of the reported uses of MIBK in cosmetics is that of a denaturant, product data indicative of this function have not been provided. However, after noting that MIBK has been approved for use as a denaturant for alcohol, in keeping with the regulations established by the Bureau of Alcohol, Tobacco, and Firearms (27CFR21.21), the Panel agreed that MIBK could be considered safe for use as a denaturant in cosmetics at concentrations up to the maximum concentration of MIBK (4%) that is listed for use as a denaturant of alcohol. It is important to note that because of the established regulations, the Panel assumes that cosmetic product formulators use MIBK as a denaturant at concentrations that do not exceed 4.0%.

The Expert Panel is aware of an ongoing carcinogenicity study on MIBK that is being conducted by the National Toxicology Program, and agreed that the results will be reviewed by the Panel after the report has been made available to the public.

CONCLUSION

Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products.

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