Amended Safety Assessment of Toluene as Used in Cosmetics

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

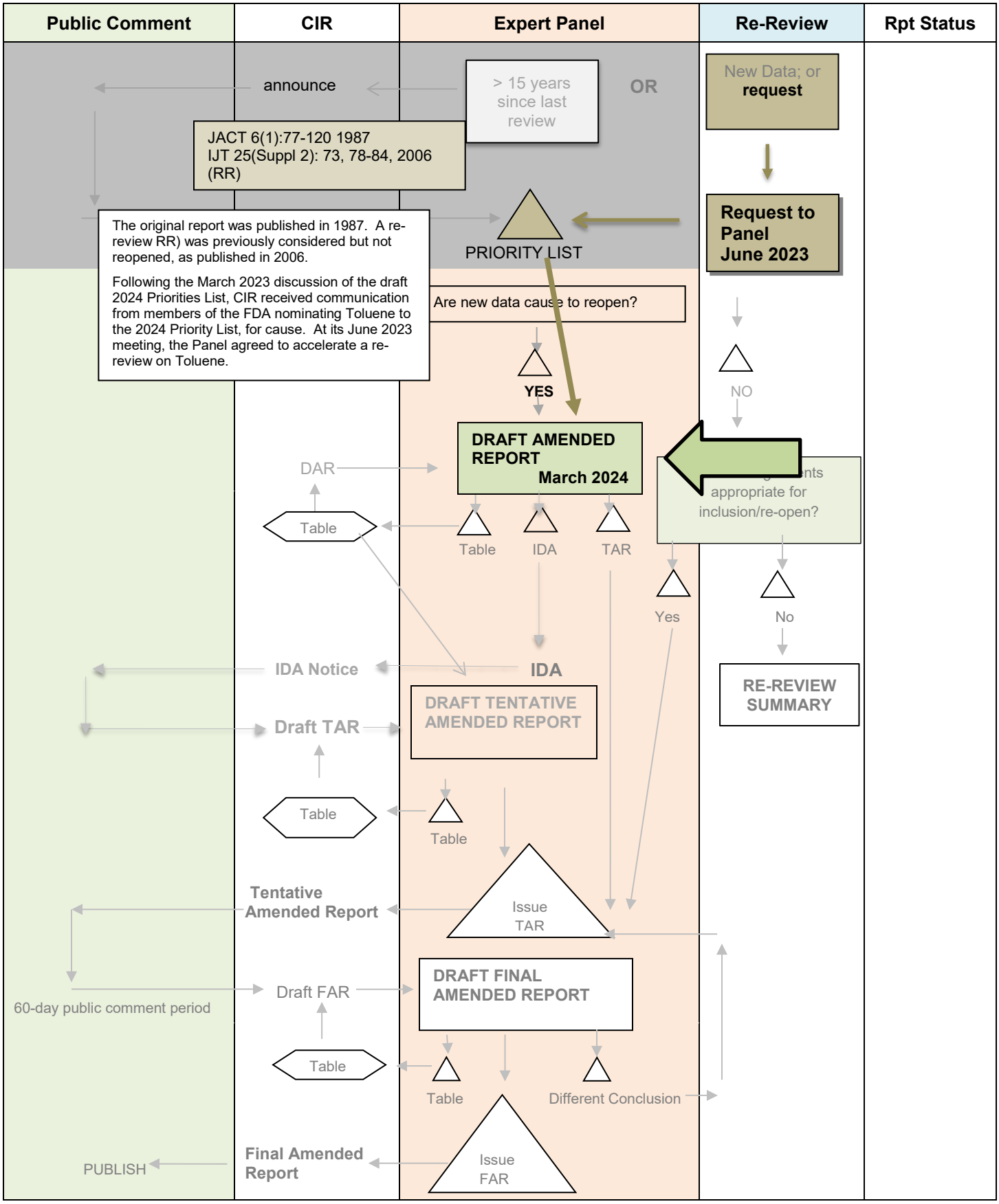
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. 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 Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651
cirinfo@cir-safety.org

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Toluene

MEETING March 2024



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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, MS, Senior Scientific Analyst/Writer, CIR
Date: March 4, 2024
Subject: Amended Safety Assessment of Toluene

The Panel previously reviewed the safety of Toluene in an assessment that was published in 1987 (identified as *originalreport_Toluene_032024)* and concluded that Toluene is safe in cosmetics in the present practices of use and concentration, as described in the safety assessment. This conclusion was re-affirmed in a re-review summary published in 2006 (*RRsum2006_Toluene_032024*). Following the March 2023 discussion of the draft 2024 Priorities List, CIR received communication from members of the FDA nominating Toluene to the 2024 Priority List, for cause. At its June 2023 meeting, the Panel agreed to accelerate a re-review on Toluene. Accordingly, enclosed is the Draft Amended Report on the Safety Assessment of Toluene as Used in Cosmetics (*report_Toluene_032024*).

Following the request for an accelerated re-review, a literature search was performed for studies dated 1983 forward, and a huge number of studies were found. At the September 2023 meeting, a strategy memo was presented to the Panel requesting guidance on how to structure and present the extensive amount of literature found. The Panel agreed to include studies published after 2005, with studies published between 1983-2005 listed in a separate data appendix, along with abstracts, for the Panel's review. As requested, this document was prepared, and will be presented to the Panel personally via Google Drive (due to the size of the document and copyright regulations). A list of these studies, without attached abstracts, have been included herein (*appendix_Toluene_032024*). Along with studies published from 1983-2005, studies published after 2005 that were not relevant to cosmetic use, or contained information that were purely cumulative to the information already presented in the report, were also included in this list. It should be noted that data from the original 1987 report and data from the unpublished re-review document evaluated by the Panel during their initial re-review deliberations (*RRdata_Toluene_032024*) have been summarized in the Draft Amended Report in *italicized text*.

According to 2023 US FDA VCRP data, Toluene has 0 reported uses. However, according to the use survey conducted by the Council in 2022 - 2023, Toluene is reported to be used at up to 20% nail products, and in other products (e.g., bath, deodorant, baby products) at low concentrations. In comparison, 59 uses were reported to the VCRP in 2002, at a maximum concentration of 26%, with all uses reported to be in nail products.

Additional supporting document included in this packet include: a flow chart (*flow_Toluene_032024*), report history (*history_Toluene_032024*), search strategy (*search_Toluene_032024*), a data profile (*dataprofile_Toluene_032024*), transcripts from the meetings at which the current re-review was discussed (*transcripts_Toluene_032024*), and the minutes from all the meetings at which Toluene was discussed during the original review and initial re-review (*originalminutes_Toluene_032024*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs an issue an Insufficient Data Announcement.

Toluene History

1987

- Final Report published with conclusion of safe as used

2006

- Re-review published with conclusion of safe as used

June 2023

- Panel agrees to accelerate re-review on Toluene in response to FDA request

September 2023

- Strategy memo presented to Panel regarding large amount of studies found in literature search
- Comments received from WVE

March 2024

- Panel reviews Draft Amended Report

Toluene Data Profile* – March 2024 – Writer, Priya Cherian																															
	Use				Toxico-kinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	New Rpt	Old Rpt	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Oral	Inhalation	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Toluene	**X**	**X**	**X**	XO	O	XO	XO	O	O	O		O	XO	O	XO	XO	XO	XO	XO		O	O		O	O	O		O	X	XO

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

Toluene

CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
108-88-3	x	x			x	x		x	x	x	x	x	x	x		x

Search Strategy

PUBMED:

- INCI name searched along with "Typical Search Terms" listed below
- CAS numbers searched
- Searches performed from 1983-2023

Typical Search Terms

- metabolism
- dermal
- skin
- toxicity
- developmental
- reproductive
- cancer
- carcinogenicity

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
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- 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

JUNE 2023 PANEL MEETING – FDA NOMINATION

Belsito Team – June 12, 2023

DR. BELSITO: So, since our March meeting we received communication from the FDA nominating ingredients for cause, specifically Toluene and Dibutyl Phthalate. So, we're going to be doing accelerated re-reviews on those. And then there was something here that I just want Monice or someone to clarify. So, it basically said that instead of just doing a re-review summary, we're going to fully open this or something?

MS. FIUME: So, are you talking about Toluene?

DR. BELSITO: Yeah.

DR. SNYDER: We never reviewed it before.

MS. FIUME: Well, it is on our list of items to be re-reviewed. It's currently on Christina's docket. Right, you have Toluene?

DR. BELSITO: Right. We reviewed both of them before.

MS. BURNETT: I think so. I don't know.

DR. SNYDER: Oh, that's the TPO. I was talking about TPO. Yeah.

DR. BELSITO: Right.

DR. SNYDER: I'm sorry, TPO is what I was talking about.

MS. FIUME: Right. TPO is the only on. Dibutyl Phthalate was just re-reviewed in 2017.

DR. BELSITO: Right.

MS. FIUME: But Toluene was scheduled for consideration for re-review this year, so you will be seeing that soon.

DR. BELSITO: Right. But it says, "The CIR will present the panel with a draft amended report on this ingredient instead of an abbreviated re-review document."

MS. FIUME: Okay. So instead of getting the table that you have been --

DR. BELSITO: Right. We are actually going to get a written document?

MS. FIUME: Assuming that you were going to accept FDA's request to reopen it.

DR. BELSITO: I think if FDA comes to us with a request for cause, we have to -- I don't know -- yeah.

MS. FIUME: Which is why you'll get an actual report person versus do you want to reopen? Here's the table of data that we found and then -- just taking that step out.

DR. BELSITO: Right, okay. So, we're going to -- yes, we're reopening Dibutyl Phthalate and Toluene for cause. And I think the third ingredient -- I mean, this is the type of stuff that I want to see happening. Something's going on in Europe, there's a concern about this material for reproductive toxicity, we need to be looking at it, number one. Number two, we've never even reviewed it. So, yes, I personally would like it added to the 2024 priority list.

DR. SNYDER: Agreed.

DR. KLAASSEN: It would be interesting to know why they wanted these first two chemicals. We don't -- why they want us to do Dibutyl Phthalate?

DR. BELSITO: Because it's a huge issue in endocrine disruption --

DR. KLAASSEN: Right, right.

DR. BELSITO: -- and --

DR. KLAASSEN: But I don't think there's any new data since the last time we did it, but maybe there is. And how about Toluene? I mean, I'm not against doing it, I'm just wondering. It'd be nice if they said why.

MS. FIUME: So, I'm looking at the memo and the email that was originally sent on March 20th, it's PDF Page 26. It just says that they're proposing it.

DR. BELSITO: Yeah. This is from Prashiela.

DR. KLAASSEN: Yeah. It says nothing really.

DR. BELSITO: Right.

MS. FIUME: Sorry, Priya has Toluene. So, Priya will be bringing that back probably in September.

MS. BURNETT: And Phthalates.

MS. FIUME: Yeah.

DR. BELSITO: I mean, both of them have gotten a lot of press, you know, bad press.

DR. KLAASSEN: Yeah, I know about the phthalates always do.

DR. BELSITO: Well, Toluene for carcinogenicity.

DR. RETTIE: So, the phthalates are the less (inaudible) issues, right?

DR. BELSITO: Right. I'm surprised that they are supposedly only one reported use because they used to be used in a lot of nail enamels. But I guess now everyone's using acrylic, so I don't know.

DR. KLAASSEN: Well, let's do them.

DR. SNYDER: Been there, done that.

DR. BELSITO: They're also used in a lot of fragranced products to hold the fragrance on the skin as a fixative, I think.

MS. KOWCZ: No.

DR. BELSITO: No?

MS. EISENMANN: Diethyl.

MS. KOWCZ: The Diethyl.

DR. BELSITO: Yeah, diethyl. Okay. Lunch time. Back at 1:00?

DR. KLAASSEN: Back at 1:00.

Cohen Team – June 12, 2023

DR. HELDRETH: I think that the main point was that FDA had actually asked for three additions to our prioritization. Two of these are request for accelerated rereviews, so Toluene and the Dibutyl Phthalate.

Now Toluene was actually already in our in-house pipeline. We were already working on it, so that one's definitely coming back your way. Dibutyl Phthalate, we haven't started working on yet. But now that FDA has requested it, we've went ahead and added it, unless the Panel has an objection to accelerating that be reviewed.

So, the only real question, I think, for the panel is do they want to add this Trimethybenzoyl Diphenylphosphine Oxide to the prioritization list for next year?

DR. SLAGA: I think we should accelerate it.

DR. COHEN: Yeah. That's a question to the Panel. We should add them.

DR. TILTON: Yeah, I agree.

DR. ROSS: New data. I agree.

DR. HELDRETH: Okay. That's easy.

DR. ROSS: Bart, could I ask you, what was the reason for -- or maybe you don't know -- why FDA nominated Toluene and the Dibutyl Phthalate? Was there a specific reason?

DR. HELDRETH: Prashiela stepped out?

DR. ANSELL: Our FDA person just --

DR. COHEN: We can ask her when she comes back.

DR. ROSS: Ah, okay.

DR. COHEN: These are plastics, the phthalates, right?

DR. HELDRETH: Plasticizer, yeah.

DR. ROSS: Yeah, they're phthalates. Toluene is a little different.

DR. COHEN: Yeah, Toluene is going to be a bit different.

DR. HELDRETH: Well, we've looked at the phthalates before.

DR. ROSS: Yeah.

DR. BERGFELD: And there's a lot of endocrine disruption with that group.

DR. COHEN: So, it's interesting. In 2017, the panel reaffirmed it, so this would be a real short cycle.

DR. HELDRETH: Right.

DR. COHEN: Prashiela, a question. No, no, no, it's okay. For the priority list, the FDA nominated some items, one was Toluene. Do you know why Toluene was nominated?

DR. MANGA: I'm going to have to get back to you on that one. Let me take a quick look at what we --

DR. COHEN: And the phthalates, the dibutyl phthalate?

DR. MANGA: I think there's just a lot of interest in phthalates right now. It's come up quite a bit. The Toluene is being used in a lot of nail products.

DR. ANSELL: Historically.

DR. MANGA: Historically.

DR. COHEN: Are you talking about the Toluene sulfonamide resins or just Toluene?

DR. ANSELL: No Toluene is a diluent.

DR. ROSS: I think Toluene is being reviewed quite a bit at IARC on its own, but also in connection with Benzene.

DR. ANSELL: Right. Also not used anymore, so.

DR. ROSS: Yes.

DR. ANSELL: But we fully support accelerating anything FDA ask us to.

DR. COHEN: We're good. Yeah. So are we.

DR. HELDRETH: Which is a question, I just wondered why they --

DR. BERGFELD: Actually, we really like it when they ask.

DR. ANSELL: Yes. More than support it, encourage it.

DR. MANGA: We appreciate that.

DR. COHEN: No, it’s nice we’re being paid attention to. And the other one was -- Annex 3 was a little more self-explanatory.

DR. BERGFELD: What was that?

DR. COHEN: The Trimethybenzoyl Diphenylphosphine Oxide.

DR. ROSS: Yeah it's more data. Yeah.

DR. HELDRETH: Yeah, it looks like there may be some repro concerns with that one.

DR. COHEN: Some? I didn't hear what you said.

DR. HELDRETH: Repro -- DART issues with that ingredient.

DR. COHEN: Repro. Okay. All right, so I think we're aligned on the priorities.

DR. BERGFELD: I think when we present this, it would be nice if you, the FDA, presented the reasons for bringing them forth.

DR. COHEN: Just like a sentence.

DR. BERGFELD: It would be very nice.

Full Panel – June 13, 2023

So the FDA has asked us to move Toluene and Phthalates up for cause. And I would agree with doing that. And also, it was brought to our attention that a material that we haven’t reviewed, trimethylbenzoyl dimethyl phosphine oxide, is being looked at by the European Chemical Agency, ECHA. And they’re very concerned about the safety of this. It’s a substance of very high concern (SVHC), and I think we should move that up on our Priority List as well.

And I think this is the type of thing that needs to be done, where we’re monitoring what other safety organizations are looking at, perhaps, flagging ingredients that we weren’t aware of. And we should continue to do this type of thing.

DR. BERGFELD: Any comments, Dr. Cohen?

DR. COHEN: No, I thought we might had heard from the FDA a little more why they were nominated.

DR. BERGFELD: Jan, do you want to talk about the nominations?

DR. HELDRETH: We also have Dr. Manga online.

DR. BERGFELD: Manga too?

DR. HELDRETH: She had to return to the office.

DR. MANGA: Hi, this is Prashiela. So these three ingredients came up because we've had a couple of inquiries about these being used in nails -- I'm sorry, I'm getting a bit of feedback from the room.

DR. BERGFELD: We can hear you.

DR. MANGA: So these ingredients have been noted particularly for the use in nail products. And that was why we were interested. And then, as Don mentioned, at least for the TPO, that is coming up as a new ingredient. We were concerned that it be reviewed given the other reviews that are going on.

Toluene is now one of the California Department of Toxic Substances Control products that effective January 1, 2023, nail products containing Toluene will become priority products. And, so, we felt that this was also one that needed to be looked at once again.

In terms of Dibutyl Phthalate, this is one which was included when FDA amended the food-additive regulations, to no longer provide for 25 plasticizers in various foods contact applications. They did this because the uses were abandoned, but given that this one was included in these amendments, we felt that it would be timely for CIR to review it as well.

DR. BERGFELD: Thank you very much. We're really appreciative of the FDA coming in and suggesting these particular ingredients.

DR. MANGA: Sure. Can I just make one other announcement quickly, please? Dr. Jannavi Srinivasan is there in person today, she was there yesterday. She will be serving as the FDA liaison when either Linda or I are not available. I just wanted to let you know that she'll be representing FDA when we're not available. Thank you.

DR. HELDRETH: Thank you, Prashiela.

DR. BERGFELD: Thank you. Now we've come to the end of all the things we were to cover. As you know, this year we'll be having two live -- I call them live, face-to-face -- meetings and two virtual. There is some consideration for the next year that we do more lives than virtual. And that is under consideration by Bart at this time.

But, at this point in time the next live meeting will be September 11th and 12th, here at the Melrose. And we look forward to seeing you all and having a good summer. And, of course, some of this will be sort of summarized and some of it will have to be previewed by the Panel before it goes out for review. Okay, Bart, got anything?

DR. HELDRETH: That's it. Thank you all so m

SEPTEMBER 2023 MEETING – STRATEGY MEMO

Belsito Team – September 11, 2023

DR. BELSITO: Toluene, is this under Admin?

MS. FIUME: It is.

DR. BELSITO: There was a Wave 2 to this as well.

MS. FIUME: There is a Wave 2.

DR. BELSITO: The Wave 2 was from Women's Voice for the Earth to look at inclusion of salon occupational studies and the California Air Quality Home Study. I don't think that it hurts to include them, more inclusive, the better, but how?

DR. RETTIE: When we were talking about hair dyes in the previous iteration of this, I think we took into account cosmetologist exposure. Did we do that? That might be helpful in guiding us here.

DR. BELSITO: I think we can just look at all the data, see how we feel it relevant. I don't have a problem with that.

DR. EISENMANN: For something like Toluene, I think you could also look and see if there are any recent reviews, like an ADME review or something like that, and not necessarily have to go back and pick up a lot of original studies.

DR. BELSITO: Right. There are also OSHA guidelines.

DR. EISENMANN: But I don't know if there's -- what?

DR. BELSITO: There are OSHA guidelines, right?

DR. EISENMANN: Yeah, I would think so. Yeah.

DR. RETTIE: Well, with the hair dyes, we were very specifically looking at cosmetologists as being the occupational exposure with toluene. Toluene is used for everything, so there's all types of occupations that will be brought in which might make it unwieldy. But we should look at all them too.

DR. BELSITO: But that's where I think, the OSHA guidelines, they will look at max effects. But, whatever data is in there, bring in.

DR. RETTIE: Would there be EPA data in there, too, for Toluene?

DR. BELSITO: Probably, but I wouldn't bring in every little minutiae. I would just say the OSHA guidelines are this. The EPA guidelines are this. I'm sure they have guidelines. And the California Air Quality Home study showed this. I think the major issue is going to come down to the point where, basically, we're talking about this in nail care products.

And, then, we have this study from California that suggests the home quality of the air is not quite good when individuals are using these nail products in their home. And we'll have to discuss that further once we look at all the data. Other points on Toluene?

DR. SNYDER: Just that they didn't like that Danish EPA methodology. So we'll need to take that in consideration when we look at that data.

DR. BELSITO: That's in Wave 2.

DR. SNYDER: Yeah, yeah, yeah.

DR. BELSITO: I need to go to Wave 2 on that.

DR. SNYDER: Yeah. It's Page 39, Wave 2.

DR. BELSITO: I did all the admin stuff first because I thought there would be no Waves to them. Page 39?

DR. SNYDER: Yeah.

DR. EISENMANN: One thing you should know in the exposure studies, that California paper is 1994. So toluene levels in nail products have decreased since then.

DR. BELSITO: Yeah.

DR. EISENMANN: So it would be helpful when you summarize it in a report to put the date of when it was done --

DR. BELSITO: Yeah.

DR. EISENMANN: -- for exposure studies so we know that that's an older study.

DR. BELSITO: What PDF page was that again?

DR. SNYDER: Page 39 of the Wave 2.

DR. BELSITO: Yeah. Mine were interrupted by things that my computer didn't like. It gave me these warnings for me. Give me a key word.

DR. SNYDER: WVE's comments on the strategy memo. Do strategy memo.

DR. BELSITO: Yeah.

DR. SNYDER: Good.

DR. BELSITO: Thank you.

DR. SNYDER: Mm-hmm.

DR. BELSITO: What was your point?

DR. SNYDER: In the Danish study, all these flaws and I'm assuming, incorporating it that --

DR. BELSITO: Oh, this was WVE with a risk assessment.

DR. SNYDER: Yeah.

DR. BELSITO: Yeah. Okay. Okie doke. Anything else with Toluene?

DR. BELSITO: Okay. Sodium Dehydroacetate.

MS. CHERIAN: Can we come back to Toluene before we move on?

DR. BELSITO: Yeah.

MS. CHERIAN: How do we feel about oral studies where animals are fed high quantities of Toluene? I found a lot of those that cause neurotoxic effects, behavioral effects, stuff like those. Do you want those in there?

DR. SNYDER: If there's been a rereview or a review paper that you can just cite that one paper and capture all those. It's well established that those high levels cause those. Yeah. So I think we can capture that with just one or two review papers.

MS. CHERIAN: Okay.

DR. SNYDER: There should be probably in your book. Isn't it?

DR. KLAASSEN: Probably.

DR. SNYDER: Yeah.

DR. KLAASSEN: I guess the question is, is it really neurotoxic effect or anesthetic effects?

DR. SNYDER: Yeah, exactly.

DR. BELSITO: Guess it depends upon how you want to define it.

DR. SNYDER: Substance of abuse.

DR. BELSITO: Yeah.

DR. SNYDER: There are Toluene sniffers.

DR. BELSITO: Sniff your pillow.

DR. SNYDER: There are Toluene sniffers.

DR. BELSITO: Are there really?

DR. SNYDER: Oh, yeah, just like gas.

DR. BELSITO: Just like gas.

DR. SNYDER: Gas and glue, gasoline. We had a medical pathologist present a case of one who drowned.

Cohen Team – September 11, 2023

DR. COHEN: Okay, there being none we'll move onto Toluene, and I really look forward to all of your remarks on this. Someone let me know when the conversation is done so I can come back. That's a joke, for the record.

So, this is a strategy memo on Toluene Literature Selection. So, we're being asked to just comment on strategy here. Right? Following the March meeting, draft 2024 priorities list, we received communication from members of the FDA nominating Toluene to the 2024 priority list, and in June we agreed to accelerate the re-review.

The 2023 VCRP data on Toluene has zero reported uses, however, use survey conducted by the Council, in '22 to '23, had reported uses up to 20 percent in nail polish and enamel products, and ten percent in nail extenders, and considerably lower concentrations in hair formulations, bath products, some baby products. In comparison, 59 uses were reported in the 2002 VCRP at maximum concentrations of 26. We should note that Toluene is listed in Annex III in the EU, restricted to nail products at 25 percent.

So, after reading this document, there's the issue of what data ought to be interrogated, what should be included, and health outcomes that are relevant to the multiple exposures that are relevant to cosmetic use versus occupational use.

So why don't I -- and of course, we saw some interesting comparisons to formaldehyde. I think the Danish did that. So, does the Panel approve of the approach that was listed here -- there's a lot of data so I'm not going to just read through it. And does it align with our thinking? Tom, do you want to kick it off?

DR. SLAGA: Yeah. I think it's a good approach so I would approve it.

DR. COHEN: Susan?

DR. TILTON: So, my understanding is that the approach is being proposed because the past conclusions of the panel in 2005, describing adverse health effects only at concentrations that exceed use in cosmetic products. The current available data of max use is up to 20 percent. And then we have the Annex III restriction at 25 percent in nail products for adult use only.

So, in terms of reporting the data, I mean, I would at least like to be able to review the documentation that was used by the EU to establish that 25 percent limit in adults only. So presumably that does rely on data that exceeds max use at 20 percent. I

also recognize that sometimes -- you know, we don't typically rely on occupational data, but when you have some of that data you think of it as sort of the worst-case scenario in humans, and we tend to typically include that in reports.

So, I guess my question in terms of having to go through the literature and reviewing everything that's available, is -- I mean, each reference is going to have to be reviewed anyways to evaluate whether or not it would be included or not. Is it possible to include some of this information in a table format that would be similar to what we get for re-reviews, as opposed to synthesizing it and including it in the report itself? Is that a level of documentation that would be reasonable for this volume of data?

DR. ROSS: If I could just comment, I think Susan's been reading my notes, here. Are you looking over my shoulder here?

DR. TILTON: That's the fifth camera.

DR. ROSS: I had almost exactly the same comment. So, yeah, I like the Danish risk assessment, although I thought they may have one number wrong in that. But anyway I'm not going to go into that here.

DR. COHEN: I think that came up in the second wave.

DR. ROSS: Oh, really?

DR. COHEN: Yeah.

DR. ROSS: Okay. And with the literature strategy, I wasn't too keen on it because I think you're going to have to look at these reports anyway, as Susan pointed out. I don't want to miss anything because even though it's high dose, sometimes it points you in the right direction, whether that's metabolism or some other direction. So, it'd be nice to summarize the previous reports which you would do. You know, list the sections as you did in that report with a number of references, DART, for example, was 47 references. And just as Susan said, you could comment that the majority of these were too high.

But you're going to have to look at it anyway, so it would be nice to see that summary data in table. Exactly what I have here, Susan, just as you said. So, I'm sort of aligned with Susan. I think it would be nice to see that in a summary report format, even though it's high dose, just so the panel doesn't miss anything.

DR. COHEN: So, just from a procedural question. If we had agreed with the proposal to limit to collar the data, how would you do that without reviewing the article itself?

DR. ZHU: I think the first step we need to go through the abstract and the mean data. Okay. I mean, first step we need to go through the abstract of the mean conclusion and access the exposure level and whether it's related to the occupational settings, whether it's totally irrelevant to cosmetic use. So, and then we can select the (inaudible).

DR. COHEN: So, you're going to look at the abstract and if it's occupational you would stop there under this proposal?

DR. ZHU: Also based on the exposure, I mean, assessment.

DR. ROSS: So, you're going to have to look at it anyway.

DR. ZHU: Yes. We would do that, I mean, just not to incorporate those irrelevant data to the report. But we need to, I think, screen the data.

DR. ROSS: I wasn't suggesting we go into every occupational study in depth, but I think if we could just summarize it in some way so that the Panel had access to that.

DR. ZHU: Yeah.

DR. ROSS: Here in terms of tabular form. There's 50 references in this area, but 25 are at high doses, et cetera, et cetera.

DR. ZHU: Yeah. I think that's a good way because some occupational studies it's access exposure. In the air force personnel, like workers in the petroleum industry, I think, those exposure is totally irrelevant to cosmetic use, so.

DR. ROSS: No question. But there's a lot of work came out of those studies on, for example, things like metabolism which helps us out --

DR. ZHU: Yes, of course.

DR. ROSS: -- when we consider that. Yeah.

DR. ZHU: Yes, metabolism data. Yeah. ADME data. So just whether its need to be incorporated in the -- yeah. Still some data there.

DR. COHEN: I think just as it relates to the Women's Voices of the Earth comment in Wave 2, I think we're talking about two parallel issues here. One, their comment is several occupational studies of Toluene exposure in salon workers which must be included. Right? And it's different than what David is mentioning, is what information can be gleaned from high-level occupational exposures that we may be able to help inform us for cosmetic use.

The wording that I see in the WVE seems to pull us into occupational exposures in cosmetic workers, which is not the way I view our call here. Right? There's two different reasons to include it and I'm not sure cosmetic workers are the target of what we should be looking at. But certainly if there's data on cosmetic workers, I mean, we might want to review that.

DR. TILTON: David, I think that's a good point, and I made that distinction, too. That we want to make sure that we are not utilizing the document, you know, for the purpose of occupational exposures. That's not the purpose of the report.

DR. SLAGA: That's right.

DR. TILTON: But it can help to have that data available in terms of interpreting some of the other toxicological studies.

MS. CHERIAN: I can create some sort of table with the occupational studies and just a really brief summary of what happened with each of them. But when it comes to ADME studies -- and a lot of what I saw are neurotoxicity studies, totoxicity studies -- some of them might be in rats, per se, that they orally gave a large dose to.

Do you want to see oral studies when animals, especially, are given large doses of Toluene? For ADME too, do you want to see oral or just exposure via dermal penetration and inhalation?

DR. ROSS: I mean, what I had envisioned was that you would have sections at the end. Let's say the neurotox, for example, which was 108 references. And you would have a summary there, which would be just a few sentences and directing people to those references which you would put on a link if they needed to go there.

And toxicity is reported in 50 citations and the majority are not likely to be encountered in cosmetic use. Then in the Summary, lower dose XYZ resulted in ABC. You know, that sort of thing.

I mean, I don't know how realistic that is. But if you have to look at the data anyway to exclude it, it seems like a reasonable approach.

DR. COHEN: Yeah. But this is a much deeper dive. What we're suggesting, and I'm tending to agree, but there's a big difference between pulling an article, looking at the abstract, determining that it's very heavy exposure, it's a strange occupational exposure or it's a study where there's oral exposure to animals, and then stopping there; as opposed to now diving into that paper to see what it was all about.

DR. ROSS: I mean, it's going to be in the abstract. My basic point on this was I just didn't want the Panel to miss anything.

DR. COHEN: Okay.

DR. HELDRETH: So, then, what I think I'm hearing is that what would be useful is if we really didn't exclude anything that talked about any sort of biological impact from Toluene. But to categorize it as best we can, into a table, similar to what you would get in an abbreviated re-review. But instead of necessarily going through our own evaluation of each of those documents, instead, in that box simply provide the abstract. That may get something that the Panel could pair down on what actually seems useful, and be included when Priya starts to draft her draft amended report.

DR. COHEN: You're suggesting like in a re-re- -- right, I think that was a good example, the re-review summary. The abstract of the article would be in there? I think that would have to be one of the other two things we look at the whole day, right, I mean, won't it? It would be exhaustive, right?

DR. HELDRETH: I mean, even if we agreed to it and start working on it, I'm not going to see this come back in December. It's going to take a long time for Priya to be able to put that together.

DR. COHEN: Remind me how many references we're talking about?

DR. SLAGA: Many.

DR. ROSS: Yeah. I added up 300.

MS. CHERIAN: As of right now, too.

DR. COHEN: Right. You haven't dug in yet.

MS. CHERIAN: And these are including the past re-reviews, so 2005, those references (inaudible). The Panel did see them (inaudible). They took all the references that were in the re-review summary from 2005, that's in here too. If you want to make some sort of timeline -- sorry. If you want to make some sort of timeline instead to later dates, since the Panel saw the data in the re-review summary, you could do that too.

DR. COHEN: So, it would be 2005 on?

MS. CHERIAN: Yes.

DR. BERGFELD: Can I make a comment? If you look at the old document that was attached, we had dealt with some of these toxicities by confining this to nail product and a certain concentration. And now we have no FDA uses, we just have industry that has come forward. So why can't we confine ourselves to nails?

We have in the old document, regarding occupational exposure limits, a statement by the American Conference of Governmental Industrial Hygienists, when they actually limited the exposures.

DR. COHEN: Well, we probably want to see then, I think, as Susan mentioned, review the data that led the EU to Annex III with its 25 percent limit, right?

DR. BERGFELD: It's only 5 percent over the 20 percent that we had dealt with. Not 50 percent.

DR. COHEN: So where are we standing here because it sounds like we're going to have a tsunami of information coming at us that we might -- we in our hearts want to review, but I don't know if we're going to be able to.

DR. ROSS: I didn't see it as a tsunami. I think it's just a summary of numbers in these different categories that -- you know, what I was proposing, I don't think you've got to go into each article and summarize it.

But, I mean, if you're going into each article to subdivide it, oh, this is the abstract, this high concentration occupational, this is not. Then you list how many in each section, whether it's new or whether it's ADME, whether it's this, whether it's that.

And you put a link to it so if David has -- you know, he might occasionally want to look at metabolism and ADME, you never know. But he can click on the link and go to it. And so that way the Panel's not missing anything. I mean is that -- maybe I'm not articulating this clearly.

DR. COHEN: No, you are, but we're going to have 300 or 400 links. And we're still going to have to trust the -- it's always going to come down to is the writer screening that document correctly, because you're not going to open all the links, no?

DR. ROSS: No, it wouldn't be 300 or 400 links. I'm not suggesting a link for each document, I'm suggesting a link for each section, that would just then link all the papers in that section. And maybe that's not workable. Maybe we've got to test drive it and look at it, because I see some skeptical looks from either side of the table here. So maybe it's not practical.

DR. COHEN: Well, it's not that often we get a strategic memo on how a report's going to be written, right? So, I took that as, you know, Houston, we have a problem. Right? And so how are we going to do this and I'm a little concerned that, number one, we'll be including a lot of information that can be erroneously connected to cosmetic use.

DR. SLAGA: Yeah.

DR. TILTON: I would, first of all, agree to focusing just on nail use as one filter for data. I mean, however the evaluation is done for what's included, it will need to be very clearly outlined as what criteria were used in terms of what's included and what wasn't included.

So, I think David's recommendation, since you're going to have to go through and categorize the papers anyways in terms of whether it's included or it's not, we should have some kind of thinning as in these papers didn't meet the criteria for inclusion because of either high concentration or outside of use. And we should know how many papers, kind of, in each category were included for those reasons. That's essentially the kind of summary you're suggesting, referring to David Ross.

DR. COHEN: I think that's a very good compromise. So, if you were going to proceed with the proposed strategy that you had listed, you're going to exclude hundreds, perhaps, of articles, right? So instead of just discarding them, just put them on a list of discarded articles.

DR. ROSS: You're finally there. Yeah, great.

DR. COHEN: Are you insinuating that was your idea all along?

DR. ROSS: I wouldn't dream of it.

DR. COHEN: Yeah. Yeah. If it was, it's a great idea. But maybe why don't we just do that. Would that suffice? Right. Okay. I think perhaps that is what you were saying all along, but can we do that? Okay.

DR. ROSS: Can I just ask one question on this? Were you -- and this is to Bart and to writers -- were you thinking of including the margin of safety for nail use per the Danish EPA-type calculation? Were you going to do a similar calculation with Toluene in here or for MOS? Were you considering doing that?

DR. ZHU: If the Panel request it, we can do it. Yeah.

DR. ROSS: I think it would be useful.

DR. ZHU: Okay.

DR. ROSS: Bart, you got any --

DR. HELDRETH: Yeah. No. I don't see any problem with it. Honestly, I think having some sort of a more formalized risk piece in every CIR safety assessment might be useful; not only to put us more in line with most safety assessors around the world that always have a formal risk piece in their safety assessment, but it helps lay some of the groundwork for confidence in these new approach methodologies, to have that risk piece in there.

So, what I'm hearing the consensus for this, is that, yes, we kind of -- kind of and maybe -- accept this approach. But instead of discarding those that don't fit within the approach, they're put into a re-review-esque table that has the abstracts so that the Panel can go through and say, oh, actually I think this one is relevant and you should bring it back in.

DR. ROSS: Yeah. You'd have to experiment with the format you use for linking the abstracts. I mean, I don't think you need the abstracts in there but --

DR. HELDRETH: Yeah. I'm not sure we can do the linking for copyright issues, but we can certainly give the citations and discuss what the abstract said.

DR. COHEN: Citations would be okay.

DR. ROSS: Citations would be fine.

DR. COHEN: You want to articulate your comment, again, about the risk assessment that you wanted in here.

DR. ROSS: Oh, you mean the margin of safety?

DR. COHEN: Yeah.

DR. ROSS: Yeah. I mean, it just basically followed the Danish work on the formaldehyde assessment for nails and I think Wilma made some comment on that, and can we incorporate that for nail use?

DR. ZHU: We already did that in this draft memo for Toluene in nail products.

DR. ROSS: That's right.

DR. ZHU: Yeah.

DR. ROSS: Yeah.

DR. ZHU: It's in there already.

DR. ROSS: For the --

DR. ZHU: Toluene used in the nail product. We already did that risk assessment.

DR. ROSS: Okay. Yeah. That has to be in in the forthcoming ones. Yeah.

DR. ZHU: Yeah.

DR. ROSS: Yeah.

DR. COHEN: They used ten nails.

DR. ZHU: Yeah, ten nails.

DR. COHEN: But I checked, I have 20 nails. And people do paint their toenails and their fingernails, often at the same time. Right?

DR. ZHU: Yeah. Okay.

DR. COHEN: So, I thought that was odd that just ten nails were used, when I see it at home people are doing all of their nails.

DR. ZHU: Well, the exposure metrics -- the Panel can decide what exposure metrics you want to see in the risk assessment. Like the skin area surrounding the nails you want a higher percentage to be used in the risk assessment, that's fine, we can do that.

DR. COHEN: I think so. Twenty nails and what was the percentage? It was a low percentage they used for --

DR. ZHU: Twenty. Twenty percent. Twenty the highest. I mean the Toluene, right, maximum use concentration?

DR. COHEN: No, no, I meant the amount getting on the cuticle and periungual skin.

DR. ZHU: That's nine percent.

DR. COHEN: Okay.

DR. TILTON: Yeah. So, the exposure was based on the nine percent on the skin plus 12.5 percent inhaled from evaporation, so it created kind of a combined in total 21 --

DR. ROSS: 21.5.

DR. ZHU: 21.5.

DR. TILTON: -- percent.

DR. ROSS: Yeah.

DR. COHEN: Okay. So, we're okay using those parameters, but for 20 nails? Yeah, it was nine plus 12.5. Any other comments? I mean, so we'll use 20 nails and use the same parameters? Susan?

DR. TILTON: Yeah. I mean, it was discussed in the strategy memo how the inhalation exposure is expected to be an overestimate because of the lower volatility of Toluene. There really was no discussion about the amount on the skin, or where that number -- how that number was derived for the formaldehyde studies. But I'm okay using the same values as in the formaldehyde study.

DR. COHEN: Can we get further information about how they came up with 9 percent on the skin?

DR. ZHU: Oh, you mean the skin area around the nails? That's about -- I think it's about based on the -- I'll check the document for more accuracy.

DR. ROSS: Have there been other regulatory agencies which have used a similar approach as we see listed from the Danish EPA? Have there been additional regulatory agencies who have used the same approach, or is this the only one?

DR. ZHU: Oh, I think their data based on the SCCS Note of Guidance. But it is the 5th or the 6th Version, not the newest version. Because, I think, in the newest version the nail product used, I mean, they removed the category for nail product in the newest version of SCCS Note of Guidance. That's based on 2006 Version.

DR. ROSS: Well, if you could help us understand the skin exposure parameter in there that would be helpful.

DR. ZHU: Okay.

DR. TILTON: The other option is to create a range, I guess, maybe based on that one parameter if we --

DR. ROSS: That's a good idea.

DR. COHEN: It would just be nice to know how that number was even achieved.

DR. BERGFELD: Is there a document?

DR. TILTON: It's an aspect of the Danish exposure calculation that Women's Voices for the Earth also pointed out most concern about, especially as it relates to children using nail polish.

DR. COHEN: Right. I think that's a reasonable point because when kids put nail polish on, they often have polish on the tips of their fingers.

DR. BERGFELD: Smaller body surface.

DR. COHEN: Yeah. Okay, so we'll get that --

DR. BERGFELD: Can I ask a question? It was noted that the European Union has it limited to 25 percent in nail products. Is it possible to get what they base that on, so it might fulfil some of the needs that we're talking about?

DR. COHEN: Yeah. I have a comment here that we'd like to review the data that led the EU to Annex III and their max use of 25 percent in nail products. That's a request we have. Okay. Any other comments on Toluene?

MS. CHERIAN: I just wanted to make it known that in the past iterations of this report all the uses were in nail products. But in 2023, the concentration states there are other uses but they're at super, super low concentrations. So, I just wanted to make sure that was known.

DR. COHEN: Yeah. Yeah. That was in your letter to us, right --

MS. CHERIAN: Yes. Yes.

DR. COHEN: -- and it had parts per million of 0.04 parts per million, 0.01 parts per million. Look, I think we could deal with that if we have a good estimate of the skin exposure from the nail products at 25 percent. So, we'll cover a lot of that.

You know, nail products get everywhere on people. It just doesn't stay confined to the nails; it doesn't stay confined to the periungual skin. It winds up on your face, your eyelids, your neck, your mouth. So, I think the nail anchor is a good one because of high concentration. Okay, any other comments? Susan? Tom?

DR. SLAGA: No.

DR. COHEN: -- David?

Full Panel – September 12, 2023

[Insert full Panel minutes]

APRIL 1986 MEETING – TENTATIVE FINAL REPORT

Toluene

Dr. Schroeter reported that Toluene is used only in nail polishes and manicures and therefore has limited contact with the skin. However, animal studies show that undiluted Toluene is a skin irritant. He stated that no clinical photosensitization data were available and a photospectrum was requested. This was subsequently received and showed that Toluene, at three different concentrations, was not a photoreactor.

Dr. Schroeter then stated that his team was recommending the standard conclusion of "safe as used in cosmetics".

Dr. Bergfeld indicated that because Toluene was such a skin irritant, she wanted the discussion to emphasize that it is used only in nail products.

Ms. Fise recommended using "intended" use in cosmetics as some people generally do get some nail polish on the skin.

With minor editorial changes, the Panel then unanimously accepted and approved the Schroeter team recommendation.

The Tentative Final report will shortly be announced for a 90-day public comment period.

MARCH 2005 MEETING – RE-REVIEW

Day 1 – Dr. Belsito's Team

•The new data by BioResearch showed that exposures to Toluene for women using nail polish were 2-3 orders of magnitude smaller than those used by researchers in studies with animals.

•Andrew Jacques, with the American Chemistry Council, said that the systemic toxicity NOEL was 1000 ppm and that the reproductive and developmental toxicity NOEL was 2000 ppm for rats.

•Dr. McEwen said that a letter, available from the attorney general in California, states why the use of Toluene does not require a Proposition 65 warning label.

•Dr. Belsito stated that no substantive new information has entered the published literature since the CIR Final Report on Toluene was issued.

•The Team concluded that the published CIR Final Report on Toluene should not be reopened.

Day 1 – Dr. Marks' Team

In response to Dr. Bergfeld's question, Dr. McEwen said that the exposure limit for Toluene in salons is approximately 17,000 ppm.

•In response to Dr. Marks question about the salon study that was performed, Dr. McEwen stated that only 3 salons were studied and that it was not a controlled study.

Dr. Marks stated that for home use, there was probably no issue but that there may be an issue with regard to salon employees. After further discussion, the Team agreed that OSHA would responsible to any issues relating to salon exposure.

•Dr. Andersen recommended the addition of a sentence relating to developmental and reproductive toxicity to the safety assessment, stating that the findings were mixed due to a dose response.

•Mr. Jaques (American Chemistry Council) stated that a new developmental study has been completed and that he would make it available to CIR.

•The Team decided not to reopen the CIR Final Report on Toluene, and to include a statement indicating that the mixed findings relating to developmental and reproductive toxicity were due to a dose-response phenomenon.

Day 2 – Full Panel

Dr. Marks stated that a Final Report with the following conclusion was issued in 1987: On the basis of the available data presented in this report, the CIR Expert Panel concludes that Toluene is safe as a cosmetic ingredient in the present practices of use and concentration.

After reviewing data that have entered the published literature since the Final Report was issued, the Panel unanimously agreed that the published Final Report on Toluene should not be reopened and that the original conclusion should not be changed.

Dr. Belsito said that he assumes that the data on consumer exposure levels will be incorporated into the Annual Review.

Dr. Marks said that the consumer exposure data relate to the home use (not salon use) of nail products. He added that his Team is convinced that Toluene-containing nail products for home use are safe.

Amended Safety Assessment of Toluene as Used in Cosmetics

Status:	Draft Amended 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 Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651
cirinfo@cir-safety.org

ABBREVIATIONS

3β-HSD	3β-hydroxysteroid dehydrogenase
8-OHdG	8-hydroxy-2'-deoxyguanosine
17β-HSD3	17β-hydroxysteroid dehydrogenase
ACTH	adrenocorticotropic hormone
ADME	absorption, distribution, metabolism, and excretion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMP	adenosine monophosphate
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BAL	bronchoalveolar lavage fluid
CaMKIV	calcium/calmodulin-dependent protein kinase
CAS	Chemical Abstracts Service
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CREB1	cyclic adenosine monophosphate responsive element binding protein 1
CRF	corticotropin-releasing-factor
CV	coefficient of variation
DMSO	dimethyl sulfoxide
DTSC	Department of Toxic Substances Control
ECHA	European Chemicals Agency
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FACS	fluorescence activated cell sorter
EU	European Union
FDA	Food and Drug Administration
GD	gestation day
GDF9	growth differentiation factor-9
GGT	gamma-glutamyl transaminase
HCIS	Hazardous Chemical Information System
HPA	hypothalamus-pituitary-adrenal
HPT	hypothalamus-pituitary-thyroid
HQ	hazard quotient
HR	hazard ratio
IARC	International Agency for Research on Cancer
IFN	interferon
Ig	immunoglobulin
IGF-1	insulin-like growth factor 1
IL	interleukin
Insl3	insulin-like 3
LC3	light-chain 3
LCR	lifetime cancer risk
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
ln	natural logarithm
LOAEL	lowest-observed-adverse-effect-level
log K_{ow}	n-octanol/water partition coefficient
MADL	maximum allowable dose level
MI	multiplicative interaction
MOS	margin of safety
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MTT	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NHANES	National Health and Nutrition Examination Survey
NMDA	N-methyl-D-aspartate
NOAEC	no-observed-adverse-effect-concentration

NOAEL	no-observed-adverse-effect-level
NR	none reported
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OEL	occupational exposure limits
OR	odds ratio
OVA	ovalbumin
P450c17	cytochrome P450 17α-hydroxylase/c17-20 lyase
P450scc	cytochrome P450 cholesterol side-chain cleavage
Panel	Expert Panel for Cosmetic Ingredient Safety
PCR	polymerase chain reaction
PEL	permissible exposure limit
PGN	peptidoglycan
PND	post-natal day
PVN	paraventricular nucleus
REL	recommended exposure limit
RERI	relative excess risk due to interaction
RfC	reference concentration for chronic inhalation exposure
RfD	reference dose for chronic oral exposure
RT-PCR	reverse transcription–polymerase chain reaction
SCCP	Scientific Committee on Consumer Products
SCE	sister chromatid exchange
SED	systemic exposure dose
SKF525A	2-diethylaminoethyl-2,2-diphenylvalerate-HCl
STEL	short-term exposure limit
TG	test guideline
TH	helper T-cell
TNF-α	tumor necrosis factor – alpha
TSH	thyroid-stimulating hormone
TUNEL	terminal deoxynucleotidyl transferase dUTP nick-end labeling
TWA	time weighted average
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

INTRODUCTION

Toluene, which according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*) is reported to function in cosmetics as an antioxidant and a solvent,[1] was previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) in a safety assessment that was published in 1987.[2] At that time, the Panel concluded that Toluene is safe as a cosmetic ingredient in the present practices of use and concentration, as stated in that report. The Panel first considered a re-review of this report in March 2005,[3] and the Panel re-affirmed the original conclusion, as published in 2006.[4] Subsequently in 2023, the US Food and Drug Administration (FDA) nominated Toluene for an accelerated re-review; in accord with Cosmetic Ingredient Review (CIR) procedures, the report was re-opened and a Draft Amended Report has been prepared.

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 January 2024. 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 CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Numerous studies were found during this process, many of which were cumulative to the information already provided in this report, or were not relevant to cosmetic safety, and were therefore not included herein. Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Excerpts of data from the original 1987 safety assessment are summarized throughout the text of this document, as appropriate, as are summary excerpts of the original re-review document[3] considered by the Panel in March 2005. These data are identified using *italicized* text. (This information is not included in tables or the Summary section.) For complete and detailed information, the original 1987 report can be accessed on the CIR website (https://www.cir-safety.org/ingredients).

CHEMISTRY

Definition and Structure

Toluene (CAS No. 108-88-3; molecular weight = 92.13 g/mol; log K_{ow} = 2.73), an ubiquitous volatile organic compound, is a homolog of benzene in which one hydrogen atom has been replaced by a methyl group.[2,5,6] According to the *Dictionary,*[1] Toluene is an aromatic compound that conforms to the structure:

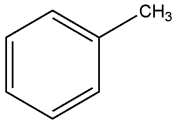

Figure 1. Toluene

Chemical Properties

Toluene is a clear, refractive liquid with an aromatic odor similar to benzene that is both volatile and flammable.[2] *No significant absorption was noted above 300 nm when the ultraviolet absorption spectrum of 300 g/l of Toluene diluted in hexane was measured.*

Toluene is miscible in several organic solvents, and has a water solubility of 526 mg/ml.[6] Since Toluene has a low molecular weight, is a liquid at room temperature, and has an octanol/water partitioning coefficient between -1 and 3, systemic exposure resulting from topical application cannot be easily mitigated. Chemical properties of Toluene are summarized in Table 1.

Method of Manufacture

Three major sources of Toluene production include petroleum refining processes, as a by-product of styrene production (via the dehydrogenation of ethylbenzene), and as a by-product of coke oven operation (high-temperature carbonization of coal).[2] *Petroleum refining processes to isolate Toluene are either performed via catalytic reforming or pyrolytic cracking. Catalytic reforming involves the catalytic dehydrogenation of selected petroleum fractions, resulting in a mixture of aromatics and paraffins. Toluene is isolated from the reformate via distillation, washing with sulfuric acid, and re-distillation. Toluene can be purified via various extraction and distillation processes (Udex extraction, sulfur dioxide extraction, sulfolane extraction). The grade of Toluene (e.g., pure, commercial, solvent) is defined in terms of boiling ranges.*

Impurities

Commercial Toluene may contain benzene as an impurity.[2] *Toxicological and clinical studies involving Toluene should specify the purity of Toluene used for experimentation to determine if observed effects were caused by Toluene, and not benzene as an impurity.*

According to the Food and Agriculture Organization the United Nations, Toluene should not contain more than 5 mg/ 100 ml non-volatile residues, 0.2% non-aromatic substances, 0.5% benzene, or 2 mg/kg lead.[7] The ingredient should have a purity of no less than 99%, and should be negative for hydrogen sulfide and sulfur dioxide. In addition, an ECHA dossier on Toluene reported potential impurities of ethylbenzene, *m*-xylene, *o*-xylene, *p*-xylene, and benzene.[8]

Reactivity

Toluene undergoes substitution reactions (halogenation, chloromethylation, nitration, acetylation, benzoylation, mercuration, sulfonation, brosylation, methylation, and isopropylation) on the aliphatic side group (-CH_3) and on the benzene ring at the ortho- and para- positions.[2] Toluene can be oxidized with air under catalytic conditions to yield benzoic acid. In the presence of heat (or catalyst) and hydrogen, Toluene undergoes dealkylation to produce benzene. Under water chlorination, Toluene may undergo hydrolysis to produce benzaldehyde. In the presence of solvents (e.g., paraffins, naphthenics, and alcoholic hydrocarbons), Toluene may produce azeotropes. Toluene may also undergo photo-oxidation and other photochemical reactions. Toluene is reported to be chemically-stable and unreactive under conditions of use in cosmetic preparations.

A study was performed to evaluate the pyrolysis products of Toluene. Toluene vapor was passed through nitrogen through a silica tube filled with porcelain chips at Toluene at 700° C. Reported pyrolysis products included some known or suspected carcinogenic aromatic hydrocarbons (e.g., 1,2-benzathracene, benzene, 3,4-benzofluoranthene).

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover their 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.

No uses were reported for Toluene according to 2023 FDA VCRP data; however, according to a concentration of use survey performed in 2023, Toluene is used at up to 20% in nail polish and enamel (Table 2). It should be presumed that there is at least one use in every category for which a concentration is reported.[9,10] In 2002, Toluene was reported be used in 59 total formulations, at up to 26% in other manicuring preparations (according to 2003 concentration of use survey).[4] All uses and concentrations provided in 2002/2003 were in nail products. Current (2023) concentration of use data indicate that Toluene is used in nail products, as well as other product categories (e.g., baby products, hair conditioners and tints, bath soaps and detergents).

According to 2023 concentration of use data, Toluene is used in baby lotions/oils/creams at up to 0.000001%.[9] In addition, according to the California Safe Cosmetics Program Product Database, Toluene is also used in lip glosses at concentrations of 0.00005% (which may result in incidental ingestion; database updated in 2024).[11] This database also reported the use of Toluene in perfumes at up to 0.0042%.

Toluene is used at up to 0.000002% in deodorant sprays, and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Although products containing this ingredient 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.

The use of Toluene in cosmetics in the European Union (EU) is restricted to nail products at maximum concentrations of 25%.[12] In addition, according to the EU, caution statements should be used informing users to keep products containing Toluene out of the reach of children.

Cosmetic Use Exposure

Nail products containing Toluene may be applied several times a week over an extended period of time.[2] Areas directly exposed to Toluene include the fingernails, toenails, cuticles, and skin surrounding the nail area. Other areas of the body (e.g., eye region, face) may come in contact with the ingredient prior to the drying of the wet polish. In addition, Toluene may come in contact with the eyes and nasal mucosa during product application due to evaporation.

The amount of Toluene in the breathing zone before, during, and after exposure to nail product application was evaluated in 15 female subjects under simulated-use conditions (conducted following principles of Good Laboratory Practice and Good Clinical Practice).[13] Each subject applied a base coat, two enamel coats, and top coat (all products formulated with 25% Toluene; minimum of 1 min drying time between applications). Sampling began 1 min before application and was continuous throughout the application period until the subject declared her nails to be dry after the 4th application. When nails were dry after top coat application, participants were asked to leave the room until Toluene concentrations returned back to a stable baseline. This procedure was performed 3 times, on 3 consecutive days. Toluene concentrations in the breathing zone were measured using an infrared gas analyzer connected to a recirculation pump with flexible tubing, within a 16 m^3 that maintained an air flow of about 1.0 changes per hour. The sampling rate was set at 30 l/min. Exposure per subject was calculated by multiplying the mean Toluene concentration by application duration and a pre-calculated minute inhalation constant for women. The mean total of the nail polish applied was 0.5276 g (coefficient of variation (CV)% = 20.60%). The estimated average Toluene exposure amount, which was measured in the breathing zone of all subjects was 0.6 mg on study days 1 (CV% = 33%) and 2 (CV% = 50%), and 0.5 mg (CV% = 40%) on study day 3, with a mean application duration of 15 min.

Analytical air measurements of Toluene content were taken on 178 professional nail technicians who were working with Toluene-containing cosmetic nail products with their clients.[14] The mean Toluene exposure from inhalation was 0.236 ppm, or 0.260 ppm at the 90% upper confidence level. In the same study, the results of air sampling for the customers of the nail technicians were 0.149 ppm (mean) or 0.166 ppm at the 90% upper confidence level.

In 2011, the Department of Toxic Substance Control (DTSC) measured Toluene concentrations in nail polish products available in the San Francisco Bay Area. Toluene was detected in 83% of nail products that claimed to be Toluene-free at concentrations up to 190,000 ppm.[15] Dermal and inhalation exposure to a salon patron, nail technician, and home user was evaluated. The maximum daily exposure (dermal and inhalation) in salon patrons, nail technicians, and home users was determined to be 2160, 28,200, and 7760 µg/d, respectively.

According to the Scientific Committee on Consumer Products (SCCP), cosmetic nail product application is typically less than 30 min, and although products may come in contact to the keratin of the nail plate, penetration of Toluene through the nail plate is nil or minimal due to the hydrophobicity and low vapor pressure of the substance.[14,16] In addition, although products come into contact to the skin, this contact is also typically nil or minimal. In 2008, the SCCP concluded that the occasional exposure to Toluene present in nail cosmetics where the exposure may be within the range of 1 – 4 ppm can be considered safe.

Exposure to Toluene as An Impurity in Personal Care Products

While Toluene is commonly an intentionally added cosmetic ingredient, in consideration of total aggregate exposures, it may be worth noting that it has also been reported to be present as an impurity in several products, including hand sanitizers (in amounts of 0.074 – 20,700 ng/g), feminine hygiene products (in amounts of up to 4538 ng/g in feminine sprays and powders), and sunscreen (in amounts of 0.006 – 470 ng/g).[17-20] The mean dermal exposure dose of Toluene was calculated to be 133 ng/kg bw/d (in children) and 94.6 ng/kg bw/d (in adults) in subjects exposed to sunscreens containing Toluene as an impurity, and were calculated to be 14.4 ng/kg bw/d (in children) and 10.3 ng/kg bw/d (in adults) in subjects exposed to hand sanitizers containing Toluene as an impurity. Feminine hygiene products containing Toluene as an impurity were associated with a higher calculated cancer risk (largely due to presence of benzene in products).

Non-Cosmetic

Toluene may be used as an indirect food additive, gasoline additive, ink thinner, non-clinical thermometer liquid, suspension solution for navigation instruments, extraction solvent for plant materials, and as a solvent for many industrial substances (e.g., adhesives).[2] Toluene is also used a s a starting material for the product of several chemicals (e.g., benzene), polyurethane resins, detergents, dyes, and drugs.

Several CFR citations have been found regarding the use of Toluene in the food and drug industry. A listing of these CFR citations can be found in Table 3. According to these citations, Toluene may be used as an indirect food additive, a denaturant, and as an ingredient in veterinary pharmaceuticals. Toluene is commonly used in glue and spray paint products; it should be noted that according to Directive 76/769/EEC on certain dangerous substances and preparations, Toluene is banned in glue and spray paint concentrations above 0.1% in products of the general public (in the EU).[14]

TOXICOKINETIC STUDIES

Dermal Penetration/Percutaneous Absorption

In vitro penetration of Toluene through excised rat skin was estimated to be 8.5 nmol/min per cm^2.[2] Blood concentrations of Toluene were determined to be 1.1 and 0.60 µg/ml in guinea pigs dermally exposed to 1 ml Toluene after 0.5 h and 6 h, respectively. The rate of absorption of undiluted Toluene (0.2 ml) through the skin of the hands and forearms of humans was estimated to be 14 - 23 mg/cm^2/h after a 10 - 15 min exposure. When the hands and forearms were immersed for 1 h in an aqueous solution containing 180 - 600 mg Toluene per liter, the rate of absorption was determined to be 0.16 - 0.60 mg/cm^2/h. Study authors estimated that exposure of both hands in a saturated solution of Toluene for 1 h would be

equivalent to inhalation exposure to an atmosphere containing 26.6 ppm Toluene for 8 h. Between 2050 and 3370 mg of Toluene was absorbed in volunteers wearing respiratory protection (volunteers emerged hands in pure Toluene for 10 min). Percutaneous absorption was estimated to be about 0.9% of the amount that would be absorbed from the respiratory tract during a 3.5 h exposure to 600 ppm Toluene (masked subjects intermittently exercised during study period to enhance percutaneous absorption).

A physiologically-based pharmacokinetic model was used to evaluate the dermal absorption of Toluene in human subjects.[3] The average dermal permeability coefficient of Toluene was 0.012 ± 0.007 cm/h.

The dermal penetration/percutaneous absorption studies summarized here can be found in Table 4. Maximum Toluene concentrations of 3.07 ± 0.40 µg/ml (in samples exposed for 15 min) and 5.38 ± 0.92 µg/ml (in samples exposed for 240 min) were reported in receptor fluid in an in vitro dermal penetration assay using rat skin exposed to 100% Toluene.[21] The effect of tape stripping and pre-treatment with topical products (barrier creams) was also evaluated in this study. Neither tape stripping nor topical product usage induced a significant change in dermal penetration of Toluene or *o*-cresol content. A steady-state flux of 0.00038 g/cm^2/h was determined in an in vitro percutaneous absorption study performed using split-thickness pig skin (skin exposed to undiluted Toluene).[22] In a study performed in humans evaluating the effect of temperature on dermal absorption, 5 masked (to prevent inhalation exposure) subjects were exposed to Toluene (50 ppm) in inhalation chambers for 4 h.[23] Venous concentrations of Toluene were not statistically different between 25 and 30°C. The maximum mean venous concentration reported in this study was 6.21 ± 0.076 µg/l (at 30° C, measured at 4 h).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Toluene is absorbed by the respiratory tract, gastrointestinal tract, and skin, is rapidly distributed to all tissues, and readily passes through cellular membranes.[2] The amount of Toluene absorbed is proportional to the concentration in inspired air, length of exposure, and pulmonary ventilation. Toluene can be detected in human blood as soon as 10 sec post-exposure. Toluene (100 ppm) absorption via inhalation was determined to be 1.6 mg/min in a study performed in humans. Pulmonary absorption of Toluene in cross-bred dogs within 1 h of exposure to 700, 1500, and 2000 ppm Toluene was determined to be 25, 56, and 74 mg/kg, respectively. Because Toluene is lipophilic, it accumulates in tissues with high fat content. In one study, the half-life of Toluene in human adipose tissue ranged from 0.5 to 2.7 d. Following a 3-h inhalation exposure to 3950 ppm (15 mg/l) Toluene, approximately 626 mg/kg, 420 mg/kg, and 200 mg/kg Toluene reached the liver, brain, and blood of mice, respectively. In a study performed in rats, concentrations of radioactivity following a 10-min exposure to 4600 ppm 4-[^{3}H]Toluene were highest in white adipose tissue, followed in order of decreasing concentration by brown adipose tissue, adrenals, stomach, liver, kidney, brain, blood, and bone marrow. Similar distribution has been reported in rats administered Toluene via oral exposure. Radioactivity present as a volatile compound (likely unchanged Toluene) was observed in the brain and adipose tissue of rats given an intraperitoneal injection of 0.2 mg/ml Toluene. Most of the radioactivity detected in the liver and kidneys was non-volatile. Toluene is predominantly metabolized in the liver. The majority of absorbed Toluene (approximately 84%) metabolizes into benzoic acid, and is excreted as hippuric acid, benzoylglucuronic acid, benzylmercapturic acid, and cresol derivatives. In one study, approximately 16% of absorbed Toluene was expired unchanged through the lungs, whereas 80% was oxidized to benzoic acid and excreted in the urine. Urine of humans exposed to 50 and 800 ppm Toluene for 8 h contained 59% hippuric acid and 41% benzoyl glucuronide. Excretion of these metabolites increased with exposure to the higher concentration of Toluene.

Blood concentrations of Toluene in rats post-oral and inhalation (up to 867 mg/kg or 1000 ppm (6-h exposure)) were compared.[3] The relationship between the two routes of administration were described by the equation: natural logarithm (ln) (oral mg/kg) = 1.27 x ln(inhalation ppm) – 9.22. In a similar study in which rats were exposed to up to 911 mg/kg Toluene (oral) or up to 1145 ppm Toluene (3-h inhalation exposure), the relationship between the two exposures was determined by the equation: ln (oral mg/kg) = -1.44 + 0.95 ln (3 h inhalation ppm).

According to several studies, enzymes responsible for the metabolism of Toluene include CYP2B1, CYP2B2, CYP2B6, CYP2C6, CYP2C8, CYP2C11, CYP1A1, CYP1A2, and CYP2E1. Metabolism of Toluene results in the production of benzyl alcohol, o-cresol, p-cresol, and hippuric acid. A peak blood level of approximately 14 µg/g occurred 1 h after administration of 0.5 g/kg Toluene to rabbits. In a study evaluating the distribution of Toluene in rat brains, the highest concentration of Toluene was found in the brain stem. The effect of concentration and acute and chronic inhalation exposure to Toluene (up to 0.4 ml; up to 30 d) in rat pups was evaluated. Concentrations of Toluene in the brain, blood, and liver of rats increased with increased exposure levels in rat pups; however, no significant differences were observed in Toluene concentrations in tissues in rats acutely exposed versus chronically exposed. The effect of age, sex, and pregnancy on cytochrome p450-mediated metabolism was evaluated in rat livers (in vitro exposure up to 5 mM for 10 min). Production of benzyl alcohol increased in a dose-dependent manner in all liver types. Mature females had lower benzyl alcohol production compared to mature males or immature females. Day 21 pregnant rats had lower benzyl alcohol production than day 10 pregnant rats or mature non-pregnant females. Similarly, benzyl alcohol was observed in higher concentrations in males in a different study evaluating Toluene metabolism in rat livers. In studies performed in humans, increased excretion of hippuric acid and o-cresol was apparent in subjects exposed to Toluene via inhalation when exercising, versus at rest. Mean blood and alveolar air concentration of Toluene was determined to be 5.9 nmol/l and 310 nmol/m^3, respectively, in an assay in which subjects were exposed to 50 ppm radiolabeled Toluene for 2 h.

According to studies performed in humans, factors that have an effect on Toluene metabolism and excretion include drugs/other chemicals (e.g., paracetamol, acetylsalicylic acid), ingestion of ethanol, fasting/diet changes, changes in water intake, genetic polymorphisms, and mask-wearing.[3]

The ADME studies summarized here can also be found in Table 4. Toluene is rapidly absorbed via inhalation, with a total absorption of approximately 50%.[24] When Toluene is orally ingested, the gastrointestinal channel absorbs it almost completely. Toluene that is absorbed into the blood is widely distributed throughout different parts of the body. Reproductive effects may occur following Toluene exposure as it easily passes through the placenta and is secreted into breast milk. It should be noted that Toluene biotransformation may lead to the formation of Toluene epoxides, which may generate reactive oxygen species that can cause oxidative stress and DNA damage.[25]

An assay evaluating pharmacokinetic parameters following Toluene (up to 1 g/kg in corn oil; gavage) administration in rats of different ages (4, 12, and 24 mo) was performed.[26] Blood Toluene concentrations were unaffected by age; however, brain Toluene concentrations were significantly higher in 24-mo-old rats vs. 4-mo-old rats (concentrations 50% higher in 24-mo old rats). Mean blood concentrations of Toluene in male brown Norway rats following a 6-h inhalation exposure period were 0.01, 0.33, and 11.84 µg/g, after exposure to 5, 50, and 500 ppm Toluene, respectively (on day 1 of treatment).[27] Mean blood concentrations of Toluene over the 4 collection times (day 1, 5, 10 and 20) were approximately 0.04, 0.35, and 11.62 µg/g, in animals treated with 5, 50 and 500 ppm, respectively. Pregnant Sprague-Dawley rats exposed to Toluene (8000 or 12,000 ppm; gestation day (GD) 8-20; 15, 30, or 45 min whole-body exposure) displayed increased Toluene levels in saphenous blood in a concentration- and time-dependent manner.[28] Toluene levels also increased in fetal brains in a concentration-dependent manner, and in placenta and amniotic fluid in a time-dependent manner. The highest mean concentrations of Toluene in maternal saphenous blood, fetal brains, and placenta were determined to be 11, 7.3, and 10.5 ppm, respectively. The effect of temperature on absorption and excretion of Toluene (50 ppm; inhalation exposure) in humans was evaluated in 5 subjects.[23] Results suggested that absorption of Toluene is increased and elimination is decreased in the presence of heat. The maximum venous blood amount of Toluene observed in this study was 0.389 mg/l (measured 2 h into exposure; 30°C).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute dermal median lethal dose (LD_{50}) of Toluene in rabbits was determined to be 14.1 ml/kg.[2] *No deaths were observed in an acute percutaneous assay in which guinea pigs were dosed with 1.732 g/kg Toluene. Acute oral LD_{50}s of Toluene in rats ranged from 2.6 g/kg to 7.53 g/kg. No toxic effects were observed in studies performed in rats given nail products containing 33 - 33.2% Toluene via gavage. Acute inhalation LD_{50}s were determined to be 5320 ppm and 6942 ppm in two studies performed in mice (6 - 7-h exposure period). Acute inhalation studies performed in mice, rats, guinea pigs, rabbits, and dogs resulted in adverse effects including mucous membrane irritation, motor incoordination, prostration, changes in respiratory rate, changes in blood serum and enzymes, elevated blood glucose and packed cell volume, decreased body weight, and death. Effects varied according to animal species, length of exposure, and concentration of Toluene administered. Mortality was prevalent in several acute subcutaneous, intraperitoneal, and intravenous studies performed in mice, rats, guinea pigs, and rabbits (animals given 0.17 – 8.7 g/kg Toluene).*

Short-Term Toxicity Studies

Progressive symptoms were observed in several species of animals following short-term inhalation of increasingly higher concentrations of Toluene (1 – 12,000 ppm) including irritation of mucous membranes, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death. In a study in which rats were given Toluene (1 ml/kg/d) via subcutaneous injection for 21 d, adverse effects were observed (e.g., decreased body weight, decrease in erythrocyte and leukocyte counts, focal hepatic necrosis).[2] *Similarly, adverse effects (polypnea, necrosis at injection sites, hemorrhagic, hyperemic, and degenerative changes in lungs, kidneys, adrenal glands, and spleen) were observed in guinea pigs given Toluene (0.25 mg/d) subcutaneously for 30 - 70 d. Rabbits treated subcutaneously with Toluene (1 ml/kg/d) for 6 d developed granulopenia and granulocytosis.*

The effect of Toluene on body weight and pathological changes in the heart, lung, stomach, and spleen tissues of New Zealand rabbits (6/group) was evaluated.[29] Animals were exposed to Toluene (1000 mg/l) in an exposure chamber for 8 h/d for 14 d. A control group was left untreated. Body weight in the Toluene-treated groups dropped initially and recovered by day 14 post-exposure. Relative organ tissue weights were similar among control and treated groups. Adverse effects in organs observed in treated rats include slight fibrotic necrosis, lymphocyte infiltrates, congestion with local degenerative changes, lymphocyte infiltrates near the hilum pulmonis, indiscernible emphysema in alveoli, pyknotic cells in gastric pits, swelled gastric glands, congestion between mucosa and submucosa, enlarged lymphoid tissue, and lymphocyte proliferation. These effects were not seen in untreated control animals.

Subchronic and Chronic Toxicity Studies

No significant test substance-related effects were observed in a 6 mo assay in which rats were given up to 590 mg/kg Toluene per day, via gavage.[2] *No major toxicological effects were observed in chronic inhalation toxicity assays performed*

in rats exposed to Toluene (up to 1481 ppm). Toxic effects (e.g., nasal/ocular irritation, motor incoordination, lung congestion, liver hemorrhage, death) were observed in dogs exposed to Toluene (2000 – 2660 ppm) via inhalation for 6 mo.

Studies performed in mice and rats given Toluene (312 – 5000 mg/kg/d; in corn oil; 13 wk) via gavage resulted in death, increases in organ weights (e.g., liver, kidney, heart), dose-dependent necrosis of the brain, and hemorrhage of the urinary bladder.[3] Death and increased organ weights were also observed in 13-wk studies in which mice and rats were exposed to Toluene (100 – 3000 ppm) via inhalation. Hyperplasia and erosion of respiratory epithelia was observed in mice and rats exposed to Toluene (up to 1200 ppm) for 2 yr.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Teratogenicity and embryotoxicity of Toluene were assessed in hamsters, mice, and rats via various routes of exposure (dermal, oral, inhalation).[2] Minimal embryotoxic effects were observed in a dermal assay in which Toluene (concentration not stated) was applied to the clipped skin of hamsters on days 7 - 11 of gestation. Toluene was teratogenic at 1.0 ml/kg and embryotoxic at 0.3 ml/kg in mice given Toluene via gavage on days 12 – 15 of gestation. No significant reproductive toxicity was observed in an assay in which Toluene in corn oil (10 ml/kg bw) was given to pregnant mice for 8 d (GD not stated). Rudimentary 14th ribs were observed in female mice exposed to Toluene (1000 ppm) via inhalation on GD 1 - 17. Adverse effects in fetuses, such as low weight (observed in pregnant mice treated with 133 ppm Toluene on GD 6 - 13) and skeletal abnormalities (observed in pregnant rats treated with 266 -399 ppm Toluene on days 1 - 8 or 9 – 14 of gestation), were observed. Toluene was not teratogenic in fetuses of rats exposed to up to 400 ppm Toluene vapor on days 6 - 15 of gestation or in fetuses of rats exposed to 266 ppm Toluene on days 7 – 14 of gestation.

A no-observed-adverse-effect-level (NOAEL) for embryotoxicity was determined to be 1.46 µmol/ml in an assay in which embryos were exposed to Toluene.[3] Toluene (8.67 µg/ml) in a culture medium decreased sperm motility, inhibited in vitro fertilization, and increased preimplantation embryo degeneration. Decreased maternal weight gain and generalized growth retardation of fetuses was observed in assays in which dams were given Toluene via gavage (520 - 650 mg/kg bw; GD 6 - 19; in corn oil). Increased occurrence of pups with low birth weights and adverse effects relating to behavioral tasks in offspring were observed in an assay performed in rats and hamsters given 800 mg/m^3 Toluene via inhalation (6 h/d exposure; rats treated on GD 14 - 20 and hamsters treated on GD 6 - 11). Effects on maternal and fetal parameters were evaluated in well-nourished and malnourished rats given 1.2 g/kg Toluene in corn oil via subcutaneous injection (on GD 8-15 or GD 14 - 20). Adverse effects observed in well-nourished rats include decreased maternal body weight/weight gain, reduced pup weights, and reduced brain weights. Malnourished rats displayed extensive adverse effects (e.g., decreased number of ossification centra, increased fetus deaths, death during labor). No effect was observed on sperm motility of male offspring of pregnant rats exposed to 1200 ppm Toluene via inhalation on GD 7 throughout gestation, and daily after parturition to postnatal day 8. Absolute and relative testes weights were significantly reduced in the pups of Toluene-exposed maternal rats (1800 ppm; GD 7 - 20). A significant increase in the number of apoptotic cells in the cerebellar granule layer of the hippocampus was also observed in this study. Decreases in the volume of the granule cell layer, the hilus, and the commissural-association zone of the hippocampus were observed in rat pups exposed to Toluene via inhalation (100 - 500 ppm; exposed on postnatal days 1 - 28). The neurosomatic development and behavioral effects of Toluene exposure via maternal milk in rat pups was evaluated (lactating rats given injection of 1.2 g/kg Toluene on lactation day 2 to 21 (type of injection not stated)).

According to the Scientific Committee for Toxicity and Ecotoxicity and the Scientific Committee on Consumer Products, Toluene is considered a reproductive category 3 toxicant (possible risk of harm to unborn child).[14] Similarly, Toluene is classified as hazardous with hazard category 'reproductive toxicity category 1A (may damage fertility or the unborn child)' in the Hazardous Chemical Information System (HCIS) of Safe Work Australia.[30] Toluene is also regulated by the State of California as a developmental toxicant under Proposition 65.[31] A maximum allowable dose of 7000 µg/d was established based on protection against adverse developmental effects.

The developmental and reproductive toxicity studies summarized here can be found in Table 5. Gravid female rats were dosed with 1250 mg/kg Toluene in peanut oil by gavage on days 16 – 19 of gestation, and killed on GD 20.[32] Maternal and reproductive parameters were not affected, and there was no significant difference in the number of fetal external or skeletal malformations between the treatment and the control group. However, a pattern of accelerated development in the upper mid-turn in the treated fetal cochleas was observed.

Gravid female rats (GD 14) and male offspring (post-natal day (PND) 2 or 8) were treated with 5 or 50 ppm Toluene for 5 d.[33] Up-regulation of N-methyl-d-aspartate (NMDA) receptor subunits, cyclic adenosine monophosphate (AMP) responsive element binding protein (CREB1), calcium/calmodulin-dependent protein kinase (CaMKIV), and apoptotic-related genes were observed in treated offspring, but not in maternal rats. A no-observed-adverse-effect-concentration (NOAEC) of 600 ppm was determined in male rats and an F1 generation in a study evaluating fertility.[8] Anti-nociception, and effects on memory and locomotion were observed in rats subjected to a pre-natal and post-natal exposure to Toluene (6000 ppm).[34] In an inhalation study in which mice were exposed to 8000 ppm Toluene for 30 min twice daily via inhalation on GD 7 - 19, neonatal death was significantly increased in the test group compared to controls.[35] In a study in which rats were exposed to Toluene (500 or 1500 ppm) for 6 h/d on days 6 - 20 of gestation and killed on day 21 of gestation, maternal

weight gain of the test animals and fetal body weights in the 1500 ppm group were decreased; no other reproductive or developmental effects due to dosing were observed.[36] In another study in which dams were exposed to up to 3000 ppm Toluene via whole-body inhalation for 6 h/d on days 6 – 15 of gestation and killed on day 20 of gestation; the maternal toxicity NOAEL was 750 ppm with a defined maternal and developmental toxicity lowest-observed-adverse-effect-level (LOAEL) of 1500 ppm.[37] There were no effects on reproductive parameters; fetal body weighs were increased in a toxicologically significant manner at 1500 and 3000 ppm.

Several inhalation studies were conducted in rats using short-duration (15 or 30 min; twice daily; various exposure times, including post-natal exposures) high-dose (up to 16,000 ppm) exposures, and effects on post-natal development were evalauted.[38-43] Generally, there were no significant maternal effects, although decreased body weight gains were observed with doses ≥ 8000 ppm Toluene. Fetal malformations were observed at some of the highest doses (> 8000 ppm), and there were indications of impaired cognitive function.

Female rats were exposed to Toluene (2000 - 8000 ppm) via inhalation, 30 min/d for 28 d, and the effect on of progesterone, estradiol, testosterone, and insulin-like growth factor 1 (IGF-1) levels and ovarian tissue was examined.[44] Progesterone levels (at 4000 and 8000 ppm) and testosterone levels (all dose groups) were statistically significantly increased and IGF-1 was significantly decreased (at 8000 ppm); no effect on estradiol was noted. A dose-dependent increase in apoptosis in ovarian tissue was observed. In another study, gravid rats were exposed to 0.09 – 9 ppm Toluene via nasal inhalation for 90 min/d on days 14.5 – 18.5 of gestation; hormone levels (all groups) and mRNA levels of steroidogenic enzymes in testicular tissues (control and low-dose group) were measured in male pups.[45] Fetal plasma testosterone concentrations were significantly reduced in males of the 0.9 and 9 ppm, but not the 0.09 ppm, groups. mRNA levels of 3β-hydroxysteroid dehydrogenase (3β-HSD) were significantly reduced after exposure to 0.9 ppm. Effects on immunological biomarkers were also examined in pups following whole-body inhalation exposure of dams to 5 or 50 ppm Toluene for 6 h/d on days 14 – 18 or 19 of gestation, followed by post-natal exposure to groups of pups at various post-natal time frames.[46,47] In one study, total plasma immunoglobulin G2a (IgG2a) levels were statistically significantly increased in the 50 ppm group, and in another study, total IgG1 levels were markedly reduced. Splenic expression of some transcription factors was suppressed.

GENOTOXICITY STUDIES

Toluene was negative for mutagenicity in a battery of mammalian cell and whole organism test systems.[2] Microbial assays were also negative for mutagenicity. Single DNA strand breakage in rat hepatocytes evaluated in vitro (exposed to 2.3 mM Toluene), and increased chromosomal aberrations/damaged chromosomes in rats exposed to Toluene via subcutaneous injection (animals exposed to up to 0.8 - 1 g/kg/d for 12 d) and inhalation (animals exposed to 80 - 300 ppm for 15 wk – 4 mo) were apparent.

Toluene did not induce dominant lethal mutations in an assay performed using male rats given Toluene in corn oil (346 and 692 mg/kg bw/d) via intraperitoneal injection.[3] Treatments were performed on 5 consecutive days.

Details of the genotoxicity studies summarized here can be found in Table 6. Positive results were observed in in vitro genotoxicity assays including an Ames assay (using *Salmonella typhimurium* strains at up to 50 µl/plate),[48] a comet assay (using human skin disks at up to 1,000,000 ppm),[49] a modified alkaline comet assay (using human lung epithelial carcinoma cells at 0.25 ppmv),[50] a mammalian cell mutagenicity assay using mouse lymphoma cells (up to 500 µg/ml). [51] Conversely, no genotoxicity was observed in other in vitro assays including an Ames assay (using *S. typhimurium* strains at up to 1000 µg/ plate), a chromosomal aberration assay (using Chinese hamster ovary cells at up to 1600 µg/ml), and a sister chromatid exchange (SCE) assay (using Chinese hamster ovary cells at up to 5000 µg/ml). [51] The majority of these in vitro assays were performed with and without metabolic activation. Similarly, mixed results were observed in in vivo genotoxicity assays. Toluene was genotoxic in an alkaline and neutral comet assay using *Drosophila* larvae given up to 100.0 mM Toluene via diet,[52] a comet assay using mice given a 5 or 15 g/kg intraperitoneal injection of Toluene,[25] and a comet assay using mice exposed to Toluene (25 ppm) via inhalation for 4 wk. Negative results were observed in a micronucleus assay using mice exposed to up to 2000 mg/kg Toluene (method of administration not stated)[51] and in a bone marrow nucleus assay using mice exposed to 100 ppm Toluene via inhalation for 15 d.[53]

CARCINOGENICITY STUDIES

No neoplasms were observed in mice treated with Toluene subcutaneously for 3 mo.[2] Similarly, no tumors were observed in several studies in which Toluene was applied topically to mice (applications length ranged from 50 wk to lifetime). Toluene was not considered to be tumor-promoting in studies in which Toluene was applied to the ears following initiation with 7,12-dimethylbenz[a]anthracene. Two of 30 animals developed skin tumors (one squamous cell carcinoma and one squamous cell papilloma) when mice were topically treated with Toluene (1 - 20 µl) for 72 wk. According to one study, tumor promotion in the skin of mice by Toluene is associated with the ability of Toluene to induce epidermal hyperplasia (no other details provided).

National Toxicology Program (NTP) researchers concluded that there was no evidence of carcinogenicity after evaluation of several studies performed in mice and rats.[3] These studies were performed via inhalation (animals treated with

up to 3000 ppm Toluene for 13 wk or 1200 ppm for 2 yr) and gavage (animals treated with up to 5000 mg/kg/d Toluene in corn oil for 13 wk).

Oral

A carcinogenicity assay was performed in Sprague-Dawley rats (40 - 50/sex/group) given Toluene in olive oil via gavage for 4 d/wk for 104 wk (2-experiment study).[54] In experiment one, animals were treated with 500 mg/kg bw, and in experiment 2, animals were treated with 800 mg/kg bw (treatment duration and methods same for both experiments). Animals were kept under observation until natural death or 130 wk. Control animals were given the vehicle only. After natural death or killing, animals underwent systemic necropsy, and histopathology was performed. In experiment 1, statistically significantly increased numbers of total malignant tumors (in both males and females), subcutaneous malignant tumors (in males), malignant mammary tumors (in females), and hemolymphoreticular neoplasias (in females) were observed in treated animals versus controls. In experiment 2, a statistically significant increased incidence of total malignant tumors and carcinomas of the oral cavity, lips, and tongue were observed in both male and female rats (compared to controls).

Inhalation

The potential carcinogenicity of inhaled Toluene was evaluated in F344/N rats (60/sex/group) and B6C3F1 mice (60/sex/group).[51] Rats were exposed to Toluene levels of 600 or 1200 ppm, and mice were exposed to Toluene levels of 120, 600, and 1200 ppm (exposure for 6.5 h/d, 5 d/wk; chamber inhalation; unexposed chamber controls). Ten animals per group (except male mice) were removed for evaluation after an exposure period of 15 mo. All other animals were exposed to Toluene for 103 wk. Animals were killed and necropsied following exposure periods. No Toluene-related neoplasms were found in mice or rats during 15-mo or 103-wk studies.

OTHER RELEVANT STUDIES

Cytotoxicity

Cytotoxicity to Toluene in several cell types was observed when evaluated in vitro.[2] These cells include rat and rabbit pulmonary alveolar macrophages, glioma (C6) cells, astrocyctes, and mouse fibroblast L929 cells. In addition to cytotoxicity, in vitro assays revealed certain toxic effects including impaired platelet agglutination, inhibition of NMDA currents in oocytes, depression of muscarinic signaling, and inhibition of acetylcholine and γ-aminobutyric acid in human IMR-32 neuroblastoma cells.

The effect of gaseous Toluene on human lung epithelial carcinoma cell line A549 was evaluated in vitro.[50] Plated cells were exposed to gaseous Toluene (0.25 ppmv; balanced with nitrogen) for 24 h. Control cells were either exposed to synthetic air (80% nitrogen, 20% oxygen) only or left in a carbon dioxide incubator. Cell viability was evaluated by quantifying the amount of lactate dehydrogenase (LDH) released from cells upon damage of the cytoplasmic membrane. Intracellular reduced and oxidized glutathione were also measured in exposed cells using a modified enzymatic recycling method. Differences in amount of released LDH were not statistically different between control groups and the treated group. In addition, effects on glutathione redox status were similar in control and treated groups.

Effects on Respiratory Tract

Two male subjects exposed to Toluene for 7 - 8 h via inhalation developed transitory mild throat and eye irritation at 200 ppm and lacrimation at 400 ppm.[2] No other details were provided.

The effect of long-term, low-level inhalation exposure to Toluene (50 ppm; 6 h/d, 5 d/wk, 6 or 12 wk; whole-body exposure chamber) on airway inflammatory responses was evaluated in female C3H mice (10/group).[55] A control group was exposed to air only. One day after the final Toluene exposure, bronchoalveolar lavage (BAL), spleen, and blood samples were collected. Lungs were also collected for histological analyses. BAL fluid was analyzed for cytokines, chemokines, neutrophins, and substance P. The total number of inflammatory cells and macrophages significantly increased in both 6- and 12-wk exposed mice compared to controls ($p < 0.05$). The production of interferon-gamma and substance P was significantly decrease in both 6- and 12-wk exposed mice compared to controls ($p < 0.05$). Nerve growth factor was not affected by Toluene treatment. Neutrophin-3 production in BAL fluid was significantly increased in 12-wk exposed mice only, compared to controls ($p < 0.05$).

The potential for Toluene to elicit microvascular leakage in rat airways was evaluated in male SPF Wistar rats (5/group).[56] Airway microvascular leakage and bronchoconstriction was evaluated in rats treated with 18, 30, 50, 135, or 450 ppm Toluene (control animals exposed to formaldehyde (positive) or clean air (negative), via inhalation, for 10 min. Airway microvascular leakage was also evaluated in rats during 3 consecutive 10-min periods of Toluene inhalation (50 or 135 ppm). Microvascular leakage was evaluated using blue dye injected into the right external jugular vein prior to provocation. The content of the blue dye that extravasated into the tissues was measured as an index of plasma leakage. Toluene exposure induced dye leakage into the trachea and main bronchi in a concentration-dependent manner. Toluene at concentrations of ≥50 and ≥ 30 ppm caused significant responses in the trachea and main bronchi, respectively, which peaked after exposure to 135 ppm for 10 min. Responses were statistically significant compared to the control groups. Further testing revealed that Toluene-induced plasma leakage is predominantly mediated by tachykinins endogenously released from airway sensory nerves.

Effect on Visual Function

Male rats were exposed to Toluene (1000 ppm; 21 h/d; 6 - 11 wk) and functions of the vestibule- and opto-oculomotor systems were tested 1 mo after exposure (via recording of nystagmus induced by vestibular or optokinetic stimuli).[3] Optokinetic gain in exposed animals were reduced compared to untreated controls. A slight reduction in gain during sinusoidal oscillatory vestibular stimulation was also observed.

Effect on Lipid Levels

Increased levels of free fatty acids, triglycerides, cholesterol, phospholipids, and blood glucose were observed in rabbits following a single dose of Toluene (0.5 g/kg) in olive oil via gavage.[3] Significant decreases in basal phospholipid methylation were observed in rats given Toluene (1 g/kg) via intraperitoneal injection ([^{3}H]methionine as methyl donor). These effects were not seen when [3H]adenosylmethionine or S-adenosylmethionine synthetase were used as methyl donors. Toluene produced no change in either phospholipid or cholesterol content of rat pulmonary microsomal membranes when evaluated using thin-layer chromatographic separation.

Toluene-Induced Endocrine Disruption

Male Wistar rats (n = 9) were treated with Toluene (1500 ppm; 4 h/d; 7 d) via inhalation.[57] Control rats were treated with air in separate chambers. Body weights and adrenal gland weights were evaluated following the last exposure. Microscopic evaluations of the adrenocortical cells, immunohistochemical analysis, analysis of mRNA levels, plasma adrenocorticotropic hormone (ACTH), and serum cortisone levels were evaluated. Body weights had increased significantly less than in controls after Toluene exposure ($p < 0.05$). In addition, a significantly increased adrenal gland weight (left and right combined) and adrenocortical cell size was observed in Toluene-treated rats compared to controls ($p < 0.05$). Hypertrophy of the cortex was observed in the Toluene-exposed group. Immunohistochemical staining revealed aldosterone-positive cells localized within the zona glomerulosa; however, a clear difference between the control and treated groups was not seen. Expansion of the corticosterone-positive area consistent with cortical hypertrophy was observed in the treated group. No obvious difference between control and treated groups were observed in anti-proliferating cell nucleus antigen-immunostaining. Enhancement of the corticotropin-releasing factor expression was seen in the paraventricular nucleus (PVN) of the hypothalamus in treated animals. ACTH concentrations were significantly increased in treated animals compared to controls ($p <0.05$), and corticosterone levels were insignificantly elevated. Cytochrome side-chain cleavage mRNA levels in the inhalation group were significantly higher (1.3-fold) compared to the control group.

Bone Mass Toxicity

The bone mineral density and content of the femoral neck of male Swiss albino mice (10/group; 1 control group untreated) exposed to Toluene (300 ppm; full-body inhalation chamber; 6 h/d) for 8 wk was evaluated via X-ray absorptiometry.[58] Bone mineral density and bone mineral content were determined to be 0.008 ± 0.005 g/cm^2 and 0.11 ± 0.006 g in the treated group, respectively and 0.190 ± 0.007 and 0.020 g/cm^2 $\pm$ 0.009 g in control animals, respectively. Bone mineral density and bone mineral content were significantly lower in treated versus control groups ($p < 0.05$).

Ototoxicity

Ototoxicity was observed in several studies using rats exposed to Toluene via inhalation (1000 - 8000 ppm; 8 d – 13 wk exposure).[3] Ototoxicity in these studies were measured via brainstem audiometry, auditory sensitivity, neurologic testing, flash evoked potential test, cortical flicker fusion test, auditory brainstem response to clicks test, auditory brainstem response to tone-pips at 10 kHz and 30 kHz test, somatosensory-evoked potentials test, caudal nerve action potentials to single and paired stimuli tests, morphological investigations of the cochlea, electrocochleographic testing, and electrophysiological testing. Permanent hearing loss was observed in a study in which guinea pigs were given 1000 ppm Toluene via inhalation (5-d exposure). Toluene did not produce ototoxic effects when evaluated in chinchillas given 2000 ppm Toluene via inhalation (10-d exposure) and a 95 A-weighted decibels 500 Hz band noise (auditory brainstem response evaluated).

The effect of Toluene exposure on the hearing of male albino guinea pigs (5 - 7/group) was evaluated.[59] Guinea pigs were evaluated following Toluene exposure alone, or along with glutathione-depletion (induced via a low protein diet), and/or cytochrome p450 inhibition (induced via 2-diethylaminoethyl-2,2-diphenylvalerate-HCl (SKF525A) injection). Guinea pigs were exposed to Toluene vapor (1750 ppm, 6 h/d, 5 d/wk; whole-body exposure) for 4 non-consecutive weeks (animals were treated every other week to allow for recovery). Control animals were left unexposed. To inhibit cytochrome P450, some groups of animals were given a 50 mg/kg subcutaneous injection of SKF525A on the Mondays of treatment weeks. Auditory function was evaluated via electrocochleography and histological analysis. A statistically significant Toluene-induced hearing loss was provoked in cytochrome p450-inhibited guinea pigs on a normal diet, and in cytochrome p-450 inhibited guinea pigs on a low protein diet. Disrupted stria vascularis and spiral fibers in the apical coil of the cochlea were observed in animals with hearing loss. Hearing loss was similar among unexposed controls and guinea pigs treated with Toluene alone or in those treated with Toluene plus a low protein diet.

Toluene Abuse

Studies have been found in the literature indicating the toxic effects of Toluene following inhalation abuse (e.g., glue/spray paint sniffing).[3] Some of these effects include impaired mentation, memory, motor strength, gait, and neuropsychological function, auditory/visual disturbances, paresis, atrophy of various areas of the brain (e.g., cerebellum,

brain stem), cardiovascular collapse, ventricular dilation, metabolic acidosis, renal injury, white matter changes, and death. Adverse effects in infants exposed to Toluene in utero due to maternal abuse have also been reported. These effects include growth impairment, developmental delays, hyperchloremic acidosis, mild language/speech impairment, dysmorphic physical features, microcephaly, and death.

Several reports of adverse effects following Toluene abuse (via inhalation) have been found in the literature.[60-69] These adverse effects include neurological symptoms (e.g., slurred speech, slowed response, confusion, uncontrolled laughing, disorientation, memory loss), tiredness, headache, blurred vision, hallucinations, tremors, dyspnea, convulsions, vomiting, renal, cardiac, and hepatic abnormalities/injury, adrenal dysfunction, metabolic alterations, hypokalemia, leukoencephalopathy, growth impairments, and death. Autopsies of fatal cases of Toluene ingestion revealed traumatic brain injury, hemorrhages, internal organ congestion, and hemorrhagic pulmonary edema.

Neurotoxicity

Many studies were found in the literature involving the effect of Toluene (administered via inhalation (50 - 3000 ppm; 2 h – 80 wk exposure)) on brain proteins and chemicals in rats.[3] These effects include a reduction of affinity and increase in density of the β-adrenergic antagonist [^{3}H]dihydroalprenolol binding sites in the frontoparietal cortex, increase of $^{45}Ca^{2+}$ uptake into unstimulated synaptosomes, inhibitory effects on acetylcholinesterase, adenosine triphosphatase, and magnesium activated adenosine triphosphatase, increase in activities of neurotransmitter-synthesizing enzymes, reduction in [^{3}H]neurotensin binding, increased binding of [^{3}H]etorphine and [^{125}I]vasoactive intestinal polypeptide, increase and decrease on activity of Ca^{2+}/Mg^{2+} adenosine triphosphatase (dependent on exposure time), increase in neuron-specific γ-enolase and glial marker proteins, increase in the number and intensity of tyrosine hydroxylase-immunoreactive fibers and terminals in the forebrain, depletion of striatal 3,4-dihydroxyphenylacetic acid, decreased glial fibrillary acidic protein in the thalamus, decreased acetylcholine release in the striatum, increased gamma-aminobutyric acid in the cerebellum, increased dopamine levels in the striatum, increase in tyrosine and tryptophan hydroxylation in certain catecholaminergic cell groups, and a decrease in the biosynthesis rate of 5-hydroxytryptophan in the ventro-median-hypothalamus. In a study performed in mice given Toluene (up to 405 mg/l in drinking water; 28 d exposure), an increase in endogenous levels of biogenic amine transmitters (norepinephrine, dopamine, and serotonin) was observed. Formation of reactive oxygen species within cortical synapses were observed in rats given 1 g/kg Toluene via intraperitoneal injection (researchers concluded that a metabolite of Toluene (possibly benzaldehyde) was responsible for this effect). Elevated levels of reactive oxygen species were also observed in mitochondrial fractions of the lung, liver, and kidney tissue, and in crude synaptosomal fractions from the cerebellum, hippocampus, and striatum of rats given 1.5 g/kg Toluene in corn oil via intraperitoneal injection.

Effects on the sleep-wake cycle were observed in several studies in which rats were treated with Toluene (80 - 2730 ppm; 2 h – 3 wk exposure) via inhalation.[3] Decreased spatial memory, increased locomotor activity and rearing behaviors, and reduced balance were observed in rats given 80 ppm Toluene for 4 wk. The test substance did not have an effect on dopamine agonist binding to dopamine receptors in this study. Increased levels of extracellular dopamine in the prefrontal cortex were observed in rats treated with 3000 ppm Toluene for 40 min. These effects were not observed in the nucleus accumbens. No neurobehavioral or gross pathological changes were observed 6 mo after rats were treated with up to1500 ppm (6 mo of treatment) Toluene. In addition, no neuronal loss was noted in this study. However, a statistically-significant neuron loss (in the hippocampus) of 16% was observed in rats treated with Toluene (1500 ppm; 6 mo of treatment), compared to untreated controls. In an in vitro assay using guinea pig hippocampal slices, Toluene (0.2 ng/ml – 20 µg/ml) had both excitatory and inhibitory biphasic effects on neurotransmission. Toluene treatment (1.3 ml/kg/d; intraperitoneal injection; 4 d) reduced immunostaining of neuropeptide Y (an appetite stimulant) in the paraventricular nucleus and increased neuropeptide Y staining in the arcuate nucleus. Increased immunostaining of galanin (appetite stimulant) was also observed in both the paraventricular and arcuate nuclei.

Several neurotoxic and behavioral effects were observed in studies performed in mice and rats treated with Toluene. These effects include decreased shock avoidance, central nervous system dysfunction, effect on gait, impaired operant/ regulating behavior, decreased rearing activity, increased narcosis, and decreased spatial memory/learning abilities (the majority of these studies were performed via inhalation at concentrations ranging from 105 - 8000 ppm). Intraperitoneal studies performed in rats suggest that Toluene may affect developing brains via the alteration in the function of NMDA and γ-aminobutyric acid$_A$ receptors (at 1 g/kg, and may increase seizure susceptibility (at 500 mg/kg).

Details of the neurotoxicity/behavioral toxicity studies summarized below can be found in Table 7.

Numerous neurotoxicity and behavioral studies performed in animals were found in the literature, and several adverse effects caused by Toluene were noted. These effects include hypothalamus-pituitary adrenal (HPA) and hypothalamus-pituitary-thyroid (HPT) axes dysfunction, up- and down-regulation of the expression of NMDA receptor subunits, poor memory retention, poor spatial and learning performance, impaired reversal learning, white matter abnormalities, changes in locomotor activity, motor incoordination, impaired swimming ability, anti-nociception, changes in anxiety levels, decreased brain chemicals (e.g., dopamine, nerve growth factor), histopathological abnormalities of the brain, and decreased hippocampal neurogenesis.[17,33-35,40,60,70-79] The majority of these studies were performed in animals exposed to Toluene via inhalation. Altered neuroplasticity was observed in an assay performed in 17 human subjects exposed to Toluene (peak of 200 ppm) via inhalation.[80] Exposure did not have an effect on cortico-spinal ability, intracortical inhibition, or learning.

Effect of Toluene on Oxidative Stress Markers in the Brain

The effect of Toluene on oxidative stress markers in the brain was evaluated in an acute (treatment with 0 or 1019 ± 14 ppm Toluene for 6 h) and subchronic (treatment with 0, 10 ± 1.4, 97 ± 7, or 995 ± 43 ppm Toluene; 6 h/d; 5 d/wk; 13 wk) inhalation studies using male Long-Evans rats (6/group).[81] Brains were dissected for evaluation within 30 min following exposure in the acute study, and 18 h after the last exposure in the subchronic study. Brain regions (frontal cortex, hippocampus, cerebellum, and striatum) were evaluated for oxidative stress parameters (total aconitase, protein carbonyls, glutathione synthetase, γ-glutamylcysteine synthetase, superoxide dismutase, total antioxidants, nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase-1, and nicotinamide adenine dinucleotide (NADH) ubiquinone reductase activities). A significant increase of NADH ubiquinone reductase, γ-glutamylcysteine synthetase, and glutathione reductase in the cerebellum and total antioxidants in the frontal cortex ($p < 0.05$) was observed in Toluene-treated animals in the acute assay. In the subchronic assay, dose-dependent increases in NADPH quinone oxidoreductase-1 activity were observed in the cerebellum. A similar increase was observed in NADH ubuquinone reductase activity in the frontal cortex, hippocampus, and cerebellum. Adverse effects of Toluene exposure on superoxide dismutase and total antioxidants were most prevalent in the striatum compared to other brain regions. Total aconitase activity was inhibited in the striatum and cerebellum, increased in the hippocampus, and unchanged in the frontal cortex. Protein carbonyls increased significantly in both the frontal cortex and cerebellum.

Immunoreactivity in the Brain

c-Fos immunoreactivity in the brain following whole-body exposure to 5000 ppm Toluene vapor for 0, 5, 10, or 30 min was examined using groups of 7 - 8 male Sprague-Dawley rats.[82] Quantitative analyses revealed increases in c-Fos immunoreactivity in about one-third of the brain structures examined, with most of these structures significantly activated only after prolonged Toluene exposure. The majority of brain structures activated by Toluene were found in the forebrain and midbrain, with particularly pronounced activation in nuclei implicated in the processing of rewarding, emotional, and olfactory stimuli, and those controlling motor output.

Immunotoxicity

Immunotoxicity (impaired function of splenocytes and adversely affected interleukin-2 (IL-2) synthesis) was observed in male mice given 405 mg/l Toluene in drinking water for 4 wk.[3] These effects, however, were not observed at lower dose levels (up to 80 mg/l). Spleens of 10 mice were observed in an assay in which animals were treated with 600 mg/kg Toluene in vegetable oil (method of administration not stated). The test substance was observed to stimulate splenic mast cell populations and inhibit other amino-containing structures 6 h post-dose.

To investigate the effect of Toluene inhalation on immune responses to ovalbumin (OVA), groups of 6 C3H/HeN mice were exposed to 0, 5, 50, or 500 ppm of Toluene for 6 h/d, 5 d/wk for 3 or 6 wk.[83] The allergic mouse groups were immunized with OVA intraperitoneally on days 0 and 7 and as an aerosol on days 21 and 23. One day after the final exposure, mice were killed and BAL fluid and lung, spleen and blood samples were obtained. Real time polymerase chain reaction (PCR) was used for analysis of expression levels of mRNA in the lung, fluorescence activated cell sorter (FACS) analysis was used to examine splenic cell immunophenotypes, and enzyme-linked immunosorbent assay (ELISA) was used to analyze lung samples and determine plasma Ig levels.

Allergic mice exposed to Toluene for 3 wk did not exhibit any changes in their plasma, lung, or spleen samples. The lungs of mice exposed to 50 ppm Toluene for 6 wk (with and without OVA) were examined microscopically. As compared to controls, slight hyperplasia was observed in the bronchial epithelial cells of the 50 ppm non-allergic group; there was no accumulation of macrophages and neutrophils observed. In the allergic 50 ppm group, an increase in the hypertrophy and hyperplasia of the epithelial cells and mucus secretion in the lung was observed; no marked accumulation of inflammatory cells in the alveoli was noted. Histological changes and increased amounts of fibronectin were observed in the lungs of 50 ppm allergic mouse group. Exposure to Toluene for 6 wk did not increase the number of inflammatory cells in BAL fluid of non-allergic mice; however, the number of BAL cells was increased in the 50 (but not 500) ppm allergic mouse group. Exposure to 500 ppm significantly increased the expressions of transcription factors STAT3, STAT4 and STAT5a mRNAs in the spleens of non-allergic mice. In allergic mouse group, the expressions of splenic STAT3, STAT4, STAT5a, STAT6, GATA3 and Foxp3 mRNAs were significantly enhanced following exposure to 50 ppm Toluene for 6 wk, but the expression of T-bet mRNA was not increased. Regarding the Th1/Th2 balance, the expressions of IL-4 and IL-12 mRNAs were enhanced in the spleens of the 50 pm allergic mouse group. Splenic immunophenotypes were not affected in any of the mice exposed for 6 wk. Total IgG1 antibody production in the plasma was significantly increased in the 50 ppm allergic mouse group, but not the other groups; IgE and IgG2a levels were not affected.

The pathological effects of inhaled Toluene (0 (control), 1000, 2000, or 4000 ppm) exposure to the lung and brains of male Swiss-Webster (n = 68 (number of animals per group not stated); PND 28) were evaluated.[84] Animals received a single 30-min exposure in a static vapor exposure chamber. Lung and brain tissue were extracted 24-h post-exposure, and histology and immunochemistry were evaluated. Morphological abnormalities of the lung tissue (e.g., irregular cellular architecture) were observed in the 2000 and 4000 ppm groups. Markers of immune system activity and cellular proliferation in the lung revealed no significant differences between control and treated groups. Animals treated with 2000 ppm Toluene showed

significantly increased astrogliosis in the striatum compared to controls ($p < 0.05$). This effect was also seen in animals treated with 4000 ppm; however, this was statistically insignificant ($p = 0.08$).

Effect of Toluene Inhalation on Olfactory Parameters

Female OF-1 mice were exposed to 1000 ppm Toluene via inhalation (full-body exposure in chamber) for 5 h/d, 5 d/wk, for 4 wk, and evaluated for olfactory changes each week during treatment and for each week for 1 mo. after treatment.[85] Structural modifications (density of cells and thickness of olfactory epithelium) were observed soon after the start of exposure. The number of cells did not change at the beginning of exposure (week 1 and 2), decreased markedly later (week 3 and 4), increased significantly the first week of the recovery period and stayed stable during the following weeks. A decrease in the thickness of neuroepithelium was observed at week 1, followed by an increase at week 2 and 3.

Hepatotoxicity

Hepatotoxic effects were evaluated in male Wistar albino rats (8/group) given a single dose of 6 ml/kg Toluene (use of vehicle not stated) via gavage (control group given physiological serum).[86] Intracardiac blood samples were taken 3 h after administration and evaluated for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values. Liver tissues were excised and immunohistochemical and histopathological assessments were performed. In addition, a terminal transferase dUTP nick end labeling (TUNEL) assay was performed to determine if apoptosis was apparent in liver tissues. AST ($p = 0.026$) and ALT ($p = 0.005$) levels were statistically significantly increased compared to control groups. Slight degeneration of hepatocyte and mononuclear cell infiltration in liver tissue sections, and a high immunoreactivity for Bax and caspase-3 protein was observed in treated animals. Apoptotic cell numbers were statistically increased in the treated group compared to the control group ($p = 0.000$)

The hepatotoxic effects of noise and Toluene were evaluated in male New Zealand White rabbits (6/group).[87] Animals were either exposed to Toluene (1000 ± 50 ppm (inhalation)) or noise (100 ± 5 dB) alone, or in combination. An untreated control group was exposed to air and background noise (< 50 dBA). Treatment occurred for 8 h/d for 14 d. Blood samples were taken at 5 different times (immediately before exposure, immediately after exposure, and 3, 7 and 14-d post-exposure). Serum levels of AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), superoxide dismutase, antioxidant capacity, malondialdehyde, and glutathione peroxidase were determined. Histological analyses were also performed. Exposure to Toluene alone resulted in decreased levels of ALP, ALT, GGT, and superoxide dismutase, and increased levels of AST, catalase, and malondialdehyde . Exposure to both Toluene and noise resulted in increases in AST, ALT, GGT, malonaldehyde, total antioxidant capacity, and superoxide dismutase. Exposure to both Toluene and noise also resulted in a decrease in catalase and ALP levels. Histopathology revealed minor cell swelling, minor hepatic lipidosis, and eosinic cytoplasms in animals treated with Toluene only. Significant swelling and damage of the liver tissue were observed following exposure to Toluene and noise simultaneously.

Toluene-Induced Cardiotoxicity

Acute cardiotoxicity was evaluated in male Wistar albino rats (10/group) given 6 ml/kg Toluene via gavage (control animals given serum; composition of serum not stated).[88] Blood pressure, heart rate, blood samples, heart tissues, and serum troponin T levels were evaluated. Heart tissue sections were also evaluated for caspase-3-immunoreactivtiy and apoptosis in a TUNEL assay. Blood pressure and heart rate was significantly lower ($p < 0.05$), and troponin T levels were significantly increased ($p = 0.01$) in the treated versus control group. Heart tissue sections of Toluene-treated rats showed congestion and edema. Higher TUNEL positivity ($p < 0.01$) and immunoreactivity for caspase-3 protein were observed in the treated group compared to control group. In a different study, heart rate and blood pressure were evaluated in male Long-Evans rats given Toluene (0.4, 0.8, and 1.2 g/kg).[89] Toluene doses of 0.8 and 1.2 g/kg resulted in tachycardia and raised blood pressure.

Combined Effect of Toluene Exposure and Allergic Stimulation on Genotoxicity

The effect of allergic stimulation on genotoxicity in the brains of Toluene-exposed male Balb/c mice (n = 6/group) was evaluated.[25] Mice were exposed to Toluene (25 ppm) or air control for 6 h/d for 4 wk in a nose-only exposure chamber. To detect whether allergic conditions affect Toluene exposure, mice were immunized with a control (saline) or OVA on days 0, 14, 21, and 28, approximately 1 h before Toluene exposure. Aluminum hydroxide (2 mg) was also intraperitoneally administered on days 0 and 14. Mice were challenged with nebulized OVA as a booster on days 21 and 28 during the exposure period, and killed following the last exposure. A Comet assay was performed to evaluate DNA damage in different regions after the brain and in leukocytes immediately after sacrifice. A significant increase of IgG1 in immunized mice subjected to OVA sensitization and challenge were observed. Significant DNA damage was observed in the hippocampus and leukocytes of OVA-immunized mice following Toluene exposure, compared to controls ($p < 0.05$). Results of the Comet assay performed in this study on mice chronically treated with Toluene and unexposed to OVA can be found in the Genotoxicity section of this report.

In Vitro Skin Viability Following Exposure to Toluene Vapor

Human skin disks (n = 3/group) were obtained and positioned in a glass vial. Toluene (100 – 1,000,000 ppm in corn oil) were pipetted at the bottom of the vial, avoiding direct contact with the skin, and vials were sealed off and incubated for 8 h.[49] Control skin samples were incubated with corn oil only. Skin viability was assessed using a 3-(4,5-dimethylthiazol-2-

yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In vitro skin exposures to Toluene resulted in statistically-significantly reduced cell viability, at all tested concentrations, in a dose-dependent manner, compared to controls.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Undiluted Toluene produced slight to moderate skin irritation in rabbits when evaluated in 4 different assays (single patch applications; majority under occlusive conditions).[2] Slight to moderate skin irritation and slight skin necrosis was observed in a study in which rabbits were exposed to 10 - 20 applications of undiluted Toluene over a 2 - 4 wk period (level of occlusion not stated). Minimal to moderate erythema was observed in rabbits after a single-patch (occlusive treatment) of a nail basecoat containing 33.2%. No irritation was observed in a single insult occlusive patch test performed in humans using the same basecoat. No irritation was observed in a 3-patch study (semi-occlusive conditions) in which rabbits were exposed to a nail polish containing 33% Toluene. This test substance was non-irritating and non-sensitizing in assays performed in humans (under occlusive conditions). Similarly, a nail polish containing 31.23% Toluene was not considered to be cumulatively irritating or sensitizing in assays performed in humans under occlusive conditions. No phototoxic or photoallergic reactions were observed in assays performed in humans using nail products formulated with 25 and 30% Toluene.

OCULAR IRRITATION STUDIES

Ocular irritation following exposure to undiluted Toluene without rinsing, and exposure to undiluted Toluene with rinsing was evaluated in rabbits.[2] Toluene was considered an irritant in non-rinsed eyes (irritation index = 22.67/110), and was considered a slight irritant in rinsed eyes (irritation index = 13.33/110). Slight irritation of the conjunctival membrane was observed after application of 2 drops of undiluted Toluene to rabbits. Severe ocular irritation was observed in a range-finding assay in which rabbit eyes were treated with a single dose of Toluene in propylene glycol, water, and/or deodorized kerosene (concentration of Toluene reported to be higher than 15%). A nail polish containing 33% Toluene was considered mild eye irritant in an assay performed in rabbits.

CLINICAL STUDIES

Case Reports

Non-Occupational

A 22-yr-old man experience extensive chemical burns, acute renal failure, and disseminated intravascular coagulation, that eventually led to death, after spilling a sealer containing 65% Toluene on his clothing.[3] A 60-yr-old man who consistently worked with glue containing 90% Toluene was admitted to the hospital with astenia and weight loss of 6 mo duration. Abnormalities upon diagnostic testing were found (e.g., cortical atrophy). Symptoms subsided following suspension of work.

A 65-yr-old male farmer with previously diagnosed arterial hypertension presented to the hospital following accidental consumption of approximately 150 ml of an organic solvent.[90] Initial symptoms post-consumption included tiredness, confusion, weakness, drunken-like actions, and gastrointestinal symptom. The patient reported severe chest pains approximately 40 min after ingestion. Toxicological analyses of the patient's blood indicated the presence of Toluene and xylene isomers. Elevated markers of myocardial necrosis were apparent. An echocardiopgram scan showed hypokinesia within the apical segments of the lateral posterior, anterior, and inferior walls, with an ejection fraction of 38%. Angiography revealed a muscular bridge causing a 30-50% stenosis in the middle of the circumflex branch of the left coronary artery. Symptoms subsided during 4 d of hospitalization, and the patient was discharged.

A 31-yr-old woman with a history of anxiety and depression presented to the hospital with acute onset of generalized weakness and lower back and abdominal pain.[91] The patient reported worsening of the symptoms over the course of 6 d. Laboratory testing revealed hypokalemia, metabolic acidosis, and renal tubular acidosis, which were treated with oral and intravenous potassium and sodium citrate-citric acid. The patient's anion gap closed and bicarbonate level normalized upon treatment; however, the hypokalemia persisted. The patient denied paint or glue sniffing, but stated that she was exposed to paint, which was deemed the cause of symptoms. The patient's symptoms resolved during 2 d of hospitalization. A few weeks post-discharge, a Toluene test sent to an external laboratory during the patient's hospital stay reported a Toluene blood level of 4.12 mg/l.

A man in his 40s was found dead in his home shortly after spraying a wood coating varnish in a sealed off room (sized 4 x 3 x 3 m).[92] Moderate congestion and edema of the lungs and minimal steatosis of the hepatic cells were found upon autopsy/histological examination. Toxicological analyses revealed Toluene in the blood, and the death was diagnosed as acute Toluene intoxication.

A 29-yr-old woman with a history of Raynaud's syndrome, alcohol excess (reported as abstinent for months), and pancreatitis presented to the emergency department with gastrointestinal symptoms, headache, lethargy, and confusion. [93] The patients' Glasgow Coma Score was 12/15. Mild hepatorenal injury was evident upon biochemistry. The patient developed hypokalemia, hypernatremia, and hypophosphatemia, requiring management. Collateral history taken from the patient's mother revealed that the patient had been making jewelry for 2 mo using epoxy glue (containing Toluene), in a

small unventilated room in her home. After 24 h, the patient's neurological state was improved and biochemistry returned to baseline.

Occupational

Koilonychia and hapolonychia of the fingernails were observed in 6 of 16 cabinet workers percutaneously exposed to a thinner mixture containing 30% Toluene, 30% xylene, and 40% methyl alcohol. The majority of these workers had an average exposure time of about 2 yr.

No complaints of respiratory tract irritation were reported for volunteers or workers exposed to Toluene at concentrations of 800 - 1500 ppm for 8 h. Moderate conjunctival irritation and corneal damage was noted in 3 workers who were accidentally splashed in the eyes with Toluene.

A male construction worker displayed a slow reaction time, headache, nausea, and vomiting following application of an adhesive tape with a strong smell to pipes in non-ventilated underground apartments for 5 d.[94] The patient was working 10 h/d, and did not use personal protective equipment. Imagining revealed subcortical and periventricular white matter lesions plus involvement of bilateral cerebellar dentate nuclei. Benzoic acid was found in the blood and urine of the patient upon toxicology screening, and Toluene was detected in the adhesive tape. The patient was diagnosed with toxic leukoencephalopathy induced by Toluene and recovered with 6 mo of intensive care.

A 38-yr-old presented to the hospital with complaints of chronic headache and nausea.[95] Imaging revealed a T2-hyperintense cerebral white matter lesion in the left frontoparietal lobe with loss of gray-white matter differentiation, accompanied by faint T2-hyperintense lesions in the corpus splenium and right periventricular white matter. The patient was treated for possible encephalitis. Symptoms subsided; however, magnetic resonance imagining (MRI) findings remained the same. No neurological deterioration was observed during follow-up. Later, hospital personnel discovered that the lacquer thinner (composed of about 60% Toluene) was regularly used at the patient's workplace (patient reported 5 yr history at the company, and retired 3 mo prior to hospital visit). Toluene exposure was determined to likely be the cause of the patient's symptoms and MRI findings.

Occupational Toxicity/Epidemiology/Case Reports

Several occupational toxicity studies were performed comparing the prevalence of genotoxicity in printing factory workers exposed to Toluene compared to unexposed individuals.[2] In some studies, the number of chromosomal aberrations and SCEs were similar among Toluene-exposed workers versus unexposed individuals (approximate exposure: 7 - 400 ppm; average time of employment:7 - 15 yr). However, a significantly greater number of chromatid breaks, chromatid exchanges, chromatid gaps, and SCEs were observed in subjects occupationally exposed to Toluene (approximate exposure: 200 – 300 ppm; average time of employment: ≥ 16 yr) compared to unexposed controls.

According to several studies, concentrations of Toluene in the blood of workers occupationally exposed to Toluene ranged from 20 µmol/l to approximately 0.94 mg/l.[3] Blood concentrations of humans not occupationally exposed to Toluene and those who were occupationally exposed to Toluene were compared. The mean blood concentration of those occupationally exposed to Toluene was significantly higher (2785 ± 3756 ng/l) than those not exposed to Toluene occupationally (829 ± 1175 ng/l). The maximum amount of expired Toluene in the breath of Toluene-exposed workers was determined to be 4 ± 0.8 µg/l after 22.5 ± 10 min of exposure. Gasoline workers exposed to 60.3 and 527 ppb in their personal air space had exhaled breath concentrations of 4.3 to 41.8 ppb. The maximum amount of o-cresol and hippuric acid in the urine of workers occupationally exposed to Toluene was determined to be 2.8 µg/ml and 3.02 mg/ml, respectively. The release of Toluene from adipose tissue was significantly slower when compared to elimination from the blood in humans occupationally exposed to Toluene.

Several adverse effects were observed in studies on workers occupationally exposed to Toluene. These effects include changes in liver enzymes, color vision impairment, organic brain syndrome, mild chronic encephalopathy, memory loss, unstable mood, inhibition of psychomotor skills and manual dexterity, impaired cognitive functioning, headaches, eye irritation, effects on auditory and visual pathways, and a reduction in reproductive hormones.

SCE frequencies were compared in printing workers exposed to Toluene and an unexposed control group. SCE rates were higher in the exposed group versus the unexposed group. Increased risk of mortality from cancers of the bone, connective tissue, and lung were observed in a cohort study performed in workers occupationally exposed to Toluene. In a different cohort study, cancer of the respiratory tract was the only cancer type with increased incidence in workers exposed to Toluene versus unexposed controlled subjects.

Many studies regarding occupational toxicity to Toluene were found in the literature. Details of these studies can be found in Table 8. Reported adverse effects relating to occupational exposure to Toluene include an increased risk for reproductive disorders and birth defects, impaired gonad function/decreased sperm activity/quality, cardiotoxicity (e.g., decreased maximum heart rate), impaired learning/memory, genotoxicity, increased lipid peroxidation, increased liver enzymes, increased risk of cancer, increased risk of metabolic syndrome, increased oxidative stress levels, dry skin/itching, dry eyes, and increased general health risks.[96-110] No Toluene-related neuropsychological effects were observed in a cross-sectional study performed in furniture workers exposed to Toluene.[111] In a 4-yr study evaluating color perception in individuals occupationally exposed to Toluene, no significant association between adverse effects relating to color vision

perception and Toluene exposure were observed.[112] White matter lesions, headache, and nausea were reported in case reports involving workers occupationally exposed to Toluene. [94,95]

Occupational Exposure Limits

Occupational exposure limits have been placed by several organizations. A listing of these organizations, along with their regulations can be found in Table 9.

RISK ASSESSMENT

Toxicity Values and Minimal Risk Levels of Toluene

A reference concentration for chronic inhalation exposure (RfC; an estimate of an inhalation exposure, for a given duration, to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime), was determined by the US EPA.[113] This estimate was based on animal and human studies available in the literature and a calculated adjusted inhalation NOAEL of 46 mg/m^3. The RfC was determined to be 5 mg/m^3. An RfD (reference dose for chronic oral exposure) was evaluated via similar methods and determined to be 0.08 mg/kg/d. In addition, repeated-dose toxicity data (inhalation) from ECHA indicate Toluene did not cause adverse effects in the rat following inhalation exposure to 300 ppm for up to 24 mo (6 h/d, 5 d/wk).[8] The NOAEC for chronic systemic or local toxicity in this study was 300 ppm (1131 mg/m^3). Human data (reported under Epidemiological studies) demonstrate no evidence that long-term exposure to Toluene (98 mg/m^3 for 21 yr) adversely effects human neurological or cognitive function. Minimal risk levels (defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (non-carcinogenic) over a specified duration of exposure) determined by the Agency for Toxic Substances and Disease Registry (ATSDR) were determined to be 2 ppm for acute (≤ 14 d) exposure and 1 ppm for chronic (≥ 365 d) inhalation exposure.[114] In addition, oral acute and chronic minimal risk levels were determined to be 0.8 and 0.2 mg/kg/d, respectively.

Margin of Safety (MOS) Calculation of Toluene in Nail Products

In order to thoroughly assess the safety of Toluene in nail products, a tiered approach was followed in the calculation of an MOS. Tier 1 investigates exposure to Toluene via nail product use based on the most conservative parameters (100% systemic absorption via inhalation, dermal, incidental ingestion, and possible nail plate penetration exposure). Tier 2 presents a more realistic assessment of exposure, taking into account the amount of Toluene that is likely evaporated during the drying process as well as the skin area surrounding the nails, assuming 21.5% of the total content of Toluene would contribute to a systemic dose(inhalation plus dermal exposure). The third tier considers measurement of Toluene in the breathing zone of female subjects following exposure to nail polish products under simulated-use conditions[13] (Approximately 0.445% Toluene (0.6 mg) was detected in the breathing zone of women subjects in one application; other details of this assessment can be found in the Cosmetic Use Exposure section of this report).

Parameters that were the same among each calculation include the number of applications per day (1 application/d)[115], the weight of the adult (60 kg)[116], and the maximum use concentration of Toluene in nail products (20%)[9]. In addition, an NOAEL of 625 mg/kg bw/d was used for each calculation.[117] This calculation was derived from a study performed in Fischer rats given Toluene in corn oil via gavage, 5 d/wk, for 13 wk.

Tier 1

Toluene may readily volatilize into air; the primary exposure route to Toluene from nail products, as stated by the California DTSC, is through vapor inhalation.[118] Additionally, exposure to Toluene may also occur through skin contact with nail products as well as via accidental hand-to-mouth exposure. Depending on the applied concentration, some chemical substances in nail products may penetrate the nail plate and reach the tissue under the nail, particularly when the nail is thin, soft, or damaged.

In a first tier approach with conservative exposure parameters, the systemic exposure dose (SED) is calculated based on an assumption that 100% of the total content of Toluene will contribute to a systemic dose, and an applied amount of 500 mg product on 20 nails[115]. The daily SED $_{\text{inhalation + dermal + incidental ingestion + possible nail plate penetration}}$ of Toluene resulting from nail products was calculated accordingly:

$$\frac{1\ application\ per\ day\ x\ 500\ mg\ product\ x\ 20\%\ (maximum\ use\ concentration)\ x\ 100\%\ (Toluene\ content\ contributing\ to\ systemic\ dose)}{60\ kg\ (adult\ weight)} = 1.67\ mg/kg\ bw/d$$

The MOS is then calculated as follows:

$$\frac{625\ mg/kg\ bw/d\ (NOAEL)}{1.67 mg/kg\ bw/d\ (SED)} = 374$$

Of note, the amount of the nail polish applied in each application may vary depending on different use habits and practices. The in silico tool VERMEER Cosmolife estimates that the amount of nail polish use per application for 10 nails is 300 mg/kg bw/d.[119] In an unpublished study submitted by the Council in 1991,[13] it was found that the average amount of nail polish applied was 0.5276 g (with a coefficient of variation of 20.60%), which involved applying four coats (one base coat,

two enamel coats, and top coat) on 10 nails in one application. Based on this exposure scenario, adopting a cautious estimate for applying polish to 20 nails would result in a usage of 1.0552 g (or 1055.2 mg), which results in an MOS of 177.7, corresponding to a SED of 3.517 mg/kg bw/d.

Tier 2

In the second tier, employing a probabilistic approach, a more realistic exposure assessment was carried out to refine the exposure model.

In a safety assessment of formaldehyde applied in nail hardeners, the Danish Environmental Protection Agency considers formaldehyde in nail hardeners may reach the skin surrounding the nail and evaporate during the drying process of the nail hardener.[115] Additionally, it is presumed that formaldehyde reacting on the nail plates will not contribute to the systemic dose as there is no specific data available regarding this potential contribution. The daily SED of formaldehyde is calculated based the following model parameters:[115]

- It is estimated that about 25% of the total content of formaldehyde in the product will evaporate during the drying process. Furthermore, it is assumed that formaldehyde is released in the close area around the person (1 m^3), and half of formaldehyde is absorbed via inhalation or dermal absorption, while the remaining 75% will coat the nail plate and the skin surrounding the nail.
- The nails surface area (10 nails) amounts to a maximum 40 cm^2; the skin surrounding the nails amount to 4 cm^2, which corresponds to about 9% of the total area of nail and skin. Hence, it is presumed that 9% of the nail hardeners applied will coat the skin around the nail, potentially contributing to the systemic dose.
- Consequently, when calculating the SED, only the portion covering the skin surrounding the nail (9%) and half of the portion that evaporates during the drying process (12.5% of the total product content) are included. In total, 21.5% (9% + 12.5%) of the formaldehyde content in the product will contribute to the systemic dose.

As Toluene is less volatile than formaldehyde (formaldehyde has a higher vapor pressure than Toluene at room temperature), a similar risk assessment was conducted for Toluene as used in nail polish and enamel products, based on the following exposure parameters and assumptions:

- It is assumed that about 25% of the total content of Toluene in nail products will evaporate during the drying process, and half of Toluene is absorbed via inhalation or dermal absorption.
- It is presumed that around 9% of the applied nail polish and enamel products will cover the skin around the nail.
- In total, 21.5% (9% +12.5%) of the total content of Toluene will contribute to a systemic dose.
- It is assumed that a negligible amount of Toluene can penetrate the nail.
- Applied amount of 500 mg product on 20 nails[115]

Daily SED $_{\text{inhalation + dermal}}$ of Toluene resulted from nail product use:

$$\frac{1\ application\ per\ day\ x\ 500\ mg\ product\ x\ 20\%\ (maximum\ use\ concentration)\ x\ 21.5\%\ (Toluene\ content\ contributing\ to\ systemic\ dose)}{60\ kg\ (adult\ weight)} = 0.358\ mg/kg\ bw/d$$

MOS calculation:

$$\frac{625\ mg/kg\ bw/d\ (NOAEL)}{0.358\ mg/kg\ bw/d\ (SED)} = 1745.8$$

It should be noted that in the second-tier approach, 12.5% of the total content of Toluene in nail polish products is assumed to be inhalable per application per day, which equals to 12.5 mg [= 500 mg (daily applied amount of nail polish) × 20% (maximum use concentration) × 12.5% (inhalable fraction)]. In comparison, a study indicated that personal inhalation exposures to Toluene during the application of nail lacquers in residences ranged from approximately 1.03 to 2.82 mg/person/d (the mean Toluene levels measured in the breathing zone during the nail lacquer application ranged from 0.85 to 2.4 ppm).[120,121] Therefore, the assumption that 12.5% of the total Toluene content in nail polish products is inhaled remains a conservative estimation.

Tier 3

In the third tier, the measurement of Toluene exposure in the breathing zone of women under simulated-use conditions has been considered.

According to an unpublished study, the amount of Toluene in the breathing zone before, during, and after exposure to nail product application was evaluated in 15 female subjects under simulated-use conditions: each subject applied 4 coats of nail polish product per application (each coat formulated with 25% Toluene) within a 16 m^3 room that maintained an air flow of about 1.0 changes per hour.[13] The mean total of the nail polish applied was 0.5276 g (CV% = 20.60%). The estimated average Toluene exposure amount, which was measured in three applications in the breathing zone of all subjects,

was 0.6 mg (CV% = 33%, the mean Toluene levels measured in the breathing zone during the nail lacquer application ranged from 1 to 4 ppm) and the grand mean application duration was 15 min. Therefore, only 0.6 mg Toluene was detected in the human breathing zone during application of nail polish products. Considering the Toluene concentration in nail polish products of this study is 25%, it indicated that around 0.455% of Toluene [0.455% = 0.6 mg ÷ (527.6 mg ×25%) ×100%] was available for inhalation. The following parameters were used for this MOS derivation:

- Approximately 9% of the applied nail polish and enamel products will cover the skin around the nail.
- 0.455% of Toluene is available in the breath zone under simulated-use conditions
- In total, 9.455% (9% +0.455%) of the total content of Toluene will contribute to a systemic dose.
- Applied amount of product: 0.5276 g (or 527.6 mg)

$$\frac{1\ application\ per\ day\ x\ 527.6\ mg\ product\ x\ 20\%\ (maximum\ use\ concentration)\ x\ 9.455\%\ (Toluene\ conteent\ contributing\ to\ systemic\ dose)}{60\ kg\ (adult\ weight)} = 0.166\ mg/kg\ bw/d$$

MOS calculation:

$$\frac{625\ mg/kg\ bw/d\ (NOAEL)}{0.166\ mg/kg\ bw/d(SED)} = 3765$$

Values of MOS calculations for all three tiers are greater than 100. This figure threshold is generally considered to be protective. The standard of MOS value of 100 is derived from multiplying two factors: a 10-fold factor accounts for the extrapolating data from test animals to human being (interspecies extrapolation), and an additional 10-fold for accommodating differences among the human population (intra-species extrapolation).[122]

EPIDEMIOLOGICAL STUDIES (NON-OCCUPATIONAL)

A cross-sectional study including 3011 US adults from the National Health and Nutrition Examination Survey (NHANES) was performed to evaluate the association of urinary exposure biomarkers of volatile organic compounds (including Toluene) with liver injury biomarkers and risk of non-alcoholic fatty liver disease.[123] NHANES surveys were released every 2 yr and evaluated from 2011 - 2016. Throughout this period, urinary volatile organic compound metabolites were measured. The presence of the Toluene metabolite *N*-acetyl-*S*-(benzyl)-L-cysteine in the urine was associated with increased AST, GGT, ALP, albumin, AST/ALT ratio, and Hepamet fibrosis scores.

The association between exposure to certain chemicals (including Toluene) in ambient air during pregnancy and cases of acute lymphoblastic leukemia and acute myeloid leukemia was evaluated in a case-control study. A total of 69 cases of acute lymphoblastic leukemia (2994 controls) and 46 cases of acute myeloid leukemia (19,209 controls) were ascertained from the California Cancer Registry records of children (< 6 yr of age) between the years of 1990 and 2007. Information on chemical exposures was taken from community air monitors (monitors collected 24-h air samples every 12 d). Exposure to Toluene during third trimester was associated with an increased risk for acute lymphoblastic lymphoma (adjusted odds ratio (OR): 1.22; 95% confidence interval (CI)) and acute myeloid lymphoma (adjusted OR: 1.31; 95% CI). In addition, an increased risk of acute lymphoblastic lymphoma (adjusted OR: 1.19; 95% CI) and acute myeloid lymphoma (adjusted OR: 2.02; 95% CI) was positively associated to exposure to Toluene during the child's first year.

SUMMARY

Toluene is reported to function in cosmetics as an antioxidant and a solvent. Toluene was previously reviewed by the Panel in a safety assessment published in 1987. At that time, the Panel concluded that Toluene is safe as used in the present practices of use and concentration as stated in that report. This conclusion was reconsidered at the March 2005 Panel meeting and the conclusion was re-affirmed, as published in 2006. In 2023, members of the US FDA nominated Toluene for an accelerated rereview, and thus, according to CIR procedures, this report has been re-opened for evaluation.

No uses were reported according to 2023 FDA VCRP survey data; however, 2023 concentration of use data report that Toluene is used at up to 20% in nail polish and enamel. It is also used in other product categories (e.g., baby products, hair conditioners, bath soaps and detergents) at low concentrations. In 2002, Toluene was reported be used in 59 total formulations at up to 26% in other manicuring preparations (according to 2003 concentration of use survey). All uses and concentrations provided in 2002/2003 were in nail products.

In the EU, the use of Toluene in cosmetics is restricted to nail products at a maximum concentration of 25%. In addition, the EU requires caution statements informing users to keep Toluene-containing products away from children.

The amount of Toluene in the breathing zone before, during, and after exposure to nail product application was evaluated in 15 female subjects. The average Toluene exposure amount ranged from 0.5 – 0.6 mg, with a mean application duration of 15 min. The mean Toluene exposure via inhalation was 0.236 ppm in an assay in which analytical air measurements were taken on 178 professional nail technicians. In a different study, the maximum daily exposure (via dermal

and inhalation) in nail salon patrons, nail technicians, and home users were reported to be 2160, 28,200, and 7760 µg/d, respectively.

Toluene has been reported to be an impurity in several products including hand sanitizers, feminine hygiene products, and sunscreens. Feminine hygiene products containing Toluene as an impurity were associated with a higher calculated cancer risk (largely due to presence of benzene in products).

In an assay evaluating the effect of age on Toluene distribution in rats (4, 12, and 24 mo; exposed to up to 1 g/kg Toluene in corn oil; gavage), blood Toluene concentrations were unaffected by age; however, concentrations of Toluene in the brain were significantly higher in 24-mo-old rats vs. 4-mo-old rats. Mean blood concentrations of Toluene in rats following a 6-h inhalation exposure period were 0.01, 0.33, and 11.84 µg/g, after exposure to 5, 50, and 500 ppm Toluene, respectively (on day 1 of study). Toluene concentrations increased in a time- and dose-dependent manner in pregnant rats exposed to Toluene via inhalation (800 or 12,000 ppm; GD 8 - 20; 15 - 45 min exposure). Toluene levels also increased in fetal brains in a concentration-dependent manner, and in placenta and amniotic fluid in a time-dependent manner. An assay was performed in humans (exposed to 50 ppm Toluene via inhalation) evaluating the effect of temperature on absorption and excretion of Toluene. Increased heat increased absorption of Toluene and decreased elimination. A similar study was performed evaluating the effect of heat on the percutaneous absorption of Toluene (exposure via Toluene vapor; masked subjects). In this assay, the presence of heat did not affect percutaneous absorption levels. Maximum Toluene concentrations of 3.07 ± 0.40 µg/ml (in samples exposed for 15 min) 5.38 ± 0.92 µg/ml (in samples exposed for 240 min) were reported in an in vitro dermal penetration assay using rat skin exposed to 100% Toluene. A steady-state flux of 0.00038 $g/cm^2/h$ was determined in an in vitro percutaneous absorption study performed using split-thickness pig skin (skin exposed to undiluted Toluene).

The effect of Toluene on body weight and pathological changes in organs was observed in rabbits exposed to 1000 mg/l Toluene via inhalation for 14 d. Body weights in the Toluene-treated group initially dropped, but recovered. Organ tissue weights were similar among control and treated groups; however, abnormalities were noted in the heart, lung, stomach and spleen tissues.

Gravid female rats were dosed with 1250 mg/kg Toluene in peanut oil by gavage on days 16 – 19 of gestation and killed on GD 20. Maternal and reproductive parameters were not affected, and there was no significant difference in the number of fetal external or skeletal malformations between the treatment and the control group. However, a pattern of accelerated development in the upper mid-turn in the treated fetal cochleas was observed. Up-regulation of NDMA subunits, CREB1, CaMKIV, and apoptotic–related genes were observed in animals treated with up to 50 ppm Toluene for 5 d on PND 2 or 8. A NOAEC of 600 ppm was determined in male rats and an F1 generation in a study evaluating fertility. Anti-nociception, and effects on memory and locomotion were observed in rats subjected to a pre-natal and post-natal exposure to Toluene (6000 ppm). In an inhalation study in which mice were exposed to 8000 ppm Toluene for 30 min twice daily via inhalation on GD 7 - 19, neonatal death was significantly increased in the test group compared to controls. In a study using rats, the animals were exposed to Toluene (500 or 1500 ppm) for 6 h/d on GD 6 - 20 and killed on GD 21. Maternal weight gain of the test animals and fetal body weights in the 1500 ppm group were decreased; no other reproductive or developmental effects due to dosing were observed. In another study in which dams were exposed to up to 3000 ppm Toluene via whole-body inhalation for 6 h/d on GD 6 – 15 and killed on GD 20; the maternal toxicity NOAEL was 750 ppm with a defined maternal and developmental toxicity LOAEL of 1500 ppm. There were no effects on reproductive parameters; fetal body weights were increased in a toxicologically significant manner at 1500 and 3000 ppm.

Several inhalation studies were conducted in rats using short-duration (15 or 30 min; twice daily; various exposure times, including post-natal exposures) or high-dose (up to 16,000 ppm) exposures, and effects on post-natal development were evaluated. Generally, there were no significant maternal effects, although decreased body weight gains were observed with doses ≥ 8000 ppm Toluene. Fetal malformations were observed at some of the highest doses (> 8000 ppm), and there were indications of impaired cognitive function.

Female rats were exposed to Toluene (2000 - 8000 ppm) via inhalation, 30 min/d for 28 d, and the effect on several hormone levels and ovarian tissue was examined. Progesterone levels (at 4000 and 8000 ppm) and testosterone levels (all dose groups) were statistically significantly increased and IGF-1 was statistically significantly decreased (at 8000 ppm); no effect on estradiol was noted. A dose-dependent increase in apoptosis in ovarian tissue was observed. In another study, gravid rats were exposed to 0.09 – 9 ppm Toluene via nasal inhalation for 90 min/d on GD 14.5 – 18.5; hormone levels (all groups) and mRNA levels of steroidogenic enzymes in testicular tissues (control and low-dose group) were measured in male pups.[45] Fetal plasma testosterone concentrations were significantly reduced in males of the 0.9 and 9 ppm, but not the 0.09 ppm, groups. mRNA levels of 3β-HSD were significantly reduced after exposure to 0.9 ppm. Effects on immunological biomarkers were also examined in pups following whole-body inhalation exposure of dams to 5 or 50 ppm Toluene for 6 h/d on GD 14 – 18 or 19, followed by post-natal exposure to groups of pups at various post-natal time frames. In one study, total plasma IgG2a levels were statistically significantly increased in the 50 ppm group, and in another study, total IgG1 levels were markedly reduced. Splenic expression of some transcription factors was suppressed.

Positive results were observed in in vitro genotoxicity assays including an Ames assay (using *Salmonella typhimurium* strains at up to 50 µl/plate), a comet assay (using human skin disks at up to 1,000,000 ppm), a modified alkaline comet assay

(using human lung epithelial carcinoma cells at 0.25 ppmv), a mammalian cell mutagenicity assay using mouse lymphoma cells (up to 500 µg/ml). Conversely, no genotoxicity was observed in other in vitro assays including an Ames assay (using *S. typhimurium* strains at up to 1000 µg/plate), a chromosomal aberration assay (using Chinese hamster ovary cells at up to 1600 µg/ml), and an SCE assay (using Chinese hamster ovary cells at up to 5000 µg/ml). Similarly, mixed results were observed in in vivo genotoxicity assays. Toluene was genotoxic in an alkaline and neutral comet assay using *Drosophila* larvae given up to 100.0 mM Toluene via diet, a comet assay using mice given a 5 or 15 g/kg intraperitoneal injection of Toluene, and a comet assay using mice exposed to Toluene (25 ppm) via inhalation for 4 wk. Negative results were observed in a micronucleus assay using mice exposed to up to 2000 mg/kg Toluene (method of administration not stated) and in a bone marrow nucleus assay using mice exposed to 100 ppm Toluene via inhalation for 15 d.

In a 2-experiment carcinogenicity assay performed in rats given Toluene (500 or 800 mg/kg bw; in olive oil) via gavage for 2 yr, increased numbers of total malignant tumors and carcinomas were observed in treated rats versus controls. Conversely, no neoplasms were observed in a 2 yr study in which rats and mice were exposed to Toluene (up to 12,000 ppm) via inhalation.

A significant increase in the number of inflammatory cells and significant decrease in the production of interferon-gamma and substance P was observed in treated mice versus controls in an assay in which mice were treated with low-levels of Toluene (50 ppm) for 6 or 12 wk. Significantly increased netrophin-3 production was also observed in exposed mice.

Abnormalities in adrenal glands (increased weight) and adrenocortical size were observed in rats treated with Toluene (1500 ppm) via inhalation for 7 d. Adrenocortical hypertrophy and significant increases in ACTH serum concentrations were also observed in treated animals.

The effect of Toluene (300 ppm; 8 wk; inhalation exposure) on bone mass toxicity was examined in male mice. Bone mineral density and bone mineral content were significantly lower in treated versus control groups.

Hearing loss was evaluated in guinea pigs exposed to Toluene alone, or along with a low protein diet, and/or cytochrome p450 inhibition. A statistically significant Toluene-induced hearing loss was provoked in cytochrome p450-inhibited guinea pigs on a normal diet, and in cytochrome p-450 inhibited guinea pigs on a low protein diet. Hearing loss was similar among unexposed controls and guinea pigs treated with Toluene alone, or those treated with Toluene plus a low protein diet.

Several Toluene abuse cases have been found in the literature. These cases report a myriad of symptoms following abuse including neurological, gastrointestinal, cardiac, hepatic, pulmonary, and adrenal abnormalities and dysfunction.

According to neurotoxicity and behavioral studies performed in animals, exposure to Toluene can result in HPA/HPT axes dysfunction, up- and down-regulation of the expression of NMDA subunits, memory, learning, and motor impairments, decreased hippocampal neurogenesis, and alteration of brain chemicals. Altered neuroplasticity was observed in 17 human subjects exposed to Toluene (peak of 200 ppm) via inhalation.

Acute and subchronic assays were performed in rats evaluating the effect of Toluene (up to 1019 ppm in acute studies and up to 995 ppm (13-wk) on oxidative stress markers in the brain. Increased oxidative stress was apparent in both acute and 13-wk assays.

The effect of Toluene (up to 4000 ppm; single 30 min exposure) on lung and brain tissue inflammation was evaluated in mice. Immune system activity and cellular proliferation were similar among control and treated groups. However, treated animals displayed morphological abnormalities and increased astrogliosis in the striatum. c-Fos immunoreactivity following exposure up to 5000 ppm Toluene for up to 30 min was increased in about one-third of the brain structures examined, and the majority of brain structures activated by Toluene were found in the forebrain and midbrain.

The effect of Toluene inhalation on immune responses were investigated using groups of 6 C3H/HeN mice exposed to up to 500 ppm of Toluene for 6 h/d, 5 d/wk, for 3 or 6 wk. Low levels of Toluene exposure (50 ppm) in mice immunized with OVA might dysregulate immune responses to OVA via the activation of transcription factors. The number of BAL cells and plasma total IgG1 antibody production were increased in the 50-ppm allergic group.

The potential for Toluene (up to 450 ppm for 10 min or up to 135 for 3, 10-min sessions) to elicit microvascular leakage was evaluated in rat airways. Toluene exposure induced dye leakage into the trachea and main bronchi in a concentration-dependent manner.

Olfactory changes were assessed in female mice exposed to 1000 ppm Toluene for 4 wk. Changes in density and thickness of olfactory epithelium and neuroepithelium were observed during treatment.

Hepatotoxic effects were evaluated in male rats given a single oral dose of Toluene (6 ml/kg) via gavage. Increased AST and ALT numbers, degeneration of hepatocyte and mononuclear infiltration, high immunoreactivity, and increased apoptosis was observed in treated animals. Hepatotoxicity was also evaluated in rabbits exposed to noise (100 dB) and Toluene (up to 1000 ppm) combined or separately. Treatment occurred for 14 d. Histopathology revealed minor cell swelling, minor hepatic lipidosis, and eosinic cytoplasms in animals treated with Toluene only. Significant swelling and damage of the liver tissue were observed following exposure to Toluene and noise simultaneously.

Decreased blood pressure and heart rate, and increased troponin T levels were observed in rats given 6 ml/kg Toluene via gavage (single dose). Also observed in treated animals were cardiac congestion and edema. In a different study evaluating cardiotoxicity, Toluene (up to 1.2 g/kg; method of administration not stated) resulted in tachycardia and raised blood pressure.

The effect of allergic stimulation on genotoxicity in the brains of Toluene-exposed mice (25 ppm for 4 wk) was evaluated. Significant DNA damage was observed in the hippocampus and leukocytes of ovalbumin-immunized mice following Toluene exposure, compared to controls.

The in vitro skin viability of human skin disks exposed to Toluene vapor (up to 1,000,000 ppm in corn oil) was evaluated. In vitro skin exposures to Toluene resulted in statistically-significantly reduced cell viability, at all tested concentrations, in a dose-dependent manner, compared to controls.

A 65-yr-old man presented to the emergency department with severe chest pain 40 min after accidental ingestion of an organic solvent. Cardiotoxicity was apparent upon testing and the patient's blood indicated the presence of Toluene and xylene isomers. Laboratory testing in a 31-yr-old woman with weakness and back/abdominal pain revealed metabolic acidosis and persistent hypokalemia. The patient reported exposure to paint. The patient's Toluene level in the blood was reported to be 4.12 mg/l. A man in his 40s was found dead in his home with apparent toxicity observed in the lungs and liver shortly after spraying a wood coating varnish in a sealed off room. Toxicological analyses revealed Toluene in the blood, and the death was diagnosed as acute Toluene intoxication. A 29-yr-old woman reported to the emergency department with gastrointestinal symptoms, headache, lethargy, and confusion; laboratory analysis revealed electrolyte abnormalities. These symptoms were determined to be due to continuous exposure to epoxy glue containing Toluene (used in small unventilated room).

White matter lesions, headache, and nausea were reported in 2 case reports. Symptoms were reported to be due to occupational exposure to Toluene.

Many occupational toxicity and epidemiological assays were found in the literature. Exposure to Toluene occupationally is associated with reproductive disorders, cardiotoxicity, neurological disorders, genotoxicity, hepatotoxicity, increased risk of cancer and metabolic disorders, increased oxidative stress levels, and eye and skin irritation. No Toluene-related neuropsychological effects were observed in a cross-sectional study performed in furniture workers exposed to Toluene. In a 4-yr study evaluating color perception in individuals occupationally exposed to Toluene, no significant association between adverse effects relating to color vision perception and Toluene exposure were observed.

A reference concentration for chronic inhalation exposure and a reference dose for chronic oral exposure of 5 mg/m^3 and 0.08 mg/kg/d, respectively, were determined by the US EPA. Minimal risk levels of 2 ppm, 1 ppm, 0.8 mg/kg/d, and 0.2 mg/kg/d were determined by the ATSDR for acute inhalation, chronic inhalation, acute oral, and chronic oral exposures, respectively.

Three MOS calculations were performed according to different levels of exposure. All MOS values (374, 1745.8, and 3765) were above 100, and were thus considered to be protective.

The association between Toluene exposure (measured as urinary biomarkers) and non-alcoholic fatty liver disease was evaluated in a cross-sectional study using 3011 US adults. The presence of the Toluene metabolite *N*-acetyl-*S*-(benzyl)-L-cysteine in the urine was associated with increased aspartate aminotransferase, GGT, ALP, albumin, AST/ALT ratio, and Hepamet fibrosis scores.

The association between exposure to chemicals (including Toluene) in ambient air during pregnancy and cases of acute lymphoblastic leukemia and acute myeloid leukemia was evaluated in a case-control study. Exposure to Toluene during third trimester and during a child's first year of life were associated with a higher risk of both acute lymphoblastic leukemia and acute myeloid leukemia.

PREVIOUS DISCUSSIONS

Discussion from Original Report Published in 1987

No data were available to the Panel regarding the impurities found in cosmetic grade Toluene.[2] One possible impurity, benzene, is a carcinogen. Therefore, cosmetic products formulated with Toluene should be benzene-free.

Two studies concluded that Toluene-induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection. The significance of positive clastogenic effects attributed to Toluene in these studies is difficult to assess, since the purity of the test samples was not reported. More definitive carcinogenic studies were available. In eight studies, Toluene did not induce cancer. In one study, 1 or 30 mice developed skin cancer; however, there were no untreated controls for comparison.

Results of animal studies indicated that undiluted Toluene is a skin irritant. Thus, there is a potential for Toluene to cause skin irritation in humans. However, the sole cosmetic use of Toluene is in products intended to be applied directly to the nail. Therefore, human skin exposure to this ingredient will be minimal under conditions of cosmetic use.

Discussion from Re-Review Summary Published in 2006

Many of the newly available studies reported findings consistent with the data in the original safety assessment.[4] New findings of adverse effects included the following effects: Toluene was ototoxic in guinea pigs; interferes with performance and learning in neurotoxicity and behavior studies in animals; increased numbers of litters with low birth weight pups and adversely affected brain development; in cultured embryos exposed to Toluene, yolk sac diameter, crown-rump length, somite number, and protein concentration were significantly reduced. An NTP study concluded that there was no evidence of carcinogenic activity for Toluene in F344/N rats and B6C3F$_1$ mice.

The new adverse effects noted above appeared only at high exposures. They were found only when animals were exposed to Toluene vapor at a level of 102 to 103 ppm. Such exposures, however, were not attainable in an exposure study of human subjects using nail polish – those values ranged from 1 - 4 ppm.

The Panel recognized that other data indicate adverse effects in the brain of Toluene abusers and in children born to mothers who inhaled Toluene during pregnancy. Again, the nature of these studies suggests high exposures and are not relevant to the use of Toluene in cosmetic products.

DISCUSSION

To be determined.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	liquid	2
Odor	sweet, pungent, benzene-like	6
Color	colorless	2
Molecular Weight (g/mol)	92.13	2
Density (g/ml @ 20 ºC)	0.861 - 0.871	2
Viscosity (cp @ 20 ºC)	0.6	2
Vapor pressure (mmHg @ 25ºC)	28.4	6
Vapor Density (mmHg)	3.14	6
Melting Point (ºC)	-94.9	6
Boiling Point (ºC)	110.6	6
Water Solubility (mg/ml @ 25ºC)	0.59	113
log K_{ow} (@ ºC)	2.73	6
Index of refraction	1.4961	2
Flash Point (ºC)	4.4	113

Table 2. Frequency (2023/2002) and concentration (2023/2003) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[10]	**2002[4]**	**2023[9]**	**2003[4]**
Totals*	**NR**	**59**	**0.000001 – 20**	**20 – 26**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*NR*	*57*	*0.000001 – 20*	*20 – 26*
Rinse-Off	*NR*	*2*	*0.000001 – 0.01*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray or Aerosol	NR	NR	0.000001 – 0.000002	NR
Incidental Inhalation-Powder	NR	NR	NR	NR
Dermal Contact	NR	NR	0.000001 – 0.000004	NR
Deodorant (underarm)	NR	NR	0.000001 – 0.000002	NR
Hair - Non-Coloring	NR	NR	0.000001	NR
Hair-Coloring	NR	NR	0.01	NR
Nail	NR	59	10 – 20	20 – 26
Mucous Membrane	NR	NR	0.000001 – 0.000004	NR
Baby Products	NR	NR	0.000001	NR
as reported by product category				
Baby Products				
Baby Lotions/Oils/Creams	NR	NR	0.000001	NR
Hair Preparations (non-coloring)				
Hair Conditioner	NR	NR	0.000001	NR
Shampoos (non-coloring)	NR	NR	0.000001	NR
Hair Coloring Preparations				
Hair Tints	NR	NR	0.01	NR
Manicuring Preparations (Nail)				
Basecoats and Undercoats	NR	21	NR	NR
Nail Extenders	NR	NR	10	NR
Nail Polish and Enamel	NR	23	20	20 – 25
Nail Polish and Enamel Removers	NR	2	NR	NR
Other Manicuring Preparations	NR	13	NR	26
Personal Cleanliness Products				
Bath Soaps and Detergents	NR	NR	0.000001 – 0.000004	NR
Deodorants (underarm)	NR	NR	0.000001 – 0.000002 (aerosol and not spray)	NR

NR – not reported

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

Table 3. CFR citations on Toluene

Citation	Details
21CFR1310.02; 21 CFR1310.04; 21CFR1313.15; 21CFR1313.24	List 2 chemical; controlled import and export by Drug Enforcement Administration
21CFR175.105	Indirect food additive; ingredient may be used as in adhesives for use in food handling preparations (e.g., packaging) according to certain conditions
21CFR175.320	Indirect food additive; ingredient may be used in resinous and polymeric coatings for use on food-contact surfaces according to certain conditions
21CFR176.180	Indirect food additive; ingredient may be used as component of paper and paperboard in contact with dry food according to certain conditions
21CFR177.1010	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (acrylic and modified acrylic plastics, semi-rigid and rigid) according to certain conditions
21CFR177.1200	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (cellophane) according to certain conditions
21CFR177.1580	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (polycarbonate resins) according to certain conditions
21CFR177.1650	Indirect food additive; ingredient may be used as a component of single and repeated use food contact surfaces (polysulfide polymer-polyepoxy resins) according to certain conditions
21CFR178.3010	Indirect food additive; ingredient may be used as adjuvant in the manufacture of foamed plastics intended for use in contact with food according to certain conditions
21CFR520.580	Oral dosage animal drugs; ingredient used in combination with dichlorophene as de-worming treatment in animals under certain limitations; single dose of 120 mg Toluene per pound body weight or divided dose of 120 mg Toluene per 5 pounds body weight, daily, for 6 d
27CFR21.132; 27CFR21.151	Denaturant authorized for denatured spirits
16CFR1700.14	Solvents for paint of other similar surface-coating material containing 10% or more by weight of Toluene requires special packaging to protect children from injury and illness

Table 4. Dermal penetration/percutaneous absorption and ADME studies on Toluene

Parameter Measured	Test System or Species	No./Group	Vehicle/Dose	Protocol	Results	References
DERMAL PENETRATION/PERCUTANEOUS ABSORPTION						
In Vitro						
Dermal penetration	Male Wistar rat skin	82 total	100%; 200 µl	Three microdialysis membranes (3000 kDa) were inserted intradermally at a length of 2 cm in rat abdominal skin; perfused with albumin solution 5% at 10 µl/min; a skin area of 1 x 0.6 cm^2 above the membranes exposed to Toluene for 15 or 240 min; dialysate sampled at 20-min intervals; effects of tape stripping and pretreatment with topical products (barrier creams) also assessed in 5 - 8 samples at each evaluation period	Maximum Toluene concentrations were reached 60 min after exposure (3.07 ± 0.40 µg/ml in samples exposed for 15 min; 5.38 ± 0.92 µg/ml in samples exposed for 240 min); in 15 min exposure experiments, dermal Toluene concentrations reached baseline values after 240 min; in 240 min exposure experiments, a plateau of approximately 6 µg/ml was reached after 60 min; after 15-min and 240-min exposures, the *o*-cresol content was 8.04 ± 1.0 and 12.7 ± 1.4 µg, respectively; neither tape stripping or barrier creams usage induced a significant change on dermal Toluene penetration or *o*-cresol content	[21]
Percutaneous absorption	Split-thickness pig skin	6	100%	Six jacketed static Franz cells (orifice diameter 9 mm, corresponding to skin exposure area of 0.64 cm^2) with mounted split-thickness pig skin used to evaluated percutaneous absorption; at start of experiment, donor compartment filled with test material (1 ml; neat) and capped with glass stopper; experiments ran for 4 - 9 h; aliquots of receptor fluid sampled at predefined times; steady-state flux, permeability coefficient, and lag time calculated	Steady-state flux: 0.00038 $g/cm^2/h$ Permeability coefficient: 0.00044 cm/h Lag time: 27.4 min	[22]
Human						
Percutaneous absorption	Humans	5 subjects	50 ppm	Subjects placed in inhalation chambers (approximately 18.1 m^3) and exposed to Toluene for 4 h; subject wearing masks led to pumps that fed clean air, preventing exposure via inhalation; concentrations continuously monitored with infrared spectrophotometer every 5 - 7 min; exposure were held at 3 different temperatures: 21, 25, and 30 °C; exposures at different temperatures spaced by a at least 1 wk to minimize cumulative exposure.	Dermal exposure to Toluene did not result in statistically-significant differences in venous concentrations between 25 and 30°C (technical problems towards the end of exposure prevented accurate readings at 21°C). Mean venous concentrations of Toluene (µg/l): At 21°C 0-h exposure: 0.66 ± 0.32 2-h exposure: 6.05 ± 0.94 4-h exposure: -* 30 min post-exposure: -* At 25°C 0-h exposure: 0.73 ± 0.11 2-h exposure: 5.30 ± 0.35 4-h exposure: 6.19 ± 1.05 30 min post-exposure: 4.16 ± 0.57 At 30°C 0-h exposure: 0.13 ± 0.04 2-h exposure: 4.89 ± 0.72 4-h exposure: 6.21 ± 0.076 30 min post-exposure: 3.00 ± 0.41 *reading not reported as they may be inaccurate due to technical difficulties	[23]

Table 4. Dermal penetration/percutaneous absorption and ADME studies on Toluene

Parameter Measured	Test System or Species	No./Group	Vehicle/Dose	Protocol	Results	References
ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION						
ORAL						
Animal						
Distribution	Male brown Norway rats (4, 12, or 24 mo.old)	6 - 12/group	corn oil; 0, 0.3, 0.65, and 1 g/kg	Animals of different ages treated with test substance via gavage and killed after 45 min or 4 h post-dose; pharmacokinetic parameters including blood and brain Toluene concentrations measured	Brain Toluene concentration was significantly elevated in 24 mo old rats at 4 h after dosing with either 0.3 or 1 g/kg (concentrations were approximately 50% higher in 24-mo-old rats versus 4-mo-old rats). Blood Toluene concentrations were unaffected by age. In animals treated with 1 g/kg, 45 min post-dose, the maximum amount of Toluene in the blood and brain were approximately 35 and 90 mg/l in, respectively in 24 mo old rats (similar results observed in 12 mo old rats). Four h post-dose, in animals treated with 1 g/kg, the maximum amount of Toluene in the blood and brain were approximately 40 (in 4 mo rats) and 80 mg/ml (in 24 mo old rats).	[26]
INHALATION						
Animal						
Absorption and Excretion	Male brown Norway rats	8 - 12/group	5, 50, 500 ppm	Animals exposed to Toluene via inhalation in 200 l glass/stainless steel inhalation chambers; exposure duration of 6 h/d, 5 d/wk for 4 wk; blood was taken on days 1, 5, 10, and 20 and urine samples were taken 3 days before the study, and study days 1, 5, 10, and 20	Mean blood concentrations on day 1 of animals treated with 5, 50, and 500 ppm Toluene were approximately 0.01, 0.33, and 11.84 µg/g, respectively. Mean blood concentrations of Toluene over the 4 collection times were approximately 0.04, 0.35, and 11.62 µg/g, in animals treated with 5, 50 and 500 ppm, respectively. The amount of *o*-cresol excreted in the urine directly correlated to Toluene blood concentrations. The relationship between the amount of *o*-cresol (nmol) excreted and Toluene blood concentrations was described by the equation: ln(*o*-cresol) = 5.204 + 0.553 x ln(Toluene blood concentration) (r = 0.9795).	[27]
Distribution	Pregnant Sprague-Dawley rats	8 - 15/group	8000 or 12,000 ppm	Pregnant rats were exposed to Toluene via inhalation (full-body chamber exposure) for 15, 30, or 45 min/ exposure. Exposures occurred twice a day from GD 8 through GD 20. Immediately after the second exposure on GD 8, GD14, and GD20, blood was drawn. Animals were killed after blood draw on GD 20, and maternal tissue specimens, placenta, amniotic fluid, and fetal brain were collected for evaluation.	Maternal saphenous blood Toluene levels increased in a concentration- and time-dependent manner; the highest mean maternal saphenous blood Toluene concentration (approximately 11 ppm Toluene) was observed in animals treated with 12,000 ppm Toluene on GD14 for 45 min/ exposure. Maternal cerebellum, heart, kidney, and liver appeared to be saturated after 30 min on GD 20, suggesting extensive distribution. Toluene levels increased in fetal brains in a concentration-dependent manner, and in placenta and amniotic fluid in a time-dependent manner. The highest mean concentrations of Toluene in the placenta and fetal brain were approximately 10.5 ppm (in rats treated with 12,000 ppm Toluene; 30 min exposures) and 7.3 ppm (in rats treated with with 8000 ppm Toluene; 45 min exposures). Concentrations of Toluene in amniotic fluid were very low (less than 2.5 ppm).	[28]

Table 4. Dermal penetration/percutaneous absorption and ADME studies on Toluene

Parameter Measured	Test System or Species	No./Group	Vehicle/Dose	Protocol	Results	References
			Human			
Absorption and Excretion	Human	5 subjects	50 ppm	Subjects placed in inhalation chambers (approximately 18.1 m^3) with 4-h exposure to Toluene; concentrations continuously monitored with infrared spectrophotometer every 5 - 7 min; exposure were held at 3 different temperatures: 21, 25, and 30 °C exposures at different temperatures spaced by a at least 1 wk to minimize cumulative exposure; blood, urine, and exhaled air evaluated before, during, and after each exposure	-Mean venous blood amounts of Toluene measured 2 h into exposure -0.374 mg/l at 21°C -0.362 mg/l at 25 °C -0.389 mg/l at 30 °C -Mean exhaled air amounts of Toluene 1.5 h into exposure: -0.036 mg/l at 21°C -0.037 mg/l at 25 °C -0.042 mg/l at 30 °C -Mean amount of Toluene in urine after 4-h exposure: -10.22 µg/l at 21°C -10.23 µg/l at 25 °C -7.57 µg/l at 30 °C -Mean amount of *o*-cresol in urine after 4-h exposure -39.21 µg at 21°C -21.17 µg at 25 °C -26.78 µg at 30 °C Results suggest increased absorption and a decreased elimination of Toluene in the presence of heat	[23]

GD = gestation day; OECD TG = Organisation for Economic Cooperation and Development

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			ORAL		
Peanut Oil	Gravid female Sprague-Dawley rats; 8/group	0 or 1250 mg/kg (equiv. to 3 h inhalation of 8000 ppm)	Dams were dosed by gavage on days 16 – 19 of gestation (the time period for cochlear development) and killed on GD 20. The uterine horns and the ovaries were removed and the number of implantation sites, resorptions, and live and dead fetuses, and number of corpora lutea were determined. Two to three fetuses per litter were collected specifically for the TUNEL assay to determine apoptosis.	All maternal parameters evaluated, including weight gain, liver and kidney weights, placental weight, implantations, resorptions, pre-implantation loss, corpora lutea, and number of live fetuses, were comparable between the test and the control group. No differences were observed microscopically in the liver; however, it was noted that 75% of kidney sections in treated dams had evidence of renal pathology. Fetal weights were lower (not statistically significant) in the test group. The fetal/placental weight ratio was statistically-significantly increased in the test animals. There was no significant difference in the number of fetal external or skeletal malformations between the treatment and the control group. However, fetuses of the treatment group had an increased frequency and severity of enlarged renal pelvises. A pattern of accelerated development in the upper mid-turn in the treated fetal cochleas was observed; the researchers stated that this accelerated development suggests that Toluene may induce excessive cell death resulting in premature maturation of the cochlea. Four control and 4 treated embryos were appropriate for use in the TUNEL assay. Apoptosis was present in the mid and apical turns of treated but not control samples; results were similar in the other sections of the cochlea,	[32]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			INHALATION		
Air	18 Gravid C3H/HeJ mice; PND 2 and PND 8 male offspring (6/group)	0, 5, or 50 ppm	Dams (GD 14) and male offspring (PND 2 or PND 8) were exposed for 6 h/d for 5 d. Exposures were made in stainless streel glass chambers. On PND 21,expression levels of NMDA receptor subunits, cyclic AMP responsive binding element binding protein, CREB1, CaMKIV, and apoptotic related genes (Bax, Bcl). In addition, mRNAs in the hippocampus estimated, immunohistochemical analyses, general developmental toxicity analysis performed	NMDA receptor subunit NR1, NR2A, and NR2B mRNAs were increased significantly in the hippocampus of PND 21 male mice after exposure to Toluene at 5 or 50 ppm during PND 8-12 ($p < 0.05$). NR2B mRNA was also increased significantly in the hippocampus of PND 21 male mice after exposure 50 ppm exposure during PND 2-6 ($p < 0.05$). CaMKIV mRNAs were up-regulated in PND 21 male mice exposed to 50 ppm Toluene during PND 2-6 and PND 8-12, but not during GD 14-18 ($p < 0.05$). CaMKIV mRNA was also up-regulated in the hippocampus of PND 21 male mice exposed to 5 ppm during PND 8-12, but not during PND 2-6 or GD 14-18 ($p < 0.05$). Similar patterns of up-regulation of CREB1 mRNAs were observed in the hippocampus of PND 21 male mice. Almost all memory function-related gene mRNAs and pro-apoptotic and anti-apoptotic ratio increased significantly in mice exposed to 5 or 50 ppm Toluene on PND 8-12. Mice exposed on GD 14-18 showed no significant change. Increased active caspase-3 immunoreactive cells were found in hippocampal C1 area of male mice exposed to 5 ppm Toluene on PND 8-12. Body weight was significantly reduced in 5 ppm Toluene-exposed mice compared to controls ($p < 0.05$). Relative organ weights of the brain, thymus, liver, lung, and testis were not significantly different between groups	[33]
Air	Sprague-Dawley rats (15/sex/dose)	0, 600, or 2000 ppm	Rats were exposed to Toluene vapor at 600 or 2000 ppm for 6 h/d. Females were exposed from 14 d prior to mating until GD 7. Males were exposed for a total of 90 d (including 60 d pre-mating and during mating. Effects on fertility were evaluated.	No abnormalities were observed in mating behavior, fertility, or fetus mortality. The number of dams with dead fetuses was marginally increased in the 2000 ppm group. A significant decrease in epididymides weight and sperm count was observed in males exposed to 2000 ppm compared to controls. The NOAEC for parental males and the F1 generation was determined to be 600 ppm.	[8]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid Wistar rats (number of animals used not stated; prenatal exposure); male Wistar pups (25/group; post-natal exposure); 30-d old Wistar rats (10/group (sex not stated); acute exposure)	0 or 6000 ppm	Acute exposure: 30 d old Wistar rats were exposed to 6000 ppm or air via inhalation (30 min exposure; static chamber exposure) Prenatal exposure: Pregnant Wistar rats exposed to Toluene (6000 ppm) or air from GD 8-20 (30 min exposures, 2x/d, static chamber exposure); number of animals used not stated Postnatal exposure: Male pups (from pregnant Wistar rats exposed during prenatal exposure; 25/group) weaned on PND 21 and re-exposed to Toluene (6000 ppm) or air via inhalation, 2x/d, from PND 22-30; with this design 4 treatment groups were established: -prenatal air + postnatal air (A/A) -prenatal air + postnatal Toluene (A/T) -prenatal Toluene + postnatal air (T/A) -prenatal Toluene + postnatal Toluene (T/T) Evaluations performed: behavioral (anxiety (burying behavior), nociception (hot-plate test), long-term and short-term memory (step-through inhibitory avoidance task and object recognition test) and locomotor activity); all evaluations occurred after last exposure	Acute Toluene exposure significantly decreased burying behavior compared to controls. Chronic exposure to Toluene during adolescence, alone (A/T), or in combination with prenatal Toluene treatment (T/T) also decreased burying behavior. The T/T group received the highest number of electrical shocks in burying behavior test. The acute Toluene-treated group also produced a significant increase of shocks compared to the control group. Anti-nociception observed in the acute exposure Toluene group, and in the A/T and T/T groups during the hot-plate test. All Toluene treatments results in impaired short-term memory in the object recognition test; however, only post-natal exposure impaired long-term memory in the passive-avoidance test. Acute exposure to Toluene resulted in significant increase in locomotion compared to control rats. Groups A/T and T/T displayed significantly augmented locomotor activity	[34]
Air	Gravid Swiss-Webster mice; 14 test and 13 controls	0 or 8000 ppm	Dams were exposed for 30 min twice daily via inhalation on GD 7 until parturition (GD 19). Exposures were made in sealed 29-l cylindrical glass jars with acrylic lids equipped with injection ports; Toluene was injected onto filter paper under the lid and volatilized via a fan. Pups were examined for morphological anomalies on PND 1, and litter observations were made. (Litters of 4 pups or less were not used.) Maternal and fetal parameters were examined throughout lactation until weaning (PND 21). After weaning, a maximum of 4 pups from each group were placed in each cage and left undisturbed until PND 42; at that time, they were placed in individual cages for 1 wk prior to determining food consumption, and then the pups were weighed every week until PND 90. Dams were killed at the end of lactation, and blood was collected for measurement of serum corticosterone and prolactin levels. RNA was extracted from the hypothalamic PVN.	Maternal body weight gains decreased throughout dosing; at GD 19, body weight gain was 16.7% less in test dams as compared to controls. Food intake was not affected by dosing. The number of live litters was comparable between groups, and there were no statistically significant differences among groups in gestational length, number of live pups, or sex ratio on PND 1. Neonatal death was significantly increased in the test group compared to controls. As compared to controls, pups of the test group had statistically significantly lower body weight on PND 21, but not on the other days of lactation. No effect on body weights were observed after weaning. Dosing did not have a significant effect on serum corticosterone, thyrotropin releasing-hormone mRNA expression, or prolactin levels of dams.	[35]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid Sprague-Dawley rats; 15 low-dose and controls; 16 high-dose	0, 500, or 1500 ppm	Dams were exposed for 6 h/d using a 200 l glass/stainless-steel inhalation chambers on days 6- 20 of gestation. The dams were killed on day 21 of gestation, the uterus was removed, and various maternal and fetal parameters were assessed.	Maternal weight gain and corrected weight gain were statistically significantly decreased in test animals compared to controls. Fetal body weights were statistically significantly decreased in the 1500 ppm group as compared to controls. No adverse effects on the average number of implantations and of live fetuses or the incidence of non-live implants and resorptions were observed. There was no significant change in the occurrence of any individual external, visceral and skeletal variations. The total number with fetal variations was statistically significantly decreased in both exposure groups; this was attributed to biological variations and was not considered toxicologically significant.	[36]
Air	Gravid Sprague-Dawley rats; 12/group	0, 500, 1000, 2000, 3500 and 5000 ppm	In a range-finding study, dams were exposed via inhalation (whole-body) for 6 h/d on days 6 – 15 of gestation. All animals were observed daily for toxicological effects and mortality and killed on GD 20. A Caesarean section was performed, and all fetuses were examined and weighed.	Dose-responsive maternal and developmental toxicity at 2000 ppm and greater, including decreases in maternal and fetal body weights and post-implantation loss, was observed. Narcosis was observed in animals at 3500 and 5000 ppm. One 5000 ppm female died of unknown cause following the first exposure on GD 6.	[37]
Air	Gravid Sprague-Dawley rats; 25/group	0, 250, 750, 1500 and 3000 ppm	Dams were exposed via whole-body inhalation for 6 h/d on days 6 – 15 of gestation and were observed twice daily (before and after exposure) for signs of toxicity, changes in general appearance and mortality. A Caesarean section was performed on all animals on day 20 of gestation, and various maternal and fetal parameters were assessed.	From dose initiation until study termination, clinical signs of toxicity included ataxia and hyper-responsivity in dams of the 1500 dose group and ataxia, hyper-responsivity, increased water intake, and decreased food consumption in dams of the 3000 ppm dose group. Decreased maternal body weight gain was observed during the exposure period only for dams of the 1500 ppm group and during exposure until GD 20 for dams of the 3000 ppm group. No adverse effects on implantation, number and viability of fetuses, or fetal sex distribution were observed. Compared to controls, mean fetal weight was statistically significantly reduced in the 250, 1500, and 3000 ppm groups; the researchers stated that extensive statistical analysis indicated there was no toxicologically significant dose-related effect on fetal body weight at or below 750 ppm. Mean litter weight was decreased in the 3000 ppm group. Instances of reduced or unossified skeletal elements occurred in the 1500 and 3000 ppm groups. Low incidences (≤ 2.5%) of various malformations occurred in the 250, 1500, and 3000 ppm groups; since there was no increase in the incidence of specific or total malformations with increased exposure, these were not considered test article-related. The maternal toxicity NOAEL was 750 ppm with a defined maternal and developmental toxicity LOAEL of 1500 ppm.	[37]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid female Mol:WIST; 16/group	0 or 1800 ppm	Dams were exposed via whole-body inhalation for 6 h/d on days 7 – 20 of gestation and were observed daily following exposure. Maternal and fetal weights and fetal parameters were assessed following delivery, and developmental and neurobehavioral effects were studied during lactation and until study termination at wk 14. Litters were not culled, but litters with less than 4 pups were not included in the post-natal evaluations. On PND 10, 1 male pup/litter with a body weight just below males with median body weight was removed from litters with at least 6 pups and used for other studies. After weaning on PND 21, 1 male and 1 female from each litter having the median body weight were kept for further behavioral testing. Females that had not given birth and all the dams were killed on PND 8 and 31, respectively, and examined for macroscopical changes and the number of uterine implantation sites.	No clinical signs of toxicity were observed in the dams during the exposure period. The number of dams not pregnant, neonatal deaths, or the sex distribution were comparable among the test and control groups. No pups with external malformations were observed in any of the groups. The litter size and the number of implantations were higher and the incidence of post-implantation loss lower in the exposed group compared to the control group, but the differences were not statistically significant. Body weights of pups from the test group were statistically significantly lower than controls until PND 10. Neurobehavioral evaluation of the pups revealed no effects on motor function (rotarod), activity level (open field), acoustic startle, and prepulse inhibition. Measurements of hearing function using auditory brain stem response revealed small effects in male pups for the test group. Performance in a Morris water maze during initial learning gave some indications of impaired cognitive functions which was confirmed during further testing, especially in reversal and new learning. Effects on cognitive functions seemed most marked in female offspring.	[38]
Air	Gravid female Sprague-Dawley rats; 21 test animals/group, 16 controls	0, 8000, or 12,000 ppm	Dams were exposed for 15 min twice daily via inhalation on days 8 – 20 of gestation; daily exposures were 2 h apart. Exposures were made in sealed 36-l cylindrical glass jars with acrylic lids equipped with injection ports; Toluene was injected onto filter paper under the lid and volatilized via a fan. After delivery, litters were examined and culled to 10 on PND 1, and pups were examined daily until weaning. Litters were weaned on PND 21, at which time the dams were killed and the uterine horns excised. The teratogenic impact on prenatal and early neonatal growth, perinatal outcome and neurobehavioral development of offspring was assessed.	Three rats in each dose group were not gravid; all pregnant rats gave birth to live litters. One dam of the high-dose group died on GD 17. Six dams (1 control, 3 from the 8000 ppm group, and 2 from 12,000 ppm group) had litters with ≤ 6 pups; these litters were used for reporting birth outcome data, but not used in analyzing neonatal development. There were no statistically significant differences between treated groups and controls in maternal weights or weight gains during gestation, percent live births, mean number of implant sites litter size, or sex ratio on PND 1. Five and 9 of the dams of the 8000 and 12,000 ppm groups, respectively, had malformed, “runted,” or dead pups, as compared to 2 dams in the control group. The differences between the control and the 12,000 ppm groups were statistically significant for the percent of affected litters (12.50% vs. 52.94%, respectively), as well as the percent of affected pups/litter (0.55% vs. 2.79%, respectively). Marginal differences ($p = 0.051$) were observed for this parameter between the 8000 and the 12,000 ppm groups in the number of affected pups/litter (0.294 vs. 0.778, respectively) and the percent affected pups/litter (2.12% vs. 2.79%, respectively). Pup body weights in the 12,000 ppm group were statistically significantly less than those in the control and the 8000 ppm groups on PND 1; body weights of pups from the treated groups recovered by PND 16, and there were no statistically significant differences by PND 21. No pups from the 0 or 8000 ppm and 3 pups of the 12,000 group died during the study. A significant effect was observed for test animals in the negative geotaxis test. There was no significant main effect of exposure on surface righting time, forelimb grip strength, or hanging strength.	[39]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid Sprague-Dawley rats; 18 low-dose; 21 high-dose; 17 controls	0, 8000, or 12,000 ppm	Dosing as above, with 15 min exposure twice daily. The pups were weighed and examined on PND 1 for obvious physical abnormalities. Pups with any noticeable physical abnormalities or deemed "runts" were weighed and culled. All litters were then culled pseudo-randomly to 10 (5 males and 5 females, when possible) from the remaining pups. Two pups from each litter were selected on PND 28; spatial learning and memory were assessed in a Morris water maze.	In the 12,000 ppm group, maternal weight gain on GD 20 and overall litter weight on PND 1 were statistically significantly less than controls. There was no effect on litter size. There were no significant differences in litter mean body weighs at PND 28. There was no difference between test and control groups in acquisition initially in the Morris water maze. However, pups of the 12,000 ppm group displayed performance deficits during a probe trial and in reversal learning on PN44.	[40]
Air	Gravid female Sprague-Dawley rats; 10/group	0, 8000, 12,000, or 16,000 ppm	Dosing as above, with 15 min exposure twice daily. Two studies were performed on offspring– one examining metabolic rate and body composition post-weaning and the other examining weight gain in response to consumption of 3 different diets.	Litter weights showed a statistically significant linear decrease as a function of dose. There were no significant differences among the groups at PND 30.	[41]
	offspring (males and females); 12 test offspring/group, 11 controls		Post-weaning offspring (~30 d old) were placed in metabolic cages for 3 h. The rates of oxygen consumption and carbon dioxide output were measured. The offspring were killed after metabolic testing and fat analysis was performed.	Pups of the 16,000 ppm had statistically lower energy expenditures than control pups and those of the 12,000 ppm group; pups of the 8000 ppm group had lower energy expenditures than the controls, but the difference was not statistically significant. Respiratory quotients were higher in the test groups, but the differences were not statistically significant. Pups in the 8000 and 16,000 ppm groups had significantly greater percentage of body fat as well as total body fat than the other groups.	
	3 male and 3 female offspring/group		Starting on PND 72, rats were exposed sequentially to 3 different diets (regular chow for 16 d, purified diet for 10 d, purified high-fat diet for 18 d).	There were trends for a main effect of dose on food intake during chow and during high-fat diet consumption, with rats in the 12,000 ppm group consuming more than the 0 ppm group on both diets.	
Air	Gravid female Sprague-Dawley rats; 23 low-dose and 21/group for the control and high-dose	0, 8000, or 12,000 ppm	Dosing of dams as above, except exposure was for 30 min twice daily. Pups were assessed from PND 4 to PND 21, and teratogenic impact on prenatal and early neonatal growth, perinatal outcome, and pre-weaning neurobehavioral development of offspring was assessed.	Maternal weight gains were statistically significantly decreased in both test groups as compared to controls on GD 20. There were no significant differences in the mean overall weight of pups among the litters on PND 1; however, on PND 21, mean overall weight of pups of the treated groups were lower than controls. In the 12,000 ppm group, 20 pups in 15 different litters had malformations, including missing digits, missing limbs, and missing eyes (unilateral anophthalmia). The combined cumulative frequency of malformed, "runted," or dead pups in the control, 8000, and 12,000 ppm groups was 15.8, 33.33, and 63.16% of litters, respectively; the rate of incidence was statistically significantly increased in the 12,000 ppm group compared to controls, but the 8000 ppm group was not significantly different from either of these groups. No significant delays were observed in reaching maturational milestones.	[42]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid female Sprague-Dawley rats; 8/group	0, 8000, 12,000, or 16,000 ppm	Dosing as above, with 30 min exposure twice daily. Gross dysmorphology, skeletal defects, and soft tissue anomalies were evaluated in fetal rats.	Aside from a sedative effect and immobility initially following exposure, no abnormal behavior was observed in dams of the treated groups. Maternal weight gains were decreased in all test groups as compared to controls on GD 20. Statistically significant differences were observed in many parameters in treated rats when compared to controls. Reduced growth, including decreases in placental weight, fetal weight, and crown-rump length, was observed in all test groups. A significant increase in gross physical malformations, such as short or missing digits (most common anomaly observed) and missing limbs, was observed in all test groups. There was a significant increase in skeletal defects, including misshapen scapula, missing and supernumerary vertebrae and ribs, and fused digits, in all test groups. Ossification of the extremities was significantly reduced at all dose levels. An increase in soft tissue anomalies was also observed at all dose levels, and there was a dose-dependent increase in the number of anomalies, including cardiac defects (most common soft tissue anomaly), microcardia or cardiomegaly, microgastria or gastromegaly, caudally displaced abdominal organs, displaced or ectopic testes, and hypoplastic or distended bladder were observed in all test groups; 100% of the litters of the 16,000 ppm group had soft tissue anomalies.	[43]
EFFECT ON HORMONE LEVELS					
Air	female Wistar rats/10 group	0, 2000, 4000, or 8000 ppm	Animals were exposed via inhalation, 30 min/d, for 28 d. (Details of exposure methodology were not provided.) The animals were killed at study termination, blood samples were obtained, and the ovaries were removed and weighed. A portion of the ovaries were prepared for microscopic examination, immunostaining, and TUNEL assay, and a second portion was collected and stabilized in RNA later and stored for molecular assays. Levels of progesterone, estradiol, testosterone, and IGF-1 were measured using ELISA.	Statistically significant changes as compared to controls included increased body weights in the 2000 ppm group, decreased ovarian weights in the 4000 and 8000 ppm dose groups, a decreased number of growing follicles in the 8000 ppm dose group, and an increase in the number of abnormal ovarian follicles in all treated groups (the highest number was found in the 4000 ppm group). A statistically significant increase was observed in progesterone levels in the 4000 and 8000 ppm dose groups and in testosterone levels of all dose groups; no effect on estradiol was noted. IGF-1 was significantly decreased in the high-dose group. Compared to controls, mRNA levels of the *Insl3, Cyp19, ccnd1, Igf-1, Actb, GDF-9* and *Atg5* genes were statistically significantly decreased in all dose groups, and the expression of the *Cyp17a, Lhr, Esr2* and *Lc3* genes at 4000 and 8000 ppm and the *Esr1* gene in the 2000 ppm-group were statistically significantly increased. Expression of the GDF protein was significantly decreased, and of LC3 was significantly increased, in the ovaries of rats in all dose groups when compared to controls. A dose-dependent increase in apoptosis in ovarian tissue was observed; in test animals, this increase was statistically significantly different in the 4000 and 8000 ppm groups.	[44]

Table 5. Developmental and reproductive toxicity studies of Toluene

Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Air	Gravid female Long-Wistar rats/4 group	0.09, 0.9, or 9 ppm	Animals were exposed via nasal inhalation for 90 min/d on days 14.5 – 18.5 of gestation to determine the effect of Toluene on the synthesis and secretion of testosterone in fetal rats. Plasma testosterone levels (3–5 fetuses/sex/litter) were measured using ELISA. mRNA levels of steroidogenic enzymes in testicular tissues from control and 0.9 ppm fetuses (3 litters/group with 2–5 male fetuses/litter) were measured using real-time PCR methods.	No statistically significant effects on maternal body weight gain or total number of fetuses were observed. Fetal plasma testosterone concentrations were significantly reduced in males of the 0.9 and 9 ppm, but not the 0.09 ppm, groups. mRNA levels of 3β-HSD were significantly reduced after exposure to 0.9 ppm; mRNA levels of P450scc, P450c17, 17β-HSD3, and Insl3were not significantly altered in the 0.9 ppm group. The 3β-HSD-immunoreactive area in the interstitial region of fetal testes was significantly reduced in the 0.9 and 9 ppm groups, but not in the 0.09 ppm group.	[45]
EFFECT ON IMMUNOLOGICAL BIOMARKERS					
Air	Gravid female BALB/c mice; 6/group	0, 5, or 50 ppm	Gravid females were exposed via whole-body inhalation for 6 h/d on GD 14 to parturition (GD 19), with or without aerosolized PGN (200 µg/10 ml; GD 14, 17, and 19 via nebulizer). Pups of the Toluene- and PGN-exposed groups were dosed with PGN (100 µg; PND 7, 10, 13, 16, and 19, i.p.). Dams were killed the day following the final inhalation exposure, and spleen, lung, and blood samples were collected. Plasma total IgE, IgG1, and IgG2 antibodies and cytokine levels win the lung were measuring using ELISA, and splenic mRNA expression levels were measured using real-time PCR. Th1/Th2 balance was determined at 3 wk via ELISA and RT-PCR methods.	There were no statistically significant differences in body weights of pups from dams exposed to Toluene, with or without PGN, as compared to controls. Spleen weights at PND 21 were not affected by Toluene alone, but were increased significantly with PGN (all groups). Total plasma IgG2a levels were statistically significantly increased in the 50 ppm (alone) group, as compared to both the 0 and 5 ppm groups. Total IgE and IgG1 were not affected by exposure to Toluene alone; PGN did have some effects. Splenic expression of transcription factors T-bet, GATA-3, and Foxp3 mRNAs was statistically significantly suppressed in pups from groups given 5 or 50 ppm Toluene without, but not with, PGN. Splenic IL-4, IL-12, and IFN-γ mRNAs were not significantly affected by Toluene, with or without PGN; however, IL-12 showed a dose-dependent decrease in all groups. Cytokines in the lungs were not affected by dosing.	[46]
Air	Experiment 1: gravid female C3H/HeN mice; 2/group male pups (PND 2); 6/group male pups (PND 8); 6/group	0, 5, or 50 ppm	Gravid females were exposed via whole-body inhalation for 6 h/d on days 14 -18 of gestation; 3 male pups/dam (GD 14 - 18 group) were used for assessment at PND 21. Each of the groups of male pups were exposed for 5 consecutive days (i.e., PND 2 – 6 or PND 8 – 12) and assessed on PND 21. Immunological biomarkers in the blood and spleen of the pups were examined by ELISA, real-time RT-PCR, flow cytometry, and histological analysis.	On PND 21, body weight and weight of the thymus, left lung, and spleen were similar between the GD 14 - 18 and PND 2 – 6 exposure groups and control groups, but the weights of the thymus and spleen were decreased in the PND 8 – 12, 5 ppm group. Plasma IgE levels were similar for all test and control groups. Plasma total IgG1 levels were markedly reduced in all groups of pups exposed to 5 ppm during all developmental stages, as well as the GD 14 -18 and PND 8 -12, 50 ppm groups. IgG2a levels were not changed in the pups exposed during gestation only, were statistically significantly decreased in the PND 2 – 6, 5 ppm group, and were significantly increased in the PND 8 – 12, 5 ppm group. Splenic T-lymphocyte subsets were suppressed in the PND 8 – 12, 50 ppm group, and IL-12 mRNA, T-bet mRNA, and Foxp3 were suppressed in all PND 2 – 6 and 8 – 12 test groups. There was marked activation of extramedullary hematopoiesis in the spleen in the PND 8 – 12, 50 ppm group.	[47]
Air	Experiment 2: male offspring of 10 untreated C3H/HeN mice; 6/group	0 or 50 ppm	Pups were exposed for 6 h/d on PND 8 – 12 and killed on PND 42. The effects on plasma antibody levels, splenic lymphocyte subsets, and splenic expression of cytokines and transcription factors were evaluated.	As compared to controls, statistically significant decreases were observed in plasma total IgG2a, splenic CD19+ B lymphocyte subset, CD4+ lymphocyte subset, and T-bet mRNA. The CD3+ lymphocyte subset was increased.	

Abbreviations: 3β-HSD = 3β-hydroxysteroid dehydrogenase; 17β-HSD3 =17β-hydroxysteroid dehydrogenase; CaMKIV = calcium/calmodulin-dependent protein kinase IV; CREB1 = cyclic adenosine monophosphate responsive element binding protein 1; ELISA = enzyme-linked immunosorbent assay; GD = gestation day; GDF9 = growth differentiation factor-9; Ig = immunoglobulin; IGF-1 = insulin-like growth factor 1; IL = interleukin; Insl3 = insulin-like 3; IFN = interferon; LC3 = light chain 3; LOAEL = lowest-observed-adverse-effect-level; NMDA = N-methyl-D-aspartate; NOAEC = no-observed-adverse-effect-concentration; NOAEL = no-observed-adverse-effect-level; cytochrome P450c17 = P450 17α-hydroxylase/c17-20 lyase; P450scc = cytochrome P450 cholesterol side-chain cleavage; PCR = polymerase chain reaction; PGN = peptidoglycan; PND = post-natal day; PVN = paraventricular nucleus; RT-PCR = reverse transcription–polymerase chain reaction; TH = helper T-cell; TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling

Table 6. Genotoxicity studies on Toluene

Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VITRO		
DMSO	0.5, 2.5, 5, 25, and 50 µl/plate	*S. typhimurium* strains TA97, TA98, TA100, and TA102	-Ames assay; performed with and without metabolic activation -OECD TG 471 -Negative controls: blank and DMSO; use of positive control not stated	Genotoxic; positive results observed on the TA97, TA98, and TA102 strains without S9; after adding S9, Toluene caused further mutagenicity on the TA 100 strain; controls gave expected results; positive results observed at concentrations as low as 2.5 µl/plate	[48]
DMSO	10, 33.3, 100, 333.3, and 1000 µg/plate	*S. typhimurium* strain TA 98, TA100, TA 1535, and TA 1537	-Ames assay performed with and without metabolic activation -Negative control: DMSO -Positive control: sodium azide, 2-aminoanthracene, 2-aminoacridine, 4-nitro-*o*-phenylenediamine	Non-genotoxic; controls gave expected results	[51]
Corn oil	100 – 1,000,000 ppm	Human skin disks	-Comet assay -Skin disks obtained from 3 different subjects exposed to Toluene vapor (8 h incubation) -Control skin disks were exposed to the vehicle only	Genotoxic at concentrations of 10,000 and higher; dose-dependent responses observed; controls gave expected results	[49]
Nitrogen	0.25 ppmv	Human lung epithelial carcinoma cell line A549	-Modified alkaline comet assay -Cells incubated with test substance (gaseous Toluene) for 3 and 24 h -Comet evaluation made immediately after exposure, and after 3 and 24 h -Negative control exposures with synthetic air -Positive control: hydrogen peroxide	Genotoxic; DNA damage observed during first 3 h of exposure; effect repaired within 24 h; controls gave expected results	[50]
DMSO	Without metabolic activation (trial 1): 31.25, 62.5, 125, 250, and 500 µg/ml Without metabolic activation (trial 2): 50, 100, 200, and 300 µg/ml Without metabolic activation (trial 3): 150, 175, 200, 225, 250, and 75 µg/ml With metabolic activation (trial 4): 6.25, 12.5, 25, 50, 100, 200 µg/ml With metabolic activation (trial 5): 125, 150, 175, 200, 225, and 250 µg/ml	Mouse lymphoma L5178Y TK+/- cells	-Mammalian cell mutagenicity assay -Cells incubated with test substance for 4 h -3 trials performed without metabolic activation, 2 trials performed with metabolic activation -Negative control: DMSO -Positive control: methyl methane sulfonate	Genotoxic; genotoxicity observed at high concentrations with and without metabolic activation; controls gave expected results	[51]
DMSO	50, 160, 500, and 1600 µg/ml	Chinese hamster ovary cells	-Chromosomal aberration assay performed with and without metabolic activation -Negative control: DMSO -Positive control: mitomycin C	Non-genotoxic; controls gave expected results	[51]
DMSO	50, 160, 500, 1600, and 5000 µg/ml	Chinese hamster ovary cells	-SCE assay performed with and without metabolic activation -Negative control: DMSO -Positive control: mitomycin C	Non-genotoxic; controls gave expected results	[51]

Table 6. Genotoxicity studies on Toluene

Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VIVO		
NR	1.0, 10.0, 50.0, and 100.0 mM	Third instar larvae of wild type *Drosophila melanogaster* (Oregon R^+) (15/group)	-Larvae exposed to test substance via diet for 12, 24, and 48 h and evaluated for genotoxicity; DNA damage evaluated in gut cells of larvae -Alkaline and neutral Comet assay -Positive control: ethyl methanesulfonate and γ-irradiation -Negative control: extract from larvae exposed to control food	Genotoxic; significant increase in DNA migration after 24 and 48 h observed, when compared to control, in both alkaline and neutral Comet assays ($p < 0.01$); controls gave expected results	[52]
Corn oil	500, 1000, or 2000 mg/kg	B6C3F1 mice (sex and number of animals not stated)	-Micronucleus assay; method of administration not stated -Negative control: corn oil -Positive control: dimethylbenzanthracene	Non-genotoxic; controls gave expected results	[51]
NR	5 or 15 g/kg	Male Balb/c mice (9/group)	-Comet assay -Mice intraperitoneally injected with Toluene and killed after 2 h -DNA damage evaluated in different regions of brain, hepatocytes, and leukocytes -Negative control: corn oil -Use of positive control not stated	Genotoxic; acute exposure to Toluene induced significant levels of DNA damage in the hippocampus, cerebellum, and cortex, in a dose-dependent manner, compared to control ($p < 0.05$); no significant level of DNA damage in hepatocytes of leukocytes; controls gave expected results	[25]
NR	25 ppm	Male Balb/c mice (6/group)	-Comet assay -Animals nose-exposed to test substance or filtered air (negative control) for 4 wk (6 h/d, 5 d/wk) -One day following final Toluene inhalation, mice killed for collection of blood and brain samples -DNA damage evaluated in different regions of brain and leukocytes -Use of positive control not stated	Genotoxic; significant levels of DNA damage in the hippocampus, cerebellum, and cortex, compared to controls ($p < 0.05$); no significant DNA damage in leukocytes; controls gave expected results	[25]
NR	100 ppm	Male CD-1 mice (10/group)	-Bone marrow micronucleus assay -Animals exposed via inhalation (whole-body inhalation chambers) for 6 h/d for 15 d for a total of 8 exposures (exposures occurred on study days 1, 2, 5, 6, 7, 9, 12, 13, and 15) -Control animals exposed to air only -Use of positive control not stated -Animals killed 18 h after last exposure and bone marrow cells collected	Non-genotoxic; Percent of erythrocyte micronuclei and polychromatic erythrocytes not statistically-significantly different than control group; controls gave expected results	[53]

DMSO = dimethyl sulfoxide; NR = not reported; OECD = Organisation for Economic Cooperation and Development; SCE = sister chromatid exchange; TG = Test Guidelines

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
	ANIMAL		
	Oral		
White matter changes, immunohistochemical parameters	-F344/N rats (6/sex/group) exposed to Toluene in olive oil via oral gavage at 0, 500, or 800 mg/kg, 4 d/wk, for 104 wk -Animals killed within days of final Toluene exposure -Brains of animals exposed via inhalation were sliced at 3 coronal levels (frontal horn of lateral ventricle, anterior hippocampus, and mid cerebellum) and evaluated -In brains of animals exposed orally, immunohistochemical staining was used to detect reactive astroglial and microglial changes, neuron populations, and cytochrome p450 upregulation	-No abnormalities in the neocortex, hippocampus, brainstem, or cerebellum observed -No white-matter abnormalities were observed in orally-treated rats -A mild widespread increase in reactive microglia was detected in female rats given Toluene via gavage at 800 mg/kg; however, no significant differences were detected in neurons or astrocytes -No evidence of myelin degeneration in control or treated groups -Immunohistochemical studies did not reveal any major abnormalities in comparison to controls	[71]
	Inhalation		

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
Anxiety/withdrawal	-Male Swiss Webster mice (n = 260; number per group not stated) -Animals exposed to either 5000 ppm Toluene vapor or air for 30 min or 24 h -Mice tested in a battery of 4 behavioral tasks reflective of anxiety either immediately after or 24 or 72 h after exposure	-Mice exposed to Toluene for 30 min showed decreases in anxiety-like behaviors, whereas mice abstinent from Toluene for 24 h after a 24-h exposure displayed increases in anxiety-like behaviors -Anxiety-like behaviors not observed 72 h post-exposure	[17]
HPA and HPT axes	-Male adolescent Wistar rats (9/group) -Animals exposed to Toluene (4000 or 8000 ppm) via inhalation (30 min; 2x/d; 5 d/wk; 2 wk); control animals treated with air -HPA and HTP axes function evaluated via measurement of CRF release, CRF mRNA levels, ACTH, and corticosterone serum levels -Bloods samples collected 30 min after last exposure; brain tissues collected	-Both concentrations of Toluene significantly reduced CRF mRNA transcription in the PVN ($p < 0.001$), compared to controls -Toluene produced a concentration-dependent increase in ACTH at both concentrations ($p < 0.001$) and an increase in serum corticosterone levels at 8000 ppm ($p < 0.001$), compared to controls -Toluene significantly decreased pro-TRH mRNA levels in the hypothalamic PVN at both concentrations ($p < 0.05$) -Non-statistically significant decrease in serum TSH levels observed after exposure to 8000 ppm Toluene -8000 ppm significantly increased T_3 serum concentrations compared to control ($p = 0.001$); T_4 levels similar in control and treated groups	[35]
White matter changes, immunohistochemical parameters	-F344/N rats and B6C3F1 mice exposed to 0 or 1200 ppm Toluene in an inhalation chamber for 6.5 h/d, 5 d/wk, for 60 wk (n = 10/sex/group) or 103 wk (n = 50/sex/group) -Animals killed within days of final Toluene exposure -Brains of animals exposed via inhalation were sliced at 3 coronal levels (frontal horn of lateral ventricle, anterior hippocampus, and mid cerebellum) and evaluated -In brains of animals exposed orally, immunohistochemical staining was used to detect reactive astroglial and microglial changes, neuron populations, and cytochrome p450 upregulation	-No abnormalities in the neocortex, hippocampus, brainstem, or cerebellum observed -Focal artefactual vacuolation was evident in the white matter of approximately 10% of control and treated mice; the white-matter of exposed rats and mice was otherwise normally arranged with normal cell density -No evidence of myelin degeneration in control or treated groups -Immunohistochemical studies did not reveal any major abnormalities in comparison to controls	[71]
Learning and memory function	-Male C3H/HeJmice (number of animals not stated) exposed to 5 ppm Toluene via inhalation during PND 8 - 12, 2 control groups (0 ppm group and day-of room control group) -All exposures occurred for 6 h/d -On PND 49, animals allowed to swim freely during four 60-s trials to adapt to water; the next day, mice subjected to water maze task performed on 7 consecutive days (6 d for acquisition/training and 1 d for reversal phase to test memory retention -On 7^{th} day, after completion of reversal phase, animals subjected to a visible platform test to examine visual acuity and sensorimotor activity; escape latency evaluated	-Poor spatial and learning performance observed in treated mice -Significant prolongation of mean escape latency in the control (0 ppm; $p < 0.01$) group and the Toluene-exposed group ($p < 0.05$) on 5^{th} and 6^{th} day compared with corresponding room control -On 7^{th} day, when reversal phase was performed, a significant prolongation of the mean escape latency in the Toluene-exposed group was observed compared to room control group ($p < 0.05$)	[33]
Locomotor activity	-Adolescent (PND 28) and adult (PND 90) male Sprague-Dawley rats (n = 8/group) exposed to Toluene (0, 8000, or 16,000 ppm) via inhalation over 12 d (different exposure types per day: 2 15-min exposures separated by 120 min intervals; 2 15-min exposures separated by 30 min interval; 6 5-min exposures with 30-min intervals separating exposures) -Locomotor activity quantified during Toluene exposures and for 30 min following completion of the final daily Toluene exposure	-Compared to adults, adolescents displayed greater locomotor activity on the first day and generally greater increases in activity over days than adults during Toluene exposure -Adults displayed greater locomotor activity compared to adolescents in the recovery period following exposure -Age group differences were clearest following the pattern of brief (5-min) repeated binge exposures	[72]
Behavioral effects, motor function, memory, and visual function	-Male Long-Evans rats (20/group) -Toluene vapor inhalation (0, 10, 100, or 1000 ppm; 6 h/d, 5 d/wk, 4 wk) -10 rats from each group selected for behavioral assessments (motor activity, anxiety-related behavior, learning and performance of signal detection test) -10 rats from each group used for neurophysiological assessments of visual function	-No significant differences in motor activity, maze performance, lever-press frequencies, or visual discrimination in control and treated groups -Toluene treatment at the highest concentration reduced accuracy of signal detection at the end of training; further analysis of this effect revealed a greater influence of attentional impairment than visual or motor dysfunction	[74]
Behavioral effects, cognitive, and motor function	-Male Long-Evans rats (n = 248 total; number per group not stated) -Toluene vapor inhalation (0, 10, 100, or 1000 ppm; 6 h/d, 5 d/wk, 13 wk) -10 rats from each group evaluated for motor activity, anxiety, visual discrimination, and visual signal detection behavior -10 rats from each group selected for fear conditioning	-No significant differences in motor activity, trace fear conditioning, maze performance, or visual discrimination between control and treated groups -All rats exposed to Toluene acquired the lever-press response later than controls	[60]

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
Behavioral effects, cognitive, and motor function	-Long-Evans rats (6 - 10/group) -Rats exposed to Toluene vapor (5000 ppm) or control conditions for 30 min -Animals subjected to water maze task, swimming/ visible platform tasks (measuring sensory-motor abilities and stamina), and trials evaluating the performance of a well-learned task, reversal learning, and long-term recall	-Immediately after Toluene exposure, rats were initially severely impaired in their swimming ability and in their ability to learn and perform a visible platform task; swimming behavior mostly returned to normal about 20 min after exposure, although cognitive impairments were still evident -Rats without Toluene exposure showed normal spatial recall; Toluene-exposed rats displayed severely impaired reversal learning	[75]
Cognitive function and locomotor activity	-12 male adolescent Wistar rats (PND 35 - 40)/group and 12 adult male Wistar rats/group -Animals treated with 2000 ppm Toluene, 5 min/d, 40 d; control groups (of each age group) treated with air only -Adolescent rats and adult rats either evaluated 24 h last treatment or 90 d after last treatment -Locomotor activity, habituation to environment, object exploration/habituation to object, spatial novelty, and object change evaluated	-Adolescent animals showed recognition memory impairment 24 h after last exposure, which normalized by day 90 post-exposure -Adult animals also showed recognition memory impairment that was still evident 90 d post-exposure -Significant decreases in locomotor activity observed in adults tested both 24 h and 90 d post-exposure, compared to controls ($p < 0.05$) -Habituation indices significantly higher in adolescent animals compared to adult animals at both 24 h and 90 d post-exposure	[79]
Spatial learning and memory	-12 male adolescent Wistar rats (PND 35 - 40)/group and 12 adult male Wistar rats/group -Animals treated with 2000 ppm Toluene, 5 min/d, 40 d; control groups (of each age group) treated with air; groups based on when animals assessed post-treatment -Adolescent rats and adult rats either evaluated 24 h last treatment or 90 d after last treatment -Spatial learning and memory evaluated using water maze test	-Adolescent rats treated with Toluene showed a decrease in time and distance traveled to find hidden platform 24 h after last exposure -Adult rats treated with Toluene showed a decrease in acquisition time and distance traveled 90 d after last exposure	[76]
Intraperitoneal Injection			
NMDA receptors and memory retention	-Female C3H/HeN mice (10/group) -Mice administered Toluene (300 mg/kg) in olive oil via intraperitoneal injection; control mice injected with olive oil only -10 mice treated with Toluene 60 min before test phase (and after training phase) -Novel object recognition test performed -Hippocampus collected 24 h after injection; total RNA isolated from hippocampal samples -NMDA receptor subunit expression (18S, NR1, NR2B) evaluated	-Toluene-injected mice did not prefer novel objects and showed poor discrimination between novel and familiar objects (novel object exploration time was increased significantly in control mice $p < 0.01$, but not in Toluene-treated mice) -Toluene-treated mice showed decreased expression of NMDA receptor subunit NR2B mRNA in the hippocampus ($p < 0.05$); effect not observed for other subunits	[70]
Hippocampal neurogenesis	-Male C57BL/6 mice (n = 240 total; number per group not stated) -Mice given intraperitoneal injection of Toluene in corn oil (500 mg/kg); control mice given corn oil only -Changes in hippocampal neurogenesis evaluated using 2 immunohistochemical markers for neurogenesis: Ki-67 and doublecortin -Depression and memory tasks also evaluated after treatment to assess hippocampal neurogenesis-related behavioral dysfunction	-The number of Ki-67- and doublecortin-positive cells in the dentate gyrus of adult hippocampi declined acutely between 0 and 24 h post-treatment, and increased gradually from 2-8 d post-treatment -Ki-67 and doublecortin immunoreactivity decreased in a dose-dependent manner -Treated mice showed significant depression-like behavior and memory deficits compared to untreated controls	[78]
Locomotor activity, motor coordination, passive avoidance learning	-Male Sprague-Dawley rats (6/group) -Intraperitoneal injection of Toluene in corn oil (250, 500, or 750 mg/kg) -Locomotor activity observed, rotarod and step-through inhibitory avoidance tests performed (Toluene administered either 30 min prior to training, immediately after training, or 30 min prior to memory retention session in step-through inhibitory avoidance test)	-Toluene produced a dose-dependent increase in locomotor activity ($p < 0.01$) -Toluene at doses of 500 and 750 mg/kg produced significant motor incoordination -Toluene administered 30 min prior to training did not affect initial step-through latency during training session, but it dose-dependently reduced the latency to step-through during memory retention test session ($p < 0.001$) -When Toluene was given either 30 min before memory retention test or immediately after training session, no significant effect on the latency to step-through during the test session was observed -Further testing revealed that NMDA receptor blockade and dopamine neurotransmission may be responsible for these effects	[73]

Table 7. Neurotoxicity/behavioral toxicity studies with Toluene

Parameters Studied	Study Details	Results	Reference
Histopathological, immunohistochemical, and biochemical effects	-Male New Zealand white rabbits (10/group) -Toluene-treated group given single dose of 876 mg/kg via intraperitoneal injection; control group left untreated -Blood samples collected 5 h post-administration, and animals euthanized -Brain tissue (prefrontal cortex, hippocampus, hypothalamus, substantia nigra, and entorhinal cortex) evaluated after euthanasia for biochemical parameters (nerve growth factor, tumor necrosis factor-alpha (TNF-α)), dopamine, and glial fibrillary acidic protein levels), histopathological parameters, and immunohistochemical parameters (Bax C3 immunoreactivity)	-Statistically significant increased TNF-α levels in Toluene-treated rats compared to controls -Statistically significant decreased levels of dopamine (secreted from substantia nigra), nerve growth factor (developed from hippocampal neurons), and glial fibrillary acidic protein levels (secreted from astrocyte cells) compared to controls -Areas of focal vacuolar degeneration, gliosis, and perivascular demyelination, many pyknotic cells, and necrosis observed in Toluene-treated animals -Distinct excessive expansions of blood vessels, severe degeneration of cell structure, and dispersed cell borders observed in Toluene-treated animals -Abnormalities of the nuclei structure of oligodendrocyte cells and damage in sequential neurons of hippocampus observed in Toluene-treated animals -Cytoplasm of the cortex cell showed serious immune reactivity in Toluene-treated group (effect not observed in control group)	[77]
HUMAN			
Inhalation			
Cortical excitability, neuroplasticity, and motor learning	-Placebo-controlled, randomized, crossover study -17 healthy subjects -Subjects participated in inhalation sessions of Toluene (single peak of 200 ppm) exposure and one under placebo (clean air) exposure (at least 1 wk in between sessions to avoid interference) -Whole-body exposure in a ventilated 28 m^3 exposure laboratory -Toluene concentration in the air of the chamber was exponentially increased from 0 to 200 ppm over a period of 25 min; after reaching the plateau of 200 ppm, this concentration was kept constant for 10 min; subsequently, the jet nebulizer was turned off and within the next 20 min, Toluene concentration decreased to 0 ppm; approximately 1 h later, a second 200 peak was generated -During both peaks, participants performed light physical exercise -Subjects assessed with different transcranial magnetic stimulation measurements, motor thresholds, short-latency intracortical inhibition and intracortical facilitation, and short-interval afferent inhibition before and after clean air or Toluene exposure -Long-term potentiation-like neuroplasticity induced by anodal transcranial direct stimulation over the motor cortex evaluated, and subjects performed serial reaction time task	-Toluene abolished plasticity induced by anodal transcranial direct stimulation, attenuated intracortical facilitation, and increased inhibition in the short-latency afferent inhibition measure -Cortico-spinal excitability and intracortical inhibition not effected by Toluene exposure -Toluene exposure did not alter performance of the motor learning task (serial reaction time task)	[80]

ACTH = adrenocorticotropin hormone; AMP = adenosine monophosphate; CaMKIV = calcium/calmodulin-dependent protein kinase IV; CREB1 = cyclic adenosine monophosphate responsive element binding protein 1; CRF = corticotropin-releasing-factor; GD = gestation day; HPA = hypothalamus-pituitary-adrenal; HPT = hypothalamus-pituitary-thyroid; NMDA = N-methyl-D-aspartate; PND = post-natal day; PVN = paraventricular nucleus; TNF-α = tumor necrosis factor – alpha; TSH = thyroid-stimulating hormone

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	Reproductive Toxicity		
Study evaluating relationship between reproductive disorders and birth defects in offspring of male painters with exposure to organic solvents	-Random samples of painters and carpenters drawn from workers affiliated with the Dutch Trade Union for Construction Workers -Reproductive outcomes, occupational exposures, and lifestyle habit data collected via self-administered questionnaires -Subjects: 381 male painters (who fathered pregnancies) exposed to organic solvents in paints, thinners and cleansers and 300 carpenters (who fathered pregnancies) with little or no exposure to solvents -OR with 95% CI calculated by univariate and multiple logistic regression analysis -Quantitative exposure estimates of male painters at 3 mo prior to conception also evaluated	-Quantitative exposure estimates at 3 mo prior to pregnancy - low (0.17 - 0.38 mg/m^3); intermediate (0.38 - 1.02 mg/m^3); high (1.03 - 4.66 mg/m^3) -Adjusted ORs (95% CI) in painters exposed to Toluene at different exposure levels: -prolonged prothrombin time: -low exposure: 1.2 (0.5 - 2.5) -intermediate exposure: 1.1 (0.5 - 2.2) -high exposure: 1.1 (0.5 - 2.7) -spontaneous abortion: -low exposure: 1.3 (0.5 - 3.4) -intermediate exposure: 0.4 (0.0 - 3.2) -high exposure: none -preterm birth: 1.0 -low exposure: 1.6 (0.7 - 3.9) -intermediate exposure: 1.5 (0.7 - 3.2) -high exposure: 0.8 (0.3 - 2.0) -low birth weight: -low exposure: 1.5 (0.5 - 4.3) -intermediate exposure: 1.6 (0.7 - 3.8) -high exposure: 1.9 (0.8 - 4.7) -birth defects – all: -low exposure: 2.1 (0.7 - 5.9) -intermediate exposure: 3.0 (1.3 - 7.0) -high exposure: 2.2 (0.8 - 6.0) -congenital malformations: -low exposure: 6.8 (1.3 - 35.9) -intermediate exposure: 3.9 (0.6 - 24.5) -high exposure: 8.9 (0.8 - 95.9) -functional developmental disorders: -low exposure: 0.4 (0.0 - 3.2) -intermediate exposure: 2.9 (1.1 - 7.7) -high exposure: 1.5 (0.5 - 4.6)	[96]
Study evaluating the effect of occupational exposure to solvents (including Toluene) in shoe making, spray painting, or paint manufacturing, on semen and function of accessory gonads	-24 exposed male subjects working in either the shoemaking, spray painting, or paint manufacturing industry in Zhejiang, China (exposed for at least 1 yr) -37 age- and occupationally-matched non-exposed controls with similar physical activity used as controls -Subjects interviewed about reproductive history, tobacco/alcohol use, and occupational and medical histories -Mean concentrations of benzene, Toluene, xylene in work airborne were 103.34 (0 - 7070.3), 42.73 (0 - 435.8), 8.21 (0 - 133.1) mg/m^3, respectively -Blood and semen samples collected from each subject -Semen analysis: liquefaction time, semen pH value, sperm concentration, total sperm count, percentage vitality, sperm activity, acrosin activity, seminal fructose, seminal GGT activity, LDH C4 activity	-Toluene detected in blood and semen of exposed workers only; not detected in control group (Toluene found in blood and semen in ranges of 0.30 - 17.17 and 0.11 - 0.40 µmol/l, respectively) -Statistically significant decreased levels of sperm activity, acrosin activity, and GGT activity, and LDH C4 activity were observed in exposed workers compared to controls *It should be noted that these results refer to workers who were potentially exposed to multiple solvents, and not Toluene alone	[97]
Study evaluating the effect of occupational exposure to hydrocarbons (including Toluene) in rubber factory workers on semen quality	-48 exposed male workers in production area of rubber factory in Mexico City (exposed for ≥ 2 yr) -42 unexposed male controls from administrative offices of same company -Medical history, reproductive history, exposure to other gonadotoxic agents evaluated -Environmental concentration evaluation of aromatic hydrocarbons in the workplace performed -Weekly semen samples collected from each participant for 3 wk; spermatobioscopies performed -Samples evaluated for liquefaction, volume, pH, agglutination, viscosity, non-specific aggregation, sperm count, motility, and white blood cells	-Exposed workers in contact with a mixture of hydrocarbons that contained: 220.7 - 234 mg/m^3 ethylbenzene, 31.9 – 47.8 mg/m^3 benzene, 189.7 – 212.5 mg/m^3 Toluene, and 47 – 56.4 mg/m^3 xylene -Number of subjects with normozoospermia greater in unexposed group (76%) compared to exposed group (17%) -Statistically significant decreased sperm viscosity, liquefaction, sperm count, sperm motility, and proportion of sperm with normal morphology were observed in exposed workers compared to controls *It should be noted that these results refer to workers who were potentially exposed to multiple solvents, and not Toluene alone	[98]

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
Cardiotoxicity			
Study evaluating the effect of occupational exposure of Toluene in furniture polishing industry workers on cardiac rhythm	-40 male workers in polishing industry with more than 3 mo. exposure to solvents including Toluene evaluated -38 control subjects working in other fields; matched by age, sex, smoking habits, and living accommodations -12-lead surface electrocardiogram and 24-h Holter recordings performed to determine QRS duration, PR duration, P wave dispersion, corrected QT dispersion, and heart rate variability parameters -Hippuric acid levels studied in urine samples taken at end of shift to reflect Toluene exposure level in exposed group	-Mean hippuric acid levels in exposed group: 1141.0 ± 851.7 mg/l; mean exposure time of 198.3 ± 150.0 -Maximum heart rate significantly lower in Toluene-exposed group compared to controls (130.5 ± 15.1 vs 138.6 ± 16.0) -Corrected low frequency and corrected low frequency/corrected high frequency significantly lower in Toluene exposed group vs. control (43.6 ± 7.2 vs 50.7 ± 10.5 and 1.4 ± 0.4 vs 2.2 ± 1.0, respectively) -Mean corrected high frequency, root-mean square successive difference, and standard deviation of all 5-min normal-to-normal interval mean values statistically-significantly higher in Toluene-exposed groups -Long-term exposure to Toluene did not cause increase in arrhythmia frequency, but deteriorated cardiac function markers; long-term exposure caused significant decreased in sympathetic activity and increase in vagal activity parkers	[99]
Neurotoxicity			
Cross-sectional study evaluating the neuropsychological effects of exposure to Toluene in workers in furniture enterprises	-Exposed group occupationally exposed to Toluene via painting and varnishing furniture in Karabaglar, Izmir (n = 122 males) -Non-exposed male group engaged in other aspects of production (n = 88) -Individuals completed questionnaires (involving neuropsychological/neurological symptoms, exposure history, demographics) -Blood samples taken from individuals during the middle of the week within 2 - 4 h of completing work shift -All workers in exposed group were regularly exposed to solvents for 8 h/d	-Statistically significant difference in blood Toluene levels observed in exposed group vs. non-exposed group (levels were 6.95 times higher in exposed group) -No differences were observed in the average neurological and psychological symptoms between exposed and non-exposed groups	[111]
Longitudinal follow-up study evaluating the potential delayed central nervous system effects due to long-term occupational exposure to solvents (including Toluene) in rotogravure printers	-12 male rotogravure printers; 19 control male (refinery or carriage shop repair workers) subjects -Rotogravure printers mainly exposed to Toluene; past mean Toluene exposure estimated to be approximately 1500 mg/m^3 during the 1950s and early 1960s -By the end of the printers' working life (mid 1980s), mean levels of Toluene were approximately 43 and 157 mg/m^3, at 2 printing shops -Subjects evaluated 20 yr after occupational exposure, applying neuropsychological tests, symptoms and social interaction questionnaires, medical examinations, and exposure assessments	-More pronounced deterioration over time in printers than in referents in cognitive functioning affecting reasoning and associative learning -Printers performed significantly worse than referents in verbal memory and sustained attention at follow-up; dose-effect relationship noted for reasoning -Slightly higher depression score noted for printers vs. referents	[100]

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	Genotoxicity		
Study evaluating the potential genotoxic effects of Toluene in industrial painters occupationally exposed to Toluene	-34 male industrial painters from Rio Grande do Sul, Brazil, occupationally exposed to paints containing Toluene; 27 control male subjects with no history of occupational exposure -Subjects filled out questionnaires about general health, lifestyle, and time of occupational exposure -Urine, blood, and buccal cell samples obtained at end of work shift on last day of work week (evaluated for Toluene metabolites, creatinine, cotinine, malondialdehyde and protein carbonyl levels, ischemia-modified albumin, albumin, and hepatic enzymes) -Comet assay performed using whole blood; damage index calculated -Micronucleus assay performed using buccal cells	-Mean amount of blood Toluene, hippuric acid, and *o*-cresol levels in painters: 0.07 ± 0.01 mg/l, 0.56 ± 0.10 g/g creatinine, 0.04 ± 0.01 mg/l, respectively -Blood Toluene and *o*-cresol were not found in controls; however, a mean amount of hippuric acid of 0.41 ± 0.06 g/g creatinine was observed in controls -Damage index of controls determined via Comet assay: 39.4 ± 2.5 -Damage index of painters determined via Comet assay: 60.4 ± 3.6 ($p < 0.001$ compared with controls) -In the micronucleus assay, the frequency of abnormal cells did not show significant difference between painters and the control group ($p > 0.05$) -Malondialdehyde, the lipid peroxidation biomarker, was significantly higher in painters compared to controls ($p < 0.001$) -Painters showed higher ischemia-modified albumin concentrations ($p < 0.05$) and decreased albumin levels ($p < 0.001$) compared to controls -Statistically-significant increased liver enzyme levels were observed in painters compared to controls	[101]
Study evaluating the potential cytogenic damage in offset printing works	-14 exposed printing workers in Turkey (sex not stated) -12 unexposed male controls -Mean duration of employment: 10.36 yr; 45 h/wk -Industrial thinner used by offset printing workers contain about 65% Toluene; workers also exposed to other compounds such as cobalt and hydroquinone -Blood samples taken from all participants and SCE, chromosomal aberration, and micronuclei assays performed	-SCE, chromosomal aberration, and micronuclei frequency was significantly higher in exposed individuals compared to controls ($p < 0.001$) *It should be noted that these results refer to workers who were potentially exposed to multiple solvents, and not Toluene alone	[102]
	Carcinogenicity		
Case-control study assessing relationship between solvent exposure and acute myeloid leukemia	-Study comprised 15,332 incident cases (male and female) of acute myeloid leukemia diagnosed in Finland, Norway, Sweden, and Iceland from 1961 - 2005 and 76,600 controls matched by year of birth, sex, and country -Occupational records linked with Nordic Occupational Cancer Study job exposure matrix to estimate quantitative values for 26 occupational exposure factors -HR with 95% CI estimated using conditional logistic regression models -Cases of Toluene with exposures ≤ 42.4 (1954 controls), 42.4-61 (1602 controls), and > 612 (400 controls) ppm/yr were 424, 296, and 76, respectively	HR levels for high levels of Toluene (HR 1.35, 95% CI 0.74 - 2.46) were slightly elevated; p-value: 0.49	[103]
Case-control study assessing relationship between Toluene exposure and lung cancer	-1236 cases of lung cancer (male and female) identified from 18 hospitals in Montreal, Canada between 1996 and 2001; 1512 population-based controls matched on age and sex -Subjects interviews on lifestyle behaviors, sociodemographic characteristics, and job histories (including equipment/chemicals used during job, safety measures, and duration of job) -Team of chemists and industrial hygienists assessed exposure to 294 occupational agents (including Toluene) -Multivariate logistic regression used to estimate ORs and 95% CIs	Lung cancer was associated with exposure to Toluene (OR = 1.31; 95% CI 0.99 – 1.84)	[104]

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
Case-control study assessing relationship of maternal occupational exposure to solvents during pregnancy and childhood acute lymphoblastic leukemia	-790 cases of childhood acute lymphoblastic leukemia; 790 controls based; all cases diagnosed in Quebec, Canada between 1980 and 2000 -Maternal occupational exposure to solvents before and during pregnancy; home exposure to solvents also evaluated; exposure measured using exposure coding -Conditional logistic regression to estimated ORs and 95% CIs -Number of leukemia cases based on exposure to solvents 2 yr before pregnancy up to birth and number of leukemia cases based on exposure to solvents during pregnancy evaluated -Number of leukemia cases based on exposure level (no exposure, some exposure, greater exposure) to solvents 2 yr before pregnancy up to birth also evaluated	2 yr before pregnancy up to birth OR (95% CI): 1.88 (1.01 - 3.47); during pregnancy OR (95% CI): 2.25 (1.02 - 4.95) (increased risk); however, no indication of increased risk with increased level of exposure	[105]
Metabolic Syndrome			
Cohort study evaluating the association between occupational exposure to potential hazards (including Toluene) and metabolic syndrome	-Retrospective cohort based on 31,615 health examinees (1182 of which exposed to Toluene; male and female) in Republic of Korea from 2012 - 2021 -Demographic and behavior-related risk factors treated as confounding factors -Time-dependent Cox regression analysis used to calculate HRs; 95% CI -Adjusting for confounders (age, sex, smoking, alcohol intake, exercise frequency, and family history) -HR >1 suggests increased risk -HRs also calculated for combined exposure to Toluene and night shift (n = 157), Toluene and xylene (n = 380), Toluene with noise (n = 154), Toluene with styrene (n = 92), Toluene with copper (n = 120), and Toluene with antimony (n = 8) -If RERI > 0, the two substances had an additive effect, if MI >1, the two substances had a multiplicative effect	-Unadjusted and adjusted HRs for Toluene: 1.80 and 1.42, respectively -Toluene exposure plus night shift: -HR: 2.43 -RERI: 0.58 -MI: 1.20 -Toluene exposure plus noise exposure HR -HR: 1.65 -RERI: 0.08 -MI: 1.01 -Toluene exposure plus styrene exposure HR -HR: 1.91 -RERI: 0.42 -MI: 1.24 -Toluene exposure plus copper exposure HR -HR: 1.83 -RERI: -0.45 -MI: 0.70 -Toluene exposure plus antimony exposure HR -HR: 1.94 -RERI: -0.31 -MI: 0.75	[106]
Color Vision Impairment			
Follow-up study evaluating the potential effects of occupational exposure to Toluene on color vision	-4 yr study on color perception performed in individuals occupationally exposed to Toluene (< 50 ppm) in rotogravure printing in Germany (sex not stated) -Color vision measured 3 times throughout study period (162 participants completed all 3 examinations) -Study design based on 2 factors for stratification: intensity of exposure (high: printing division; low: end-processing division) and duration of exposure (short versus long) -Current individual Toluene exposure measured twice per year -Work history taken via questionnaire -Color discrimination abilities evaluated using a de-saturated panel test; screenings for low near vision acuity, red-green discrimination deficiencies, and contrast sensitivity also performed; color confusion index calculated -Multiple regressions performed	-Average exposure level for Toluene in breathing zone was 25.7 ± 21.0 ppm in printing area (n = 93) and 3.5 ± 3.6 ppm in the end-processing area (n = 69) -Mean exposure durations were 23 ± 6 yr for "long exposure" and 7 ± 2 yr for "short exposure" -Repeated analysis and multiple regressions did not reveal significant effects of Toluene on color vision perception with respect to intensity or duration of exposure	[112]

Table 8. Occupational toxicity and epidemiological studies of Toluene

Study Type	Study Details	Findings/Results	References
	General/Other		
Study evaluating occupational health risks of Toluene, in the automobile repair industry	-Concentrations of benzene, Toluene, and xylenes monitored at 140 operating positions in 2018 (Beijing, China) -Long-term exposure concentration range of Toluene: 0.1 – 49.7 mg/m^3 -Short-term exposure concentration range of Toluene: 0.1 – 98.7 mg/m^3 -Occupational health risks evaluated using Singaporean model -Risk rating evaluated based on hazard rating (mainly determined based on the carcinogenicity classification published by IARC, exposure level/frequency, weekly exposure, average working hours per week, reduction factor, and permissible exposure level -Risk rating based on scale of 1 - 5	Risk ratings for Toluene for individuals exposed short-term and long-term to paint mixing and paint spraying ranged from 2 - 3 (low-medium risk)	[107]
Study evaluating the health risks/effects of different cooking methods (steaming, frying, and grilling) due to exposure to aromatic hydrocarbons (including Toluene)	-Chefs from Nanjing, China answered questionnaire to provide personal information and exposure time to kitchens (sex not stated) -Morning urine, saliva, and oral epithelial cell samples collected from 6 chefs of each style of cooking (steaming, frying, and grilling) -Internal and external exposure evaluated (air concentrations of Toluene, metabolites of Toluene (S-benzylmercapturic acid) quantified in urine -Chefs wore monitoring equipment measure real-time respiratory rate, systolic blood pressure, diastolic blood pressure, and ratio between forced expiratory volume within 1 s, and forced vital capacity -Malondialdehyde and 8-OHdG concentrations in urine and saliva used as indicators of systemic oxidative stress -Samples and cardiopulmonary indicators collected and evaluated during each of the 4 seasons of 2017	-Air concentrations of Toluene in frying kitchens, grilling kitchens, and steaming kitchens were 226 ± 122.2 $\mu g/m^3$, 122.1 ± 107.0 $\mu g/m^3$, and 87.58 ± 87.42 $\mu g/m^3$, respectively -Mean urinary concentrations of S-benzylmercapturic acid in chefs in frying kitchens, grilling kitchens, and steaming kitchens were 3.31 ± 3.21, 1.56 ± 2.12, and 1.41 ± 1.19 µmol/mol creatinine, respectively -Toluene in cooking pollution had no effect on blood pressure or lung function -Toluene associated with increased oxidative stress levels	[108]
Study evaluating the health risk of exposure to Toluene in the automobile industry	-Study performed in 115 automobile workers (sex not stated) in Iran in 2021 -Vapors gathered during work hours using adsorbent tube of activated coconut charcoal; values collected for 6 different types of shops: press, body, paint, pre-delivery inspection, assembly, and material storage -Non-cancer health risk evaluated by determining the HQ; HQ calculated by dividing the exposure concentration of Toluene by the maximum acceptable daily dose of exposure (5 mg/m^3) -HQ ≤ 1 shows the absence of non-carcinogenic health effects; HQ > 1 represents the presence of non-carcinogenic health effects	-Mean concentrations of Toluene in the breathing zone of workers in press, body, paint, pre-delivery inspection, assembly, and material storage shops: 0.0790, 0.3883, 0.1240, 32.3923, 1.8572, and 0.0441 ppm, respectively -Mean HQ value in non-cancer risk assessments were higher than the acceptable limit for Toluene in the shop of pre-delivery inspection (7.832) -Mean HQ value of all shops combined: 1.396	[109]
Study evaluating the effect of long-term exposure to volatile organic compounds (including Toluene) and incidence of carcinogenic and non-carcinogenic adverse health effects	-53 beauty technicians (male and female) from Seoul, Korea recruited – these individuals delivered one or more of the following services: hair drying, hair dressing, hair coloring, haircut, epilation, nail art, facial shaving, facial steam, makeup, eyelash extensions, and waxing -Questionnaire given to assess exposure factors (total years as technician, frequency of work per week, duration in salon per day); interviews also performed -Indoor air samples collected during regular business hours over 6 business days -Personal air sampling performed using passive sampler worn during 8-h shift -HQs evaluated for non-carcinogenic assessment; HQ > 1 indicates adverse non-carcinogenic concern	-Mean indoor air concentration of Toluene: 14.99 $\mu g/m^3$ -Median personal exposure concentration: 17 $\mu g/m^3$ -Non-carcinogenic HQ mean: 0.003 -Main adverse effects reported in technicians include dry skin, skin stinging/itching, and dry eyes – technicians who experienced adverse health effects had significantly higher concentrations of acetone, benzaldehyde, and Toluene than those who did not experience adverse health effects ($p < 0.05$)	[110]

8-OHdG = 8-hydroxy-2'-deoxyguanosine ; CI = confidence interval; GGT = gamma-glutamyl transaminase; HQ = hazard quotient; HR = hazard ratio; IARC = International Agency for Research on Cancer; LCR = lifetime cancer risk; LDH = lactate dehydrogenase; MI = multiplicative interaction; MRI = magnetic resonance imaging; OR = odds ratio; RERI = relative excess risk due to interaction; SCE = sister chromatid exchange

Table 9. Exposure level regulations/recommendations of Toluene by various organizations

Organization	Regulation	Reference
Occupational Safety and Health Administration	-PEL: 200 ppm 8-h TWA; ceiling: 300 ppm; acceptable peak over ceiling: 500 ppm over 10 min -PEL in shipyard employment: 200 ppm TWA [29CFR1915.1000]	[124]
California Occupational Safety and Health Administration	-PEL: 10 ppm; ceiling: 500 ppm; STEL: 150 ppm	[125]
The National Institute for Occupational Safety and Health	-REL: 100 ppm TWA; STEL: 150 ppm	[126]
California Office of Environmental Health Hazard Assessment	-MADL – inhalation: 13,000 µg/d -MADL – oral: 7000 µg/d -REL: 5000 µg/m^3 (acute); 830 µg/m^3 (8-h); 420 µg/m^3 (chronic)	[127,128]
American Conference of Governmental Industrial Hygienists	-Threshold limit value: 20 ppm	[129]
European Union Scientific Committee on Occupational Exposure Limits	-OEL: 50 ppm 8-h TWA; 15-min STEL: 100 ppm	[130]
Lower Olefins and Aromatics Racial and Ethnic Minority Acceleration Consortium for Health Equity working group	-OEL: 20 ppm 8-h TWA; 150-min STEL: 100 ppm; and skin notation to indicate that the dermal absorption of liquid Toluene can substantially contribute to body burden	[130]

MADL = maximum allowable dose level; OEL = occupational exposure limit; OR = odds ratio; PEL = permissible exposure limit; REL = recommended exposure limit; STEL = short-term exposure limit; TWA = time weighted average

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4

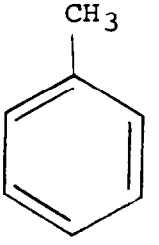
Final Report on the Safety Assessment of Toluene

Toluene has a wide variety of noncosmetic applications. However, the cosmetic use is limited to nail products at concentrations up to 50%. Toluene was practically nontoxic when given orally to rats; acute oral LD_{50} values ranged from 2.6 g/kg to 7.5 g/kg. Results of animal studies indicated that undiluted Toluene is a skin irritant. No skin irritation or sensitization was observed in subjects treated with cosmetic products containing 31–33% Toluene. No phototoxic or photoallergic reactions were noted in subjects treated with 25% or 30% Toluene. The sole cosmetic use of Toluene is in products intended to be applied directly to the nail; therefore, human skin exposure to this ingredient will be minimal under conditions of cosmetic use. On the basis of the available data and the limited user skin exposure from cosmetic products containing Toluene, it is concluded that this ingredient is safe for cosmetic use at the present practices of use and concentration.

CHEMISTRY

Definition and Structure

Toluene (CAS No. 108-88-3) is a homolog of benzene in which one hydrogen atom has been replaced by a methyl group[1]:



Other names for this cosmetic ingredient include: Methacide, Methylbenzene, Methylbenzol, Phenylmethane, Toluol, Antisal 1a, and NCI-C07272.[1-4]

Properties

Toluene is a clear, refractive liquid that has an aromatic odor similar to benzene. It is both volatile and flammable.[1,4-6] Toluene is miscible with water but is immiscible with alcohol, chloroform, ether, acetone, glacial acetic acid, carbon disulfide, ligroin, and benzene.[4,5,7-11]

The ultraviolet absorption spectrum of 300 gm/l of Toluene diluted in hexane was measured. The 300 gm/l concentration corresponded to the highest reported concentration of Toluene used in cosmetics. No significant absorption was noted above 300 nm.[12] Skin photosensitivity reactions of the immunological type occur with wavelengths greater than 320 nm.[13]

Additional chemical and physical data are presented in Table 1.

TABLE 1. Chemical and Physical Data for Toluene

Property	*Value*	*Reference*
Appearance	Colorless liquid	6, 7, 9, 10, 14
Molecular formula	$C_6H_5CH_3$	5, 10, 14
Molecular weight	92.13	1, 7, 9, 11, 14, 15
Boiling point	109–111°C	1, 4, 5, 7–11, 14–18
Melting point	−94.5–−95°C	1, 4, 5, 7, 10, 14, 17, 18
Freezing point	−93.2–−94.991°C	9, 11, 18
Specific gravity	0.8623 (15.6/15.6°C)	1
	0.865 (25/25°C)	8
	0.866	7, 10, 16
	0.866 (20/4°C)	5
	0.867 (20/20°C)	15
	0.87 (25°C)	18
Density	0.861–0.871 (20/20°C)	19
	0.8623 (g/ml at 25°C)	11
	0.866 (20/4°C)	4, 14
	0.869 (20/4°C)	1, 17
	0.871 (13°C)	9
	0.8869 (20/4°C)	18
Vapor density (air = 1)	3.1–3.2	1, 11, 14
Vapor pressure	22 mm Hg at 20°C	6
	22.4 mm Hg at 20°C	15
	28 mm Hg at 25°C	7
	28.4 mm Hg at 25°C	11
	28.7 torr at 25°C	1
	36.7 mm Hg at 30°C	14, 18
Percent in saturated air	3.94 (at 760 mm Hg and 22°C)	1, 11
Liquid viscosity	0.6 cp at 20°C	1
Viscosity (SYS)	<32.6	18
Solubility in water	0.05% (g/100 g water at 20°C)	6
Solubility in distilled water	534.8 ± 4.9 mg/l at 25°C	1, 11
Index of refraction	1.4941 (25°C)	11
	1.4961	17, 18

TABLE 1. (Continued)

Property	*Value*	*Reference*
	1.4967	4
	1.49693 (68°F)	1
	1.497 (20°C)	5
	1.496–1.497	16
Aniline equivalent	15	5
Evaporation rate (n-butylacetate = 1)	2.10	15
Flash point	Closed cup: 40°F (4.4°C)	1, 4–7, 11, 14, 18
Autoignition temperature	896°F	14
	997°F	5
	1025.6°F (552°C)	1
Flammable limits	1.4–6.7%	18
	1.17–7.10% by volume in air	1, 11
Lower explosive limit in air	1.27% by volume	5, 14
	1.3% by volume	6
Upper explosive limit in air	7% by volume	5, 14
	7.1% by volume	6
Residue after evaporation	<0.001%	16
Sulfur compounds (as S)	0.003%	16
Water	0.03%	16
Weight volume conversion in air at 25°C	1 ppm = 3.77 mg/m^3	1, 11, 18
	1 mg/m^3 = 0.265 ppm	1, 11
Specific dispersion	184.40	11
Log octanol–water partition coefficient	2.69	1
Partition coefficient (K_p) in vapor and water	5.14 at 20.06°C	11
Partition coefficient (K_D) in octanol and water	512 ± 22	11
Odor threshold		
Petrol-derived	2.14 ppm	1, 11
Coke-derived	4.68 ppm	1
Critical temperature	320.8°C	11
Critical pressure	40.0 atm	11
Critical density	0.29 g/ml	11
Critical compressibility factor (PV/RT)	0.26	11
Density of saturated air–vapor mixture	1.09 (at 760 mm and 26°C; air = 1)	1
Surface tension	28.53 dynes/cm at 20°C	1
Heat of vaporization	9.115 ± 0.50 Kcal/mol at 25°C	11
Heat of fusion	1.582 Kcal/mol	11
Heat of formation		11
Liquid	2.867 Kcal/mol	
Gas	11.950 Kcal/mol	
Entropy		11
Liquid	52.40 cal/(mol)(°C)	
Gas	76.44 ± 0.3 cal/(mol)(°C)	
Free energy of formation		11
Liquid	27.282 Kcal/mol	
Gas	29.228 Kcal/mol	

Method of Manufacture

Toluene is produced from three major sources: (1) petroleum refining processes, (2) as a byproduct of styrene production, and (3) as a byproduct of coke oven operations.[1]

Petroleum refining processes to isolate Toluene are of two types: catalytic reforming and pyrolytic cracking. Catalytic reforming involves the catalytic dehydrogenation of selected petroleum fractions that are rich in methylcyclohexane and other naphthenic hydrocarbons to yield a mixture of aromatics and paraffins. The proportions of aromatics and paraffins in the reformate depend on the feedstock used and the severity of the reforming operation. Toluene is isolated from the reformate by distillation, followed by washing with sulfuric acid and redistillation. Pyrolytic cracking of petroleum yields olefins and pyrolysis gasoline. Toluene is isolated from pyrolysis gasoline by distillation, removal of olefins and diolefins, and redistillation.[1]

The synthesis of styrene by the dehydrogenation of ethylbenzene yields Toluene as a byproduct. The Toluene derived from this method is unsuitable for chemical or solvent use; however, it may be used for gasoline blending or as feed for the manufacture of benzene.[1]

The production of coke by the high-temperature carbonization of coal yields coal tar and crude light oil, both of which contain Toluene. The production of Toluene from distillation of coal tar is minimal; however, some Toluene is isolated from crude light oil.[1]

Toluene may be purified by various extraction and distillation processes (Eldeleauno SO_2 extraction, Udex separation, sulfolane extraction).[16] The various grades of Toluene (pure, commercial, industrial, nitration, solvent, scintillation) are usually defined in terms of boiling ranges.[5]

Impurities

Commercial Toluene may contain benzene as an impurity.[1] Therefore, all toxicological and clinical studies involving Toluene should specify the quality of Toluene used for experimentation. If benzene is present in the Toluene, it should be demonstrated that the observed biological effects are not wholly or partly due to benzene.

Reactivity

Toluene undergoes substitution reactions on the aliphatic side group ($-CH_3$) and on the benzene ring at the ortho and para positions. Such reactions may include halogenation, chloromethylation, nitration, acetylation, benzoylation, mercuration, sulfonation, brosylation, methylation, and isopropylation. These substitution reactions occur at a faster rate with Toluene than with benzene.[1,11]

Toluene is quite stable in air; however, Toluene can be oxidized with air under catalytic conditions to yield benzoic acid. In the presence of heat (or catalyst) and hydrogen, Toluene undergoes dealkylation to produce benzene. In aqueous media under the conditions of water chlorination, Toluene may be chlorinated, followed by subsequent hydrolysis to benzaldehyde. In the presence

of solvents (paraffins, naphthenics, and alcoholic hydrocarbons), Toluene can form azeotropes.[1,11] Toluene also may undergo photooxidation[20] and other photochemical reactions.[1,11] For a more complete description of the types of reactions that Toluene may undergo, the reader is referred to the reviews by the Syracuse Research Corp.[1] and the National Research Council.[11]

Toluene vapor was passed with nitrogen through a silica tube filled with porcelain chips at 700°C. Reported pyrolysis products included some known or suspected carcinogenic aromatic hydrocarbons (Table 2).[11,21]

Toluene is reported to be chemically stable and unreactive under conditions of use in cosmetic preparations.[16]

Analytical Methods

Gas chromatography may be used for the analytical determination of Toluene in blood.[22,23] Methods for the determination of hippuric acid, a major metabolite of Toluene excreted in the urine,* include thin-layer chromatography,[24] high-performance liquid chromatography,[25-27] gas chromatography,[28] and colorimetric methods.[26]

USE

Noncosmetic Use

Noncosmetic applications of Toluene include use as an indirect food additive, gasoline additive, ink thinner, nonclinical thermometer liquid, suspension solution for navigation instruments, extraction solvent for plant materials, and as a solvent for adhesives, rubbers, oils, gums, resins, vinyl organosols, paints, lacquers, and coatings. Toluene also is used as a starting material for the production of benzene, benzaldehyde, benzoic acid, benzoic acid derivatives, benzyl and benzoyl derivatives, saccharin, phenol, caprolactam, explosives (TNT), toluene-diisocyanates, polyurethane resins, detergents (toluene sulfonates), dyes and drugs.[4,5,7,9,18]

Consumer products containing Toluene are listed in Table 3. Indirect food additive uses of Toluene are presented in Table 4.

Cosmetic Use

Purpose in Cosmetics

Toluene is used in nitrocellulose nail lacquer† products as a diluent and solvent.[5,15,16] Toluene also is used in nail lacquers to reduce the "blushing phe-

*In the body, Toluene is mainly oxidized to benzoic acid, which after conjugation with glycine is eliminated as hippuric acid in the urine. Although hippuric acid is often used to determine human exposure to Toluene, hippuric acid may be formed from other metabolic processes besides Toluene metabolism.[1,11]

†The term "nail lacquer" is often used to denote nail enamel, nail polish, nail varnish, top coat, and base coat.[15,29,30]

TABLE 2. Pyrolysis Products of Toluene[11,21]

Compound	Weight, % of tar formed	Compound	Weight, % of tar formed
Anthracene	0.009	4,4'-Dimethylbiphenyl	0.99
1,2-Benzanthracene[a]	0.014	Biphenyl	0.27
Benzene[a]	2.54	Fluoranthene	Trace
3,4-Benzofluoranthene[a]	0.002	Fluorene	0.085
10,11-Benzofluoranthene[a]	Trace	Naphthalene	0.042
11,12-Benzofluoranthene[a]	Trace	Phenanthrene	0.12
1,2-Benzofluorene	0.007	Pyrene	Trace
2,3-Benzofluorene	0.017	Stilbene	0.44
1,2-Benzopyrene[a]	0.002	Styrene	0.11
3,4-Benzopyrene[a]	0.002	Toluene	93.5
Chrysene[a]	0.03	*p*-Xylene	0.05
Alkylchrysene	Trace	Resins and losses	0.7
Bibenzyl	1.00		

[a]Suspected carcinogen.[11]

nomenon," which occurs as a result of excessive and rapid evaporation of low boiling solvents.[16]

Product Formulation

Data submitted to the Food and Drug Administration (FDA) in 1984 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Toluene was used in 555 cosmetic products (Table 5). Products formulated with Toluene included nail basecoats and undercoats (32 products), nail polish and enamel (501 products), and "other manicuring preparations" (22 products). Reported concentrations of Toluene in these products ranged from >10–25% (448 products) to >25–50% (107 products).[31]

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators must conform to the format of concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations.[39] Since certain cosmetic ingredients are supplied to the formulator at less than 100% concentration, the concentration reported by the formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. In addition, the fact that data are only submitted within the framework of a concentration range provides opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

TABLE 3. Consumer Products Containing Toluene[1]

Product	*% Toluene content*
China cement, solvent type	20–30
Contact rubber cement	– – –
Microfilm cement, cotton base	27–30
Model cement	Up to 20–25
Plastic cement, polystyrene	24
Shoe cement	– – –
Tire repair, bonding compounds	>80
Paint brush cleaners	– – –
Stain, spot, lipstick, rust removers	– – –
Deicers, fuel antifreeze	30
Fabric dyes	≤60
Indelible inks	– – –
Marking inks	80–90
Stencil inks	40–60
Solvent and thinners	– – –

TABLE 4. Indirect Food Additive Uses for Toluene

Use	*Limitation*	*Reference*
Component of adhesives used in articles intended for packaging, transporting, or holding food	– – –	32
Component of resinous and polymeric coatings for polyolefin films intended for food contact	– – –	33
Component of paper and paperboard in contact with dry food	– – –	34
Component of acrylic and modified plastic acrylics intended for contact with food	– – –	35
Component of cellophane used for food packaging	Residue limit of 0.1% of weight of finished cellophane packaging	36
Component of polysulfide polymer–polyepoxy resins intended for contact with dry food	Use of Toluene limited to that of a solvent	37
Adjuvant substance in the manufacture of foamed plastics intended for food contact	Use of Toluene limited to that of a blowing agent adjuvant in polystyrene at a level not to exceed 0.35% by weight of finished polystyrene foam	38

TABLE 5. Product Formulation Data for Toluene[31]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)	
			>25–50	>10–25
Nail basecoats and undercoats	47	32	18	14
Nail polish and enamel	769	501	74	427
Other manicuring preparations	66	22	15	7
1984 TOTALS		555	107	448

As noted in Table 5, the major use of Toluene is in nail polish and enamel. Most nail polish formulations typically consist of the following constituents[15,29]:

1. A film former (such as nitrocellulose, ethylcellulose, cellulose acetate, cellulose acetate-butyrate, methacrylate polymers, vinyl polymers, or sucrose acetate isobutyrate)
2. Resins to improve gloss and adhesions of the films (such as toluenesulfonamide/formaldehyde resin)
3. Plasticizers to give the film pliability, minimize shrinkage, and soften and plasticize the film former (such as camphor, or dibutyl phthalate)
4. Solvents and diluents to stabilize viscosity and to keep the film former, resin, and plasticizer in a liquid state (such as esters, glycol ethers, nitroparaffins, alcohols, xylene, or toluene)*
5. Thixotropic agents to prevent rapid settling and caking of pigments, and to provide flow properties (clay modified by quaternary ammonium compounds)
6. Coloring substances (such as fluorescent and nonfluorescent dyes, guanine, or inorganic and organic pigments)

A typical nail lacquer might contain 12% nitrocellulose, 5% n-butyl phthalate, 5% aryl sulfonamide-formaldehyde resin, 1–3% camphor, and 1–2% pigment. The solvent may consist of approximately 35% toluene, 40% butyl acetate, 15% ethyl acetate, and 10% ethanol.[40]

Basecoat is formulated in a manner similar to nail polish, but it has a lower nonvolatile content (less nitrocellulose) and a lower viscosity because a thinner film is desirable. It does not contain pigments. Basecoat may contain hydrolyzed gelatin.[29]

*These solvents may cause false-positive irritant reactions if not permitted to evaporate before the nail lacquer is applied under a patch to the skin.[29]

Suggested formulae for various nail products containing Toluene have been described in the literature.[15,41-49]

Exposure to Toluene

Nail products formulated with Toluene can be applied several times a week over a period of many years. The fingernail, the toenail, the nail cuticle, and the skin surrounding the nail are the areas directly exposed to this cosmetic ingredient. Parts of the body that come in contact with the wet nail may also become exposed. Such areas may include the eye region, the face, neck and chest, the retroauricular zone, and the vulva.[29] During application of the cosmetic product to the nail, Toluene may come in contact with eyes and nasal mucosa as a result of evaporation from the formulation.

ABSORPTION

In mammals, Toluene is absorbed by the respiratory tract, skin, and gastrointestinal tract.[11] Since Toluene can readily penetrate many of the body's protective barriers, its absorption is likely passive and dependent on the concentration gradient, so that any physiological characteristic that modifies this gradient would be expected to alter the rate of absorption.[50]

Absorption Through the Lungs

Toluene is readily absorbed through the respiratory tracts of humans and experimental animals.[51-54] The amount of Toluene absorbed (uptake) is proportional to the concentration in inspired air, length of exposure, and pulmonary ventilation (respiratory minute volume).[55-57] Total uptake (absorption) can be estimated as follows:

$$\text{Uptake} = (0.5)(V_e)(C_i)(t)$$

where V_e is the respiratory minute volume in l/minute, C_i is the inspired concentration in mg/l, and t is the length of exposure in minutes.[57,58]

Because of the dependence on respiratory minute volume, the uptake of Toluene by humans is affected by the level of physical activity.[55-57,59-61] Mild to moderate exercise can double or triple the rate of uptake compared to that at rest.[57] An individual's content of adipose tissue generally has little or no effect on the uptake of Toluene (50–150 ppm) during exposures lasting 4 h or less.[55,57]

Toluene can be detected in human aterial blood as soon as 10 seconds after exposure by inhalation. The concentration of the compound in the arterial blood quickly increases during the first 10–15 minutes. After that, the Toluene concentration increases more slowly and reaches a fairly constant concentration after about 25 minutes; during this period, the retention is 75–80%. The rate of retention decreases as the individual approaches a state of equilibrium with respect to absorption, deposition, and excretion of Toluene and its metabolites. After 2–3 h of exposure by inhalation, the rate of retention falls to an almost con-

stant level—40–50%. The average rate of retention over a 5-h period is approximately 50%.[62] Once exposure has ended, Toluene concentration in alveolar airspaces, arterial blood, and venous blood decreases rapidly.[59]

The alveolar concentrations of Toluene in humans have been measured.[63] Results of 40 measurements with three different methods and 16 persons indicated that the average Toluene absorption by inhalation at 100 ppm exposure concentrations was approximately 1.6 mg/minute.

Mature "cross-bred dogs" were exposed by inhalation for 1 h to 700, 1500, and 2000 ppm Toluene. Pulmonary absorption of Toluene within 1 h of exposure was estimated to be 25, 56, and 74 mg/kg, respectively.[64]

Other studies pertaining to the respiratory absorption of Toluene by humans and experimental animals are reviewed in detail by the Syracuse Research Corp.[1]

Skin Absorption

Lung tissues are more permeable to chemicals than is the thicker and more histologically complex dermal tissue.[50] Although Toluene is absorbed less readily through the skin than through the respiratory tract, percutaneous absorption of liquid Toluene can be significant.[1]

Undiluted, liquid Toluene was reported to be absorbed at a rate of 14–23 mg/cm^2 per h through the skin of the forearms and hands when in direct contact with about 0.2 ml (170 mg) of Toluene for 10 or 15 minutes. When the hands and forearms were immersed for 1 h in aqueous solutions containing 180–600 mg of Toluene per liter, the rate of absorption was calculated to be 0.16–0.60 mg/cm^2 per h and increased with a corresponding increase in the concentration of Toluene. Analysis of the applied solutions before and after exposure indicated that appreciable amounts of Toluene were absorbed, ranging from 41 to 100 mg (23.7–57.7%) of the undiluted Toluene applied, and from 52 to 206 mg (27.5–35.9%) in the immersion study. These authors estimated that exposure of both hands to a saturated aqueous solution of Toluene for 1 h would be equivalent to inhalation exposure to an atmosphere containing 26.6 ppm for 8 h.[65,66]

The absorption and excretion kinetics for dermal and inhalation exposures to Toluene has been reported.[67] The investigators found that the maximum Toluene concentration in the blood of subjects who immersed one hand in liquid Toluene for 30 minutes was only 26% of the concentration in blood of subjects who inhaled 100 ppm Toluene vapor for 30 minutes. Toluene was depleted from the blood much more rapidly after termination of the inhalation exposure than after the dermal exposure.

There is significant absorption of Toluene through intact skin of volunteers with respiratory protection who immersed both hands in analytically pure Toluene for 10 minutes.[68] Results of analysis of exhaled air up to 3 h after exposure indicated an exponential decline in exhaled Toluene, ranging from greater than 4 ppm at 20 minutes postexposure to less than 1 ppm after 120 minutes. The authors calculated that between 2050 and 3370 mg of Toluene were absorbed in the 10-minute exposure.

Percutaneous absorption of Toluene vapor from the surrounding air was evaluated by Riihimaki and Pfaffli.[69] Volunteers wearing respiratory protection were exposed to 600 ppm Toluene for 3–5 h. The subjects remained at rest ex-

cept for three exercise periods, each lasting for 10 minutes, which occurred at 0.5, 1.5, and 2.5 h of exposure. The exercise was sufficient to stimulate perspiration and raise the skin temperature slightly, conditions that are thought to enhance percutaneous absorption. The concentration of Toluene in peripheral venous blood, measured at the end of 1, 2, and 3 h of exposure, was constant at approximately 100 μg/l. The observed percutaneous absorption was estimated to be about 0.9% of the amount that would be absorbed from the respiratory tract during a 3.5-h exposure at 600 ppm, assuming that 60% of the inhaled Toluene is retained and 16% of the absorbed dose is exhaled.

Subjects exposed dermally to 1600 mg/m^3 (427 ppm) Toluene for 8 h had no increase in urinary excretion of a metabolite (benzoic acid) of Toluene. It was estimated that absorption of Toluene through the skin would not exceed 5% of absorption through the respiratory tract under the same conditions.[70]

The concentration of Toluene in the blood or guinea pigs was monitored following application to the skin of 1.0 ml of the solvent. At 0.5 and 6 h, blood concentrations of Toluene were 1.1 and 0.60 μg/ml, respectively.[71]

The in vitro penetration of Toluene through excised rat skin was estimated by Tsuruta[72] as 8.50 nmol/minute per cm^2.

Gastrointestinal Absorption

Absorption of Toluene from the gastrointestinal tract is nearly complete.[1,62] In studies with rabbits, 76% of an oral dose of Toluene was excreted in the urine as hippuric acid, whereas 18% of the oral dose was expired through the lungs unchanged.[73]

In rats, the concentration of radioactivity in the blood reached a maximum 2 h after gastric intubation of 100 μl 4-^{3}H-Toluene in peanut oil, whereas maximum concentrations of Toluene in blood were reached 15–30 minutes after exposure by inhalation. Although absorption from the lungs following inhalation was more rapid, the relative radioactivities in various tissues were about equal after oral and inhalation exposure.[74]

METABOLISM

Toluene is metabolized in humans and animals by the pathways outlined in Figure 1. The major site for the metabolism of Toluene in both animals and humans is the liver, where the compound undergoes sidechain oxidation to benzoic acid. Some metabolism in other tissues also may occur.[50] Most of the benzoic acid is subsequently conjugated with glycine and excreted in the urine as hippuric acid, although a large amount of conjugation with glucuronic acid also occurs, resulting in urinary excretion of benzoylglucuronic acid.[75] Oxidation of the alkyl sidechain to carboxylic acid is typically a rapid and spontaneous sequence of reactions that is catalyzed by a microsomal NADH-dependent enzyme system in tissues with a high redox potential (i.e., liver and kidneys).[76] The formation of benzyl alcohol involves an NADH-dependent alcohol hydratase or monooxygenase (oxidase). The intermediate then is rapidly converted to benzaldehyde by an NAD-dependent alcohol dehydrogenase. The aldehyde is

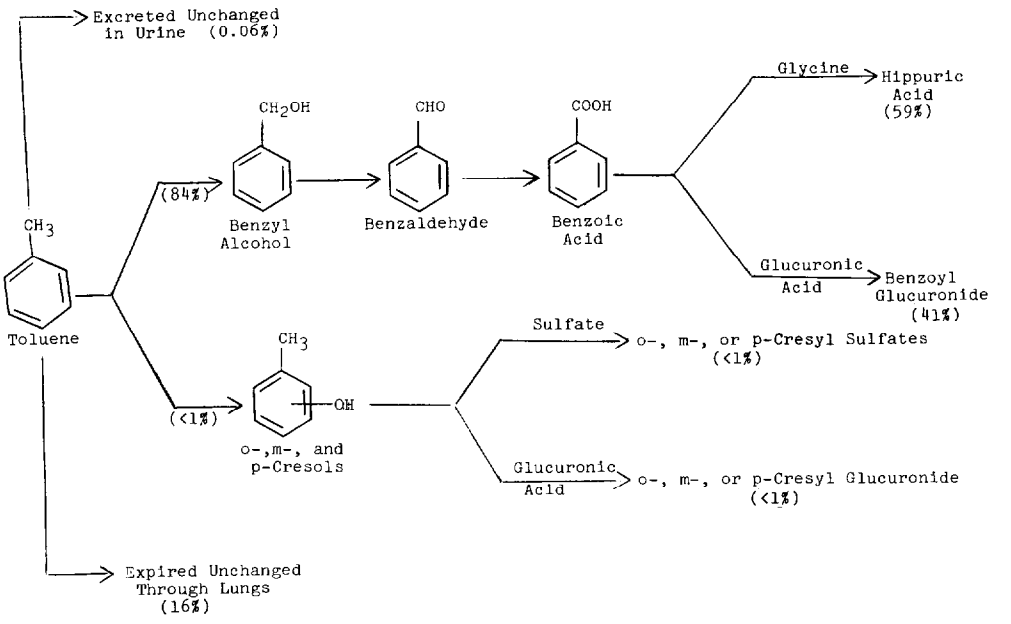


FIG. 1. Metabolism of Toluene in mammals.[50,77]

then oxidized to benzoic acid by means of an NAD-dependent aldehyde dehydrogenase.[50]

Minor amounts of Toluene undergo ring hydroxylation, probably via arene oxide intermediates, to form *o*-, *m*-, and *p*-cresol, which are excreted in the urine as sulfate or glucuronide conjugates.[78,79] The sidechain-oxidized compounds are rapidly conjugated with glucuronic acid or glycine to render them chemically inert.

DISTRIBUTION AND STORAGE

Upon entering the body, Toluene is rapidly distributed to all tissues by the circulatory system. Toluene is lipophilic, readily passes through cellular membranes, and accumulates primarily in those tissues with a high fat content. Toluene can be measured in many body tissues during and immediately after exposure. However, after exposure ends, the compound rapidly dissipates from tissues with a low fat content.[50] The quantity of Toluene absorbed by a tissue depends on the partition coefficient (tissue/blood), on the duration of exposure, and on the rate of metabolism. The rate of its absorption depends on the perfusion of the tissue and on the concentration gradient.[62]

As indicated by its partition coefficient, Toluene is highly soluble in lipid and sparingly soluble in water (Table 6). Hence, it is likely to associate with the lipid and lipoprotein components of the plasma—primarily those of the chylomicrons.[1,62] As measured in rabbits, the tissue/blood partition coefficients for fatty tissues are high (113 for adipose tissue and 35 for bone marrow); for other tissues, they range from 1 to 3. The solubility of Toluene in human blood, as indi-

TABLE 6. Partition Coefficient for Toluene at 37°C[1]

	Partition coefficient	*Reference*
Fluid/air or material/air		
Water	2.23	51
Oil, olive	492	
Blood, human	15.6	
Fat, human, peritoneal	1296	
Oil, olive	1380	54
Lard	1270	
Blood, human	15.6	
Blood, human	14.64	52, 53
Blood, rabbit	10.41	
Plasma, rabbit	16.99	
Tissue/blood (rabbit)		
Liver	2.58	52, 53
Kidney	1.54	
Brain	3.06	
Lung	1.92	
Heart	2.10	
Muscle, femoral	1.18	
Bone marrow, red[a]	35.43	
Fat, retroperitoneal	113.16	

[a]20% fat by volume.

cated by its partition coefficient (blood/air) is 14.64–15.6 (Table 6).

Table 7 presents the volumes and perfusion of four tissue groups in relation to the distribution coefficient (rabbit tissue), biological half-time, and distribution volumes, the distribution volume being:

$$V_{dist} = V \times g$$

and the biological half-time being:

$$t_{1/2} = V_{dist} \times (\ln 2)/V$$

The distribution volume indicates the amount of Toluene (expressed in millimoles) that can accumulate in the tissue at a blood concentration of 1 nmol/l. The largest quantity of Toluene accumulates in fatty tissue, with retention being positively dependent on an individual's amount of fat.[62] Carlsson and Ljungquist[60] estimated that the half-life of Toluene in human adipose tissue ranged from 0.5 to 2.7 days.

Results of animal studies indicate a relatively high distribution of Toluene on the stomach wall in the case of inhalation (partition coefficient stomach/blood = 4–5). No data are available on Toluene passing through the placenta.[62]

Mice were exposed to 3950 ppm Toluene (15 mg/l) for 3 h in an inhalation chamber. Concentrations of the compound reached 626 mg/kg in liver, 420 mg/kg in brain, and 200 mg/kg in blood by the end of the exposure.[80,81]

TABLE 7. Volumes and Perfusion of the Four Tissue Groups, Their Partition Coefficients, Biological Half-times and Distribution Volumes for Toluene[62]

Parameter	Symbol (unit)	Tissue group			
		Vessel rich[a]	Muscle[b]	Fat[c]	Vessel poor[d]
Volume in a 70-kg person	V (l)	6.0	33.0	14.5	12.5
Percentage of the minute volume of the heart to tissue group	%	75.0	18.1	5.4	1.5
Perfusion in a 70-kg person at a heart-minute volume of 7 l/minute	V (l/minute)	5.25	1.27	0.38	1.10
Tissue/blood distribution coefficient (rabbit)	λ	2.3	1.6	74.3	1.9
Tissue/blood distribution coefficient (human)	λ	– – –	– – –	81–83	– – –
Biological half-time	$t_{1/2}$ (h)	2 minutes	0.5	77	2.8
Distribution volume	V_{dist} (l)	14	53	1,189	23

[a]Vessel rich group–brain, heart, liver, intestines, kidneys, and endocrine glands.
[b]Muscle group–muscles and skin.
[c]Fat group–fatty tissue and yellow bone marrow.
[d]Vessel poor group–bones, connective tissue, lung tissue.

Rats were exposed by inhalation to 550 ppm methyl-^{14}C-Toluene for 1 h. Immediately after exposure, the amount of radioactivity in white adipose tissue was more than twice the amount in any other organ and more than six times that in brain tissues. Radioactivity in the fatty tissue continued to increase after the exposure had ended and was slightly higher 1 h after exposure than immediately after exposure. All other tissues examined had lower concentrations 1 h after the end of the exposure. Six hours after exposure, radioactivity had decreased to almost zero in the brain and adrenal tissues but was still measurable in the liver, kidneys, and fatty tissue.[59] It was suggested that the radioactivity in the kidneys and liver 6 h postexposure was likely due to Toluene metabolites and excretion products and not to the parent molecule.[50]

Pyykko et al.[74] exposed rats by inhalation to 4600 ppm 4-^{3}H-Toluene for 10 minutes. The concentration of radioactivity reached a maximum in most tissues within 15–30 minutes; the concentration in white adipose tissue reached a maximum 1 h after exposure. The highest concentration of radioactivity was found in white adipose tissue, followed in order of decreasing concentrations by brown adipose tissue, adrenal, stomach, liver, kidney, brain, blood, and bone marrow. Loss of radioactivity from adipose tissue and bone marrow occurred more slowly than the loss from other tissues.

Distribution of Toluene in tissues following oral exposure is similar to that for inhalation exposure. In rats administered 4-^{3}H-Toluene in a single gastric intubation, maximum radioactivity was reached 2–3 h in all tissues except white adipose tissue, where the maximum occurred 5 h after exposure.[74]

In mice given a single intraperitoneal injection of 0.20 mg/kg of Toluene, almost all of the radioactivity in the adipose tissues was a volatile compound that was probably unchanged Toluene. Approximately 70% of the radioactivity in the brain within 8 minutes after injection was present as a volatile material (again probably unchanged Toluene), whereas most of the radioactivity detected in the liver (64%) and kidneys (78%) was nonvolatile.[82]

A male teenager who died from sniffing glue had the following concentrations of Toluene in his tissues: heart blood, 11 mg/kg; liver, 47 mg/kg; brain, 44 mg/kg; kidneys, 39 mg/kg.[83,84]

It has been suggested that the dissipation of Toluene (radioactivity) from tissues appears to be related directly to the amount of vascularization and perfusion of the tissue as well as the presence of enzyme systems needed to metabolize the parent compound. These factors, along with the high partition coefficient for the compound, provide an explanation for the relatively fast uptake but slow release of Toluene in adipose tissue.[50]

EXCRETION

The major portion of inhaled or ingested Toluene is eliminated within 12 h of the end of exposure as free Toluene in expired air (9–18%) and as the metabolite hippuric acid in the urine (60–75%). Two percent or less of absorbed Toluene appears in the urine as benzylmercapturic acid and as cresol derivatives (glucuronides and sulfates). Metabolism of Toluene to benzylmercapturic acid suggests the formation of reactive intermediates that potentially could bind to tissue macromolecules,[1] but no such binding has been demonstrated. Small quantities of free Toluene and benzyl alcohol are excreted in the urine and feces.[62]

Srbova and Teisinger[85] reported that following inhalation exposure of humans to Toluene, approximately 16% of the absorbed compound was exhaled unchanged through the lungs, whereas 80% was oxidized to benzoic acid and excreted in the urine. A small amount (0.6%) of absorbed Toluene was excreted in the urine unchanged. Von Oettingen et al.[86] exposed humans by inhalation to 50 and 800 ppm Toluene for 8 h. Urinary metabolites consisted of approximately 59% hippuric acid and 41% benzoyl glucuronide. Urinary excretion of metabolites increased with the concentration of Toluene and was essentially complete within 14 h. In other studies, small amounts of free Toluene were detected in the expired air of humans exposed dermally to either 200 mg of liquid Toluene[65] or 600 ppm Toluene vapor for 3.5 h.[69] In the latter investigation, samples of exhaled air had detectable quantities of Toluene for at least 4 h after the exposure ended, but no Toluene was detected in samples 20 h after exposure. Respiratory protection was used in this study to preclude inhalation of the vapor.[69]

A commonly used test to determine occupational exposure to Toluene is based on the excretion of hippuric acid and/or o-cresol in the urine.[87-92]

TOXICOLOGY

Acute Oral Toxicity

Reported acute oral LD^{50} values for Toluene in rats range from 2.6 g/kg to 7.53 g/kg (Table 8), making this solvent "practically nontoxic" according to the classification system of Hodge and Sterner.[93]

In two separate studies, nail products containing Toluene were evaluated for acute toxicity. In the first evaluation, a nail basecoat containing 33.2% Toluene was administered by stomach tube to five female, albino rats. Animals were observed for 7 days following the single 15.0 g/kg dose. No deaths were reported, and all rats had normal weight gains.[99] In the second study, a nail polish formulated with 33% Toluene was given by gavage to 10 Sprague-Dawley rats (5 males, 5 females). No deaths or "toxic effects" were observed following the single 5 ml/kg dose.[100]

Acute Effects from Intraperitoneal Injection

Mortality is produced in rats and mice by a single injection of Toluene in the dose range of 0.8 to 1.7 g/kg.[101-103]

Koga and Ohmiya[82] estimated an IP LD_{50} for Toluene in male mice of 1.15 g/kg; respiratory failure was the primary cause of death in these animals. An IP LD_{50} of 1.64 g/kg was reported for female mice by Ikeda and Ohtsuji.[104]

In rats, a single IP Toluene dose of 0.65 g/kg produced apathy, whereas 1.5–1.7 g/kg produced death from respiratory failure.[103] A single 1.7 g/kg dose also was lethal to rats, mice,[102] and guinea pigs.[105]

Savolainen[106] observed that the concentration of radioactivity in the CNS was highest in the cerebrum following IP injection of methyl ^{14}C-Toluene; radioactivity was not detected in the CNS 24 h after the single exposure.

Acute Effects from Intravenous Injection

Intravenous injection of 0.2 ml Toluene/kg (0.17 g/kg) produced 100% mortality in 15 rabbits.[107]

TABLE 8. Acute Oral Toxicity

Animal tested	*Oral LD_{50}*	*Reference*
Rats	7.53 g/kg	94, 95
Wistar adult rats	7.0 g/kg	96
Sprague-Dawley rats	5.58 g/kg	97
Sprague-Dawley rats		98
14-day old	2.6 g/kg[a]	
Young adult	5.5 g/kg[a]	
Mature adult	6.4 g/kg[a]	

[a]Analytical grade Toluene.

Acute and Subchronic Effects from Subcutaneous Injection

In acute studies, a single subcutaneous injection of 1.1–1.25 g/kg and 4.3–8.7 g/kg Toluene produced mortality in rats and mice, respectively.[102,103] Braier[107] reported that a single 4 ml Toluene/kg dose injected into rabbits produced marked transient granulopenia within 24 h and marked granulocytosis and ensuing death in all animals by the end of the second day. A small area of induration was seen at the injection site.

The subchronic effects of Toluene in rats, guinea pigs, and rabbits were also evaluated. Toluene was administered to rats by subcutaneous injection at a dose of 1 ml/kg for 21 days. Treated animals had slight induration at the injection site, decreased body weights, transient decrease in erythrocyte and leukocyte counts, hyperplasia of bone marrow, moderate hyperplasia of Malpighian corpuscles in the spleen, marked pigmentation of the spleen, focal necrosis of the liver, and slight cloudy swelling in the kidneys. No lesions of the heart, testes, or lungs were noted.[103]

Guinea pigs were given Toluene by subcutaneous injection at a dose of 0.25 ml/day for 30–70 days. Necrosis developed at the injection site. Polypnea and convulsions occurred during the last days of survival (survival period: 30–70 days). Hemorrhagic, hyperemic, and degenerative changes in lungs, kidneys, adrenal glands, liver, and spleen also were noted.[108]

Subcutaneous injection of 1 ml/kg of Toluene for 6 days produced transient granulopenia and granulocytosis in rabbits; no change in the bone marrow was observed.[107]

Dermal Toxicity

The acute dermal LD_{50} of Toluene (single dose) in rabbits was 14.1 ml/kg.[94,95] In another study, a percutaneous dose of 1.732 g/kg failed to kill any guinea pigs.[105]

Increased local capillary permeability in rabbits[109] and hemoglobinuria in rats[110] were observed following application of Toluene to the skin. Application to the skin of 1 ml of Toluene for 16 h produced karyopyknosis, karyolysis, spongiosis, perinuclear edema, and cellular infiltration in the dermis; no hepatic or renal damage was noted.[111] Reduced weight gain was noted for 1–3 weeks in guinea pigs treated percutaneously with 2.0 ml of Toluene. However, body weights were comparable to those of control animals at week 4.[105]

Skin Irritation

Undiluted Toluene produced slight to moderate skin irritation in rabbits when tested by four different procedures. Slight skin necrosis also was observed in one study in which Toluene was repeatedly applied to rabbit skin over a 2–4-week period (Table 9).

Two nail products were evaluated in separate studies for skin irritation. In the first evaluation, a nail basecoat (0.5 ml) containing 33.2% Toluene was applied under an occlusive patch to the clipped skin of each of nine albino rabbits. After 24 h, the patches were removed and the treated sites graded for erythema and edema. Five of the nine rabbits developed skin reactions following the single

TABLE 9. Skin Irritation

Material tested	*Animals tested*	*Method*	*Results*	*Reference*
Undiluted Toluene	6 male albino rabbits	*Journal Officiel de la Republique Francaise*[112,113]: 0.5 ml applied under occlusive dressing to clipped skin (intact and abraded) for 23 h	Moderate skin irritation (PII = 3.25/8.0)	117
Undiluted Toluene	6 male albino rabbits	Association Francaise de Normalisation[114]: 0.5 ml applied under occlusive dressing to clipped skin (intact and abraded) for 4 h	Moderate skin irritation (PII = 3.42/8.0)	117
Undiluted Toluene	3 albino rabbits	Organisation for Economic Cooperation and Development[115]: 0.5 ml applied under occlusive and semiocclusive dressing to intact clipped skin for 4 h	Slight skin irritation (occlusive dressing: PII = 2.94/8.0) Slight skin irritation (semiocclusive dressing: PII = 2.13/8.0)	117
Undiluted Toluene	Unspecified number of albino rabbits	Adams et al.[116]: 10–20 applications were repeatedly made to ear and shaved abdomen over a 2–4-week period	Slight to moderate skin irritation and slight skin necrosis	96

exposure. Of these five, three rabbits had minimal erythema and two had moderate erythema. The remaining four animals had no skin irritation.[118] It was not reported whether the product was applied with or without solvents.

In the second study, a nail polish containing 33% Toluene was applied "dry" under a semiocclusive patch (open) to the clipped, intact skin of six female, albino rabbits. Applications of the product (0.5 ml) were made every other day for a total of three exposures. Skin reactions were evaluated both 24 and 48 h after each application. No skin irritation was observed.[119]

Ocular Irritation

Results of studies with rabbits indicate that Toluene is an ocular irritant. However, the results have varied as to the degree of irritation produced. These studies are summarized below and in Table 10.

The ocular irritation potential of undiluted Toluene was assessed in male albino rabbits. A single 0.1 ml dose of the test material was instilled into one eye of each of 12 rabbits; the untreated eye served as control. Treated and control eyes were either given no rinse (6 rabbits) or were rinsed with a solution of boric acid, sodium borate, sodium chloride, and phenylmercury borate 30 seconds after instillation (6 rabbits). Lesions of the conjunctiva, iris, and cornea were scored over a 7-day observation period by means of the numerical system of Kay and Calandra.[120] For rabbits given no rinse, the highest irritation index at any one evaluation was 22.67 (max = 110), indicating that Toluene was an "irritant." For

TABLE 10. Ocular Irritation

Material tested	*Animals tested*	*Method*	*Results*	*Reference*
Undiluted Toluene	12 albino rabbits	Single application to one eye of each rabbit; eye received either no rinse (6 rabbits) or a borate solution rinse (6 rabbits)	No rinse: "irritant" With rinse: "slight irritant"	121
Undiluted Toluene	Unspecified number of albino rabbits	2 drops instilled into right eye with no further treatment; eyes observed up to 7 days thereafter	Slight irritation of conjunctival membrane; no corneal injury	96
"Excess of 15%" Toluene in propylene glycol, water, and/or deodorized kerosene	5 albino rabbits	Single application of 0.005 ml to cornea; no water rinse given	Severe ocular irritation	94, 122

rabbits given an eye rinse, the highest irritation index was 13.33, indicating that the test substance was a "slight irritant".[121]

Slight irritation of conjunctival membranes was observed after application of 2 drops of undiluted Toluene to the right eye of an unspecified number of albino rabbits. No corneal injury was noted.[96]

In a range-finding study with rabbits, a single 0.005 ml dose of Toluene produced severe ocular irritation when instilled into the cornea at concentrations in excess of 15%. Vehicles used included propylene glycol, water, and deodorized kerosene. A total of five eyes were treated; the treated eyes received no water rinse.[122] Similar results were reported by Smyth et al.[94]

A nail polish formulated with 33% Toluene was evaluated for ocular irritation. The product was instilled in a single 0.1 ml dose into the conjunctival sac of one eye of each of nine albino rabbits. The untreated eyes served as the control. Three of the nine female rabbits received no water rinse after treatment, a second group of three rabbits received a water rinse in the treated eye 4 seconds after exposure, and a third group of three received a water rinse in the treated eye 2 seconds after product instillation. In the "no rinse group," all three animals developed erythema of the conjunctivae, which cleared by day 9. One of these rabbits also developed chemosis of the conjunctiva, which cleared by day 6. In the "4 second group," one of three rabbits had erythema and chemosis, which cleared by day 7. No rabbits in the "2 second group" developed irritation. The investigator concluded that the nail polish was a "mild eye irritant."[123]

Acute Inhalation Toxicity

In studies with mice, acute inhalation LD_{50} values for Toluene of 5320 ppm (<0.1% benzene) and 6942 ppm (99.5% purity) were estimated by Svirbely et al.[124] and Bonnet et al.,[125] respectively. The exposure period was 6–7 h.

Inhalation of 4000 ppm technical grade Toluene for 4 h produced death in one of six rats.[94,95] Inhalation of 4000 ppm Toluene (purified by distillation) for 4 h was lethal to two of three guinea pigs within a few days; the third animal was severely prostrated.[126] Inhalation of 55,000 ppm was lethal to six rabbits within 24–62 minutes.[127] Von Oettingin et al.[128] reported that inhalation of 850 ppm Toluene (0.01% benzene) for 1 h by six dogs produced an increase in respiratory rate and a decrease in respiratory volume.

RD_{50} values for Toluene of 5300 ppm[129] and 3373 ppm[130] were estimated for Swiss mice. The RD_{50} is the concentration necessary to depress the respiratory rate by 50%.

Male Sprague-Dawley rats were exposed by inhalation to 0 or 2000 ppm Toluene for 48 h. Compared to nontreated controls, the treated rats had impaired psychomotor performance, elevated blood glucose, elevated serum alanine aminotransferase and aspartate aminotransferase, increased packed cell volume, and decreased body weight.[131]

Inhalation of 4000 ppm Toluene (99.9% pure) for 3 h by male ICR mice had no effect on blood lactate dehydrogenase activity. However, a significant increase in serum glutamic-oxaloacetic transaminase activity was observed 24 h after the single exposure.[132]

Mucous membrane irritation and incoordination were noted in rats exposed by inhalation to 1250 ppm Toluene for 18–20 h.[103]

Subchronic Inhalation Toxicity

Progressive symptoms typically observed in experimental animals after subchronic inhalation exposure to increasingly higher concentrations of Toluene include irritation of the mucous membrane, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death.[1] Results of selected subchronic inhalation studies are summarized in Table 11.

Chronic Oral and Inhalation Toxicity

Results of four studies indicated no major toxicological effects in rats following chronic oral or inhalation exposure to Toluene. However, toxic effects were observed in a fifth study in which dogs were exposed by inhalation to $\geq$2000 ppm Toluene for 6 months. These studies are discussed below.

The chronic oral toxicity of Toluene was assessed by Wolf et al.[96] Three groups of 10 female Wistar rats were given an olive oil solution of Toluene emulsified with a 5–10% aqueous solution of acacia by oral intubation. Toluene doses were either 118, 354, or 590 mg/kg per day 5 days a week for 6 months (193 days). The total volume of the test solution administered daily was never greater than 2–3 ml. A group of 20 rats served as controls and were given doses of 2.5 ml of olive oil emulsified in the acacia solution. No adverse effects were noted with respect to general appearance, behavior, growth, body and organ weights, blood urea nitrogen, total erythrocyte and leukocyte counts, differential leukocyte counts, or hemoglobin concentration. At necropsy and microscopic examination, no treatment-related changes were found in lungs, heart, liver, kid-

ney, spleen, testes, adrenals, pancreas, and femoral bone marrow. No deaths were reported.

The chronic toxicity of Toluene (>99.98%) by inhalation was assessed in Fischer-344 rats. Four groups of 240 animals (120 males and 120 females per group) were exposed for 6 hours/day, 5 days/week, for up to 24 months to Toluene concentrations of 0, 30, 100, or 300 ppm. The calculated time-weighted average concentrations for the 24 months of exposure were 0.0, 30.1, 99.7, and 299.0 ppm, respectively. Randomly selected rats were killed after 6, 12, or 18 months to determine progression of toxic effects; all remaining animals were killed for study after 24 months. Males in the Toluene treatment groups were heavier than control males throughout the study. However, there was no dose–response relationship demonstrable within the treatment groups. The 300 ppm group had increased mean corpuscular hemoglobin concentration. Female rats exposed to 100 and 300 ppm for 24 months had reduced packed cell volumes. Other hematological parameters were not significantly different from controls. There were no significant differences among control and treated groups with respect to clinical appearance, blood chemistries (BUN, SGPT, SAP), urinalysis (pH, specific gravity, microscopic and biochemical constituents), absolute organ weights (brain, heart, kidneys, liver, lungs, gonads), or gross and microscopic lesions. The authors concluded that Toluene caused no chronic toxicity or oncogenicity at the concentrations tested. A partial list of the large battery of tissues and organs examined included brain, pituitary, heart, lungs, esophagus, adrenals, lymph nodes, kidney, bladder, ovaries, stomach, thymus, skin, mammary gland, bone marrow, nasal turbinate, eyes, and testes.[138] The National Toxicology Program has determined that this study was inadequate for carcinogenicity evaluation. The several factors that preclude a definite conclusion of noncarcinogenicity are outlined by the Syracuse Research Corp.[1]

Groups of 15 Sprague-Dawley rats of each sex were exposed by inhalation to Toluene concentrations of 1, 100, or 1481 ppm Toluene 6 h per day, 5 days per week for 26 weeks. Initially, the high-dose group was exposed to 2000 ppm, but the dose was lowered to 1500 ppm after seven exposures because CNS depression was apparent. A battery of blood and clinical chemistry tests (BUN, SGPT, SAP, glucose), urinalysis, and neurohistological examination of tissue were performed. The only treatment-related effects observed were an increased incidence of dry rales and staining of the anogenital fur in the high-dose treatment group. Significant changes in the values obtained for tests of blood and urine were not found, with the exception of a dose-related decrease in blood glucose values and a dose-related increase in SGPT activities in female rats. Body weights were significantly greater in the high-dose male rats than in the control rats, but this was not considered a toxic effect. Treatment-related neurohistopathological changes were not found.[139]

Exposure of 24 OFA rats to 1000 ppm Toluene by inhalation for 6 h a day, 5 days a week for 6 months produced no treatment-related effects. No differences were observed between treated and nontreated control rats with respect to body weight gain, hematological parameters (RBC and WBC counts, hemoglobin, mean corpuscular volume, packed cell volume, sedimentation rate), and tissue histology (lungs, liver, spleen, kidneys, gonads, and other unspecified "principal" organs).[140]

TABLE 11. Subchronic Inhalation of Toluene

Animal	*Toluene tested*	*Results*	*Reference*
Male Sprague-Dawley rat	1000 ppm 8 h/d × 7 d × 13 wk	Retarded weight gain during exposure; however, weight gain similar to controls by end of experiment. Hematocrit values, psychomotor performance, blood glucose, serum ALAT, and serum ASAT also similar to controls	131
Rat	1600 ppm 18 to 20 h/d × 3 d	Mild twitching; drop in body temperature; death. Histology: severe cloudy swelling of kidneys; no effect on liver, heart, or testes	103
Rat	3184 ppm 4 h/d × 30 d	Increased activities of SGOT, SGPT, and increased concentrations of β-lipoproteins. Decreased activities of catalase, peroxidase, and decreased concentrations of glutathione and total cholesterol	133
Rat	2500 ppm or 5000 ppm 7 h/d × 5 d × 5 wk	Transient decrease in body weight, hyperactivity, marked incoordination, recovery after cessation of exposure; mortality in 5000 ppm group (18/25); increased bleeding time; reduced leukocyte count after each exposure; pulmonary lesions; casts in renal tubules in all rats within 2 wk of exposure	128
Rat	5 d/wk × 15 wk	Cytochrome P-450, ethoxycoumarin o-deethylase increased; UDP glucuronsyltransferase increased only at end of exposure	128
Male Sprague-Dawley rat	7 consecutive cycles daily, 5 d/wk × 8 wk: each cycle, 10 min of 12,000 ppm followed by 20 min toluene-free recovery interval	Depression of body weight gain; increased SGOT, serum LDH activities; no effect on BUN levels. Depression of kidney, brain, and lung weights. No lesions of brain, lung, liver, heart, or kidney; no indication of hepatic lipid vacuolation	132
CFY rat (both sexes)	265 ppm 6 h/d × 5 d/wk for 1, 3 or 6 mo	Bromsulphthalein retention decreased; cytochrome P-450 increased independent of period of exposure; SGOT and SGPT activities unaffected	134
CFY rat (both sexes)	929 ppm 8 h/d × 5 d/wk for 1 wk, 6 wk, or 6 mo	Cytochrome P-450 increased independent of exposure period; no effect on SGOT or SGPT; aniline hydroxylase and aminopyrine N-demethylase activity increased; cytochrome b_5 concentrations increased. Dilation of cisternae of rough endoplasmic reticulum; increase of autophagous bodies, which was dose and time dependent; retarded growth of females but not males; glycogen content decreased	134
Male CFY rat	398, 796, 1592 ppm 8 h/d × 5 d/wk × 4 wk	Cytochrome P-450 increased with dose	134
Rat, guinea pig, dog, monkey	107 ppm continuously for 90 d, or 1085 ppm 8 h/d, 5 d/wk × 6 wk	No effect on leukocytes, hemoglobin, or packed cell volume. No lesions of liver, kidney, lungs, spleen or heart; no effect on brain or spinal cord of dogs and monkeys	135

Male ICR mice	7 consecutive cycles daily, 5 d/wk × 8 wk: each cycle, 10 min of 12,000 ppm followed by 20 min toluene-free recovery interval	Depression of body weight gain; no effect on serum LDH; decreased BUN concentrations; SGOT activities increased (not significantly). Depression of kidney, brain and lung weights. Histology: no lesions of brain, lung, liver, heart or kidneys; no indication of *hepatic lipid vacuolation*	132
Male ICR mice	4000 ppm for 3 h/d × 1, 3, or 5 d	SGOT activities increased after 1 and 3 days of treatment; no effect 24 h after 5 d	132
Male ICR mice	4000 ppm for 3 h/d × 5 d/wk × 8 wk	Depression of body weight gain during first 7 wk; increased liver-to-body weight ratio after 4 wk exposure, no effect at 1, 2, or 8 wk. No increase in kidney, brain, or lung weights. SGOT activity increased after 4 wk of exposure and 2 wk postexposure, but not after 2 wk or 8 wk of exposure; no change in BUN. No lesions of heart, lung, kidney, brain or liver	132
Mice	1, 10, 100, or 1000 ppm 6 h/d × 20 d	No effect on body weight; 1 and 10 ppm produced increase in RBC count on 10th day, recovery on day 20; 100 ppm and 1000 ppm produced decrease of RBC count; all doses produced increase (40–70%) of WBC count on day 10, recovery for all doses except 1000 ppm; 10 ppm to 1000 ppm produced slight decrease in density of bone marrow cells and in megakaryocytes and red cell elements; 1000 ppm produced slight hypoplasia of red cell elements, slight to moderate disturbance in maturity of neutrophils and thrombocytes, moderate increase of reticulocytes. No lesions in brain, lung, liver, spleen, or kidney	136
Guinea pig	1250 ppm 4 h/d × 6 d/wk (18 exposures)	*Prostration, marked liver and renal degeneration, marked pulmonary* inflammation	126
Guinea pig	1000 ppm 4 h/d × 6 d/wk (35 exposures)	Slight toxic degeneration in liver and kidney	126
Dogs (2 experimental, 1 control)	2000 ppm 8 h/d × 6 d/wk × 4 mo, and then 2660 ppm 8 h/d, 6 d/wk × 2 mo	Death on days 179 and 180; slight nasal and ocular irritation; motor incoordination and paralysis of extremities during terminal phase, congestion in lungs, hemorrhagic liver, reduced lymphoid follicles and hemosiderosis in spleen; hyperemic renal glomeruli; albumin in urine	137
Dogs	200, 400, or 600 ppm: three 8 h exposures for 1 wk then five 7 h exposures for 1 wk and finally 850 ppm for 1 h	No effect on circulation or spinal pressure; increase of respiratory rate, small increase of minute volume, decrease of respiratory volume	128
Dogs	400 ppm 7 h/d × 5 d	Moderate temporary lymphocytosis	128

h, hour; d, day; wk, week; SGOT, serum glutamic oxalacetic transaminase; SPGT, serum glutamic pyruvic transaminase; ALAT, serum alanine aminotransferase; ASAT, serum aspartate aminotransferase; WBC, white blood cell; RBC, red blood cell; UDP, uridine 5'-phosphate; BUN, blood urea nitrogen; mo, *month.*

Two dogs were exposed 8 h a day, 6 days a week for 4 months to 2000 ppm Toluene. Following this 4-month exposure, the Toluene concentration was increased to 2660 ppm for 8 h a day, 6 days a week for an additional 2 months (6 months total exposure). Slight nasal and ocular irritation occurred at the lower concentration, and motor incoordination that preceded paralysis of the extremities occurred in the terminal phase. Death occurred on days 179 and 180, respectively. There was no effect on gain in body weight, on the bone marrow, or on the adrenal, thyroid, or pituitary glands. Congestion in the lungs, hemorrhage in the liver, a decrease of lymphoid follicles, and hemosiderosis in the spleen were observed. Glomeruli of the kidneys were hyperemic, and albumin was found in the urine.[137]

Genotoxicity

Toluene was negative for mutagenicity in a battery of microbial, mammalian cell, and whole organism test systems. However, there were several reports of increased chromosome aberrations in the bone marrow of rats exposed by inhalation or by subcutaneous injection to Toluene,[141–143] as well as reports of increased sister-chromatid exchanges and chromosome aberrations in the lymphocytes of workers chronically exposed to Toluene.[144,145] These studies are summarized below.

No mutagenicity was observed when Toluene was tested in the Ames *Salmonella* assay with strains TA1535, TA1537, TA1538, TA98, and TA100 or in the *Escherichia coli* WP2 reversion to trp^+ prototrophy assay (Table 12).[146–150] These reverse mutation assays were all performed in the presence and absence of Aroclor 1254-induced rat liver hemogenate (S-9) and employed positive and negative controls. It should be noted that there may have been significant losses of Toluene from the culture media during incubation in all but one of the aforementioned studies.[150] Snow et al.[150] conducted plate incorporation assays in sealed plastic bags and chambers as well as vapor exposures in desiccators to prevent excessive evaporation.

Toluene was tested with and without metabolic activation in *S. cerevisiae* for the (1) induction of reversions to isoleucine independence in strain D7,[147] (2) induction of mitotic gene conversion to tryptophan independence in strains D4 and D7,[146] and (3) induction of mitotic crossing-over at the ade2 locus in strain D7.[147] The compound did not produce any positive mutagenic response in any of these assays (Table 12).

The photochemical formation of mutagens from various aromatic compounds was studied by Suzuki et al.[151] An aqueous solution containing Toluene and an aqueous nitrate solution containing Toluene were both irradiated with a 100 W high pressure mercury lamp (250–577 nm). The reaction mixtures were then evaluated for mutagenicity in a modified Ames assay, using *S. typhimurium* (TA98) in both the presence and absence of liver microsomal fraction (S-9) of PCB-induced rats. A positive mutagenic response was observed in bacteria exposed to photolytic products of the Toluene/nitrate solution. Mutagenic responses were greater in those assays in which there was an absence of metabolic activiation, as compared to those assays in which S-9 activation was present. No mutagenicity was observed in bacteria exposed to photolytic products in the nitrate-free solution.

TABLE 12. Microbial Mutagenicity Assays

Type of assay	*Strain*	*Metabolic activation*[a]	*Toluene dose*	*Application*	*Mutagenic response*	*Reference*
Reverse mutation						
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.001 to 5.0 μl/plate	Plate incorporation	Negative	146
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.004 to 0.031%[b]	Liquid suspension	Negative	146
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.01 to 10 μl/plate	Plate incorporation	Negative	147
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	5 μl/plate	Plate incorporation	Negative	148
S. typhimurium	TA98, 100, 1535, 1537, 1538	Yes and no	0.115–2.3 μl/plate	Plate incorporation	Negative	149
S. typhimurium	TA98, 100	Yes and no[c]	0.3 to 100 μl/plate	Plate incorporation[d]	Negative	150
		Yes and no[c]	11 to 3764 ppm	Vapor exposure[e]	Negative	
E. coli	WP2	Yes and no	0.01 to 10 μl/plate	Plate incorporation	Negative	147
S. cerevisiae	D7	Yes and no	0.001–0.5%[f]	Liquid suspension	Negative	147
Mitotic gene conversion						
S. cerevisiae	D4	Yes and no	0.001–5.0 μl/plate	Plate incorporation	Negative	146
		Yes and no	0.138–1.1%[b]	Liquid suspension	Negative	
S. cerevisiae	D7	Yes and no	0.001–5.0%[f]	Liquid suspension	Negative	147
Mitotic crossing-over						
S. cerevisiae	D7	Yes and no	0.001–5.0%[f]	Liquid suspension	Negative	147

[a]Aroclor 1254-induced rat liver homogenate S-9 fraction.
[b]50% mortality at the highest dose.
[c]The Toluene was tested with Toluene-induced S-9 as well as with Aroclor-induced S-9.
[d]The plates were incubated in sealed plastic bags or chambers for part of a 72-h incubation period. In the Aroclor-induced S-9 tests, the plates were removed from the bags after 48 h, counted, incubated an additional 24 h, and recounted. In the experiments with Toluene-induced S-9, the plates were removed after 24 h to prevent moisture and spreading problems, and then incubated an additional 48 h before counting.
[e]The assays were run in a sealed incubation chamber with a second glass plate (open) that contained the Toluene; after 24 h the chambers were opened and the plates incubated for an additional 48 h.
[f]100% mortality at 0.1% and 0.5%.

Inhibition of growth and induction of DNA damage by Toluene were evaluated in two studies by comparing differential toxicity to wild-type and DNA repair deficient bacteria. Two species were tested with and without metabolic activator with negative results: *E. coli* (W3110 pol A^+ and p3478 pol A^-) and *S. typhimurium* (SL4525 rfa rec^+ and SL4700 rfa rec^-).[147,152]

Breaks in single DNA strands were observed by Sina et al.[153] in rat hepatocytes exposed in vitro for 3 h to 2.3 mM Toluene. Toluene vapor was a potent mitostat in studies with intact grasshopper embryos (*Melanophus sanguinipes*). Arrested metaphases of the exposed embryos had a c-mitotic appearance and highly contracted and scattered chromosomes.[154] Roots of *Vicia faba* seedling exposed to Toluene developed chromosomal alterations and a longer than normal mitotic cycle.[155]

Drosophila melanogaster males were fed 500 or 1000 ppm Toluene for 24 h. No significant increase in recessive lethals was noted in the total of 3281 X-chromosomes examined.[156]

The ability of Toluene to induce dominant lethal mutations in sperm cells was evaluated by Litton Bionetics.[157] CD-1 mice were exposed by inhalation to 100 or 400 ppm of the compound 6 h per day, 5 days per week for 8 weeks. No increase in pre- or postimplantation loss of embryos or reduction in fertility of treated males was observed.

Toluene failed to induce specific locus forward mutation in the L5178, thymidine kinase mouse lymphoma cell assay. The compound was evaluated at concentrations of 0.05–0.30 μl/ml, with and without mouse liver S-9 activation.[146]

In the micronucleus test, Toluene doses of 250, 500, and 1000 mg/kg were given by IP administration to groups of 32 Swiss male mice. No increase was observed in micronucleated polychromatophilic erythrocytes of the bone marrow.[158] In a second micronucleus test, Toluene induced no clastogenic activity when administered in two oral doses of either 860 or 1720 mg/kg to male and female CD-1 mice.[159]

Two reports concluded that Toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection.[142,143] In an analysis of 720 metaphase cells from the bone marrow of five rats that had been subcutaneously injected with 0.8 g/kg per day Toluene for 12 days, Dobrokhotov[142] found 78 (13%) with chromosomal aberrations. Sixty-six percent of the aberrations were chromatid breaks, 24% were chromatid "fractures," 7% were chromosome "fractures," and 3% involved multiple aberrations. The frequency of spontaneous aberrations in 600 marrow metaphase cells from five control rats injected with vegetable oil averaged 4.16% (65.8% were breaks and 32.4% were chromatid aberrations; no "fractures" or multiple lesions were recorded). The significance of the positive clastogenic effects attributed to Toluene in this study is difficult to assess, since the purity of the sample employed was not stated and because the distinction between chromatid breaks and fractures was unclear.

Lyapkalo[143] administered 1 g/kg per day Toluene to six rats by subcutaneous injection for 12 days. Treatment with Toluene resulted in chromosome aberrations in 11.6% of the bone marrow cells examined (84 aberrant metaphases/724 cells) compared with 3.9% (40/1033) in olive oil injected controls. The types of aberrations that were observed consisted of "gaps" (60.5%), chro-

matid breaks (38.4%), and isochromatid breaks (1.2%). The purity of the Toluene used in this study was not stated.

Dobrokhotov and Enikeev[141] reported that rats exposed to 80 ppm (610 mg/m^3) Toluene by inhalation 4 h daily for 4 months had damaged metaphase chromosomes in 21.6% of the bone marrow cells analyzed. The percentage of metaphase cells with damaged chromosomes in bone marrow cells from air-exposed control rats was 4.0%. The number of cells evaluated and the purity of the Toluene were not specified.

In contrast to the aforementioned cytogenetic studies, Litton Bionetics[160] found that IP injection of Toluene into Charles River rats did not induce bone marrow chromosomal aberrations. The compound was injected at doses of 22, 71, and 214 mg/kg in two different experiments. In one study, five rats were killed at 6, 24, and 48 h following injection of each dose; in a second study, five rats were treated daily at each dose for 5 days, and the rats were killed 6 h after injection of the last dose. Approximately 50 cells per animal were evaluated for damage. Dimethyl sulphoxide (DMSO, the solvent vehicle) administered IP at 0.65 ml/rat was used as the negative control, and triethylenemelamine (TEM) in saline at 0.3 mg/kg was used as the positive control.

Male Wistar rats exposed by inhalation to Toluene (300 ppm, 6 h/day, 5 days/week for 15 weeks) did not have more chromosome aberrations in the bone marrow cells than nonexposed control animals. The frequency of sister chromatid exchanges (SCEs) was analyzed in cultured bone marrow cells of the exposed animals only after 11, 13, and 15 weeks of exposure. There was a statistically significant increase of SCEs in rats exposed for 11 and 13 weeks, but the frequency was in the control range after 15 weeks of Toluene exposure.[156]

Evans and Mitchell[161] reported that Toluene did not alter SCE frequencies in cultured Chinese hamster ovary (CHO) cells. In this study, CHO cells without rat liver S-9 homogenate were exposed to 0.0025–0.04% Toluene for 21.4 h, and CHO cells with S-9 homogenate were exposed to 0.0125–0.21% for 2 h.

In vitro exposure to Toluene at concentrations of 1.52 mg/ml, 15.2 μg/ml and 152 μg/ml had no effect on the number of sister-chromatid exchanges (SCEs) or number of structural chromosomal aberrations in cultured human lymphocytes. However, cytotoxicity was observed at the highest dose.[162] The data from this study cannot be adequately evaluated, since the purity of the Toluene was not indicated, no positive control experiments were performed, no metabolic activation system was employed, and the type of scoring system for chromosome damage was not specified.

Lymphocytes from 32 rotogravure workers with occupational exposure to Toluene were studied for chromosome aberrations and SCEs. The frequencies of these did not differ from the corresponding values of 15 unexposed control subjects. However, a significant increase of SCEs was observed in smoking subjects, both occupationally exposed and unexposed.[156]

Peripheral blood lymphocytes of 34 workers from a rotogravure printing plant and of 34 matched controls from outside the plant were compared for chromosomal aberrations. Ten of the workers were exposed daily to benzene (131–532 ppm) for 2–7 years and subsequently to Toluene (200–400 ppm) for 14 years. Twenty-four workers were exposed only to Toluene for 7–15 years. No significant differences were found between the Toluene and control groups in

frequencies of stable and unstable chromosome aberrations or in chromosome counts. Approximately 100 metaphase cells from each subject or control were scored. The proportion of chromosome changes was significantly higher statistically in the benzene/toluene group compared with controls and in the benzene/toluene group relative to the Toluene group.[163]

Maki-Paakkanen et al.[164] reported no evidence of clastogenicity in cultured peripheral blood lymphocytes from 32 workers from two different rotoprinting factories who had a history of exposure to Toluene (benzene concentrations, ≤0.05%) at 8-h, time-weighted average concentrations of 7–112 ppm. The average age of the workers was 34.2 years, and the average length of employment was 14.6 years. Results of analyses indicated that the frequencies of chromosome aberrations and sister-chromatid exchanges were not significantly different from those of 15 unexposed workers. Similarly, no significant deviations were observed in the frequencies of aberrations in relation to duration of exposure.

Bauchinger et al.[144,145] performed cytogenic analyses on peripheral lymphocytes from 20 male rotogravure plant workers exposed for ≥16 years to Toluene (<0.3% benzene). A group of 24 workers from the same plant, but without exposure to Toluene, served as controls. Toluene concentrations in the workroom air ranged from 200 to 300 ppm. Small amounts of liquid Toluene also were used by the workers to wash the hands. The measured concentrations of Toluene in the blood were reportedly between 0.001 and 0.01%. There was no exposure to other chemicals. As compared with the 24 nonexposed controls, exposed workers had a significantly greater number of chromatid breaks, chromatid exchanges, and chromatid gaps. The number of SCEs also was significantly increased in smoking and nonsmoking Toluene-exposed workers compared with the corresponding control group. The authors suggested that Toluene or its metabolite may induce a weak clastogenic effect in human lymphocytes in vivo.

Carcinogenicity

A 2-year inhalation study with rats and mice is being conducted by the National Toxicology Program at Research Triangle Park. The investigation is currently in the tissue evaluation phase, and no publication date has been established.[165] No neoplasms were observed in mice given a subcutaneous exposure to Toluene. Results of skin painting studies in mice were negative for carcinogenicity. These studies are summarized below.

Toluene suspended in an aqueous gel was applied to the surface of a filter disc. The filter disc was then implanted subcutaneously into the dorsolumbar region of 10 male and 10 female Alderley Park Swiss mice. Each filter disc contained 0.02 mmol of Toluene. Three months after implantation, the surviving mice were killed, and the implant site tissue was removed for histopathological evaluation. No tumors were observed.[166]

Toluene was used as a vehicle control in a study in which benzo(a)pyrene was tested for carcinogenicity. Toluene was applied to the shaved interscapular skin of 20 SWR, 17 C3HeB, and 17 A/He female mice three times a week for life. Mice were 10–14 weeks old on the initial exposure. No skin tumors developed in the Toluene-only treated mice.[167]

Benzo(a)pyrene (20 nmol) or 15,16-dihydro-11-methylcyclopent(a)phenan-

thren-17-one (20 nmol) in a vehicle of 10 μl of Toluene/croton oil (99:1 v/v) was applied twice weekly to the shaved dorsal skin of T.O. mice. The number of exposed mice with skin tumors at 75 weeks was 14/20 and 19/20, respectively. No skin tumors were observed at 75 weeks in the 20 mice topically treated twice weekly with the Toluene/croton oil vehicle.[168]

Groups of Swiss male mice were treated on the ears with either (1) 1.5% DMBA in mineral oil (one exposure), (2) 1.5% DMBA in mineral oil (one exposure) followed after 1 week by two weekly exposures of Toluene for 20 weeks, (3) Toluene twice a week for 20 weeks, or (4) DMBA in mineral oil (one exposure) followed by biweekly applications of mineral oil. Of the 23 surviving mice treated once with DMBA, 1 tumor was observed at 20 weeks. Of the 35 surviving mice treated with DMBA and Toluene, 7 tumors were observed. None of the 14 surviving mice treated with Toluene developed tumors. The negative control group (DMBA and mineral oil) had 8 tumors in 53 survivors at 20 weeks.[169]

Frei and Kinsley[170] examined the promoting effect of Toluene in Swiss mice following initiation with 7,12-dimethylbenz[a]anthracene (DMBA). The ears of the mice were topically treated once with 0.1 ml of 1.5% DMBA in mineral oil. One week after DMBA initiation, mineral oil or Toluene was applied twice a week for 20 weeks. Eleven of 35 mice developed tumors (6 permanent, 5 regressing) following exposure to DMBA and Toluene, whereas, 8 of 53 negative control animals (DMBA and mineral oil) developed tumors (all permanent). Fourteen mice topically treated for 20 weeks with Toluene alone (no DMBA initiation) developed 2 tumors (1 permanent, 1 regressing).

Doak et al.[171] applied Toluene (0.05–0.1 ml) to the backs of CF_1, C_3H, and CB_9H mice twice weekly for 56 weeks. For each strain, approximately 50 mice (25 male, 25 female) were tested. No difference was observed between treated and control mice with respect to frequency of skin or systemic tumors. It was not clear in this study if the Toluene was applied under an occlusive dressing or if it was allowed to evaporate.

Toulene was applied twice a week for 50 weeks to the clipped dorsal skin of 20 TO albino mice. Animals were observed for a period of 1 year after treatment. A second group of 20 mice served as untreated controls. No skin tumors developed in either group; however, survival was only 35% (7 of 20) in the treatment group.[172]

No skin tumors were observed in skin painting studies in which 50 mg of Toluene was applied twice a week for 80 weeks to 50 male C3H/HeJ mice[173] or in which Toluene was applied twice weekly for 50 weeks to 10 male and 10 female TO albino mice.[174]

Lijinsky and Garcia[175] used Toluene as a vehicle control in the carcinogenicity testing of various polynuclear hydrocarbons. Toluene (1–20 μl) was applied twice a week for 72 weeks to the clipped interscapular skin of 30 Swiss female mice. Two mice developed skin tumors. One animal developed squamous cell carcinoma, and one developed squamous cell papilloma. The "average latent period" of the first tumor was 58 weeks. Twenty-four and fifteen mice survived to 60 and 80 weeks, respectively.

In a brief abstract, Frei and Ritchie[176] reported that tumor promotion in the skin of mice by Toluene and other "irritant solutions" was associated with the ability of these agents to induce epidermal hyperplasia. No other details were specified.

Teratogenicity and Embryotoxicity

The teratogenicity and embryotoxicity of Toluene were assessed in hamsters, mice, and rats by various routes of exposure (skin, oral, inhalation).

Overman[177] reported that Toluene produced "minimal embryotoxic effects" in hamsters following applications to clipped skin. Applications were made every day for 2 h between days 7 and 11 of gestation. Animals were killed at day 15 of gestation. A decrease in fetal size and weight and an increase in the incidence of prenatal death were noted. Observed malformations included fetal hemorrhage and gastroschisis. No malformations appeared in the control groups. The dose and vehicle were not specified.

Toluene was administered to CD-1 mice by gavage from days 6 through 15 of gestation at 0.3, 0.5, and 1.0 ml/kg body weight per dose. The compound was also given by gavage from days 12 through 15 of gestation at 1.0 ml/kg per dose. The vehicle used was cottonseed oil (0.5% of maternal body weight/dose). Maternal toxicity was not observed after exposure to Toluene (days 6–15) at any dose. However, an increase in embryonic deaths at all doses and a reduction in fetal weight in the 0.5 and 1.0 mg/kg groups was noted. An increased incidence of cleft palate was observed after exposure to 1.0 ml/kg on days 6–15 of gestation. The same dose given on days 12–15 of gestation produced decreased maternal weight gain. The authors concluded that Toluene was teratogenic at 1.0 ml/kg and embryotoxic at 0.3 ml/kg.[178]

Toluene was dissolved in corn oil and administered by gavage for 8 days to pregnant CD-1 mice. The daily oral dose was 10 ml/kg body weight, which corresponded to 2350 mg of Toluene per kg of body weight. No differences between test and control groups were observed in terms of number of maternal deaths, mean maternal weight, number of animals producing litters, incidence of resorbed fetuses, litter size, number of live or dead fetuses, and mean litter weight. The investigators concluded that Toluene caused no significant reproductive toxicity.[179]

Female ICR mice were exposed by inhalation to 100 or 1000 ppm Toluene 6 h a day from the first to seventeenth day of gestation. No differences were observed between control and treated groups with respect to litter size, incidence of resorbed fetuses, number of implantation sites, number of live or dead fetuses, fetal body weight, external malformations, eye or ear opening, weaning, and incidence of body hair. The incidence of skeletal abnormalities was similar between treated and control groups, with the exception of extra 14th ribs and rudimentary 14th ribs in the 1000 ppm dose group. The authors stated that the high incidence of 14th ribs "suggested the possible teratogenicity of Toluene."[180,181]

Hudak and Ungvary[182] studied the teratogenic and embryotoxic effects of Toluene in CFY rats and CFLP mice. The mice were exposed by inhalation to 500 mg/m^3 (133 ppm) Toluene for 24 h/day from days 6 to 13 of pregnancy. Rats were exposed to Toluene in one of three dosage regimens: (1) 1500 mg/m^3 (399 ppm) for 24 h/day from day 9 to 14 of pregnancy, (2) 1500 mg/kg (399 ppm) for 24 h/day from day 1 to 8 of pregnancy, or (3) 1000 mg/m^3 (266 ppm) for 8 h/day from day 1 to 21 of pregnancy. Exposure of mice to 133 ppm Toluene was associated with decreased average fetal weights and an increased incidence of weight-retarded fetuses. Irregular sternebrae and extra ribs were observed in the fetuses of rats treated with 399 ppm Toluene on days 9–14 of pregnancy. Re-

tarded skeletal growth and decreased weights were noted in fetuses of rats exposed on days 1–8 of pregnancy to 399 ppm Toluene. Retarded skeletal development was also observed in the fetuses of rats treated with Toluene at 266 ppm.

Pregnant CFY rats were exposed by inhalation to benzene (400 mg/m^3), Toluene (1000 mg/m^3), or a combination of the two solvents from day 7 to day 14 of gestation. Exposure to benzene or benzene plus Toluene was associated with decreased fetal weight, whereas exposure to Toluene or benzene plus Toluene was associated with increased incidence of extra fetal ribs. Exposure to benzene, Toluene, and benzene plus Toluene caused skeletal retardation in the fetuses but did not produce increases in the rates of external, internal, or skeletal malformations.[183]

In other studies with CFY rats, Toluene potentiated the maternal and embryonic toxicity of acetylsalicylic acid.[184]

No evidence of teratogenicity was observed in the 20-day old fetuses of Charles River rats that were exposed to 100 or 400 ppm Toluene vapor for 6 h/day on days 6–15 of gestation. At microscopic examination, no unusual incidence of visceral or skeletal abnormalities was observed. Unusual skeletal variations were observed in a small but comparable number of fetuses from both the exposed and control groups, but these changes were in most cases attributed to retarded bone ossification and were not considered to be malformations as such. No maternal deaths occurred during the study, and the sex ratio of the offspring did not differ significantly between the treated and control groups.[160]

Groups of 20 CFY rats were exposed to 266 ppm (1000 mg/m^3) Toluene, 125 ppm (400 mg/m^3) benzene, or a combination of these concentrations of Toluene and benzene vapor for 24 h/day on days 7–14 of gestation. A group of 22 rats inhaling air served as controls. Fetuses were examined on day 21 of pregnancy. Continuous exposure to 266 ppm Toluene was not teratogenic (no external, internal, or skeletal malformations were reported), although the exposures were associated with evidence of skeletal retardation (not detailed) and an increased incidence of extra ribs. Also, it was reported that the incidence of extra ribs was higher in the group exposed to Toluene in combination with benzene than in the groups exposed to Toluene alone. Maternal loss, maternal weight gain, number of litters, mean implantation/dam, placental weight, fetal loss, and fetal weight loss were not significantly affected by the Toluene exposures. Exposure to 125 ppm benzene did cause decreases in maternal weight gain, placental weight, and fetal weight, but these effects appeared to be inhibited by concurrent exposure to 266 ppm Toluene. Further, it was reported that postinhalation fetal loss (the number of dead and resorbed fetuses relative to the number of total implantation sites) was significantly increased in the group exposed to benzene in combination with Toluene. Fetal loss was not, as indicated earlier, affected by exposure to the Toluene (or benzene) alone.[185]

CLINICAL ASSESSMENT OF SAFETY

Effects on Skin and Nails

Toluene's degreasing action removes natural lipids of the skin, which in turn may cause dryness, fissures, and contact dermatitis.[186]

Koilonychia and hapalonychia of the fingernails were observed in 6 of 16

cabinet workers exposed percutaneously to a thinner mixture containing 30% Toluene, 30% xylene, and 40% methyl alcohol. Most of the affected workers had an average exposure of 2 years.[187]

No skin irritation was observed when 20 subjects were exposed in a single insult occlusive patch test to a nail basecoat containing 33.2% Toluene.[188] The length of the exposure period was not reported.

A nail polish containing 31.23% Toluene was assessed for its cumulative skin irritation potential. Applications of the product (0.3 ml) were made under closed patches everyday for 21 consecutive days to the skin of the back of 10 subjects. Contact periods were for 23 h, and applications of the product were made to the same site. The composite total score for the 10 subjects treated with nail polish was 16 out of a maximum possible score of 630, indicating minimal irritation with "essentially no evidence of cumulative irritation." Composite scores of 7/630 and 569/630 were reported for baby oil (negative control) and deodorant (positive control), respectively, indicating "no evidence of cumulative irritation" and "strong potential for cumulative irritation."[189]

A repeated insult patch test was used to evaluate the skin irritation and sensitization potential of a nail polish containing 33% Toluene. Occlusive "dry patches" containing the test material were applied to the upper back of 148 subjects (59 males, 89 females) every Monday, Wednesday, and Friday over 3 consecutive weeks for a total of nine induction applications. Following a 2-week nontreatment period, two consecutive 48-h challenge patches were applied to a site adjacent to the original induction site. No skin reactions were observed.[190]

The Maximization Test described by Kligman[191] and Kligman and Epstein[192] was used to assess the sensitization potential of a nail polish containing 31.23% Toluene. The product (0.3 ml) was applied under an occlusive dressing to the forearm of 25 subjects for five 48-h periods. Since the product contained volatile ingredients, the product was applied to the patch and allowed to air-dry prior to application. Throughout the induction phase, the test sites were pretreated with 24-h patches containing 1.5% sodium lauryl sulfate in aqueous solution. Following a 10-day nontreatment period, a challenge patch of the nail polish was applied under occlusion for 48 h to a previously untreated site. The challenge site was pretreated for 1 h with a 10.0% aqueous solution of sodium lauryl sulfate. Evaluations were made 48 and 72 h after treatment. No reactions were observed during the induction or challenge phases.[193]

In separate studies, two nail products were evaluated for phototoxicity and photoallergenicity. One product contained 30% Toluene and was evaluated on 28 subjects.[194] The second product was formulated with 25% Toluene and was assessed on 30 subjects.[195] In each instance, the light source was a xenon Arc Solar Simulator (150W), which was filtered to produce a continuous emission spectrum in the UVA and UVB region (290–400 nm). Prior to testing, each panelist's "minimal erythemal dose" was determined according to the procedures outlined in the Federal Register.[196] For the induction phase, each product (0.1 ml) was applied to the skin of the back under an occlusive patch. Twenty-four hours later, the patch was removed, and the sites were irradiated with three times the individuals' minimal erythemal dose using the full xenon lamp spectrum. Forty-eight hours later, the sites were evaluated. The procedure of product exposure and light exposure was repeated twice weekly for a total of six induction exposures. Following a 10-day nontreatment period, the product was applied under an occlusive patch to a previously untreated site adjacent to the in-

duction site. Twenty-four hours later, the challenge patch was removed, and the sites were irradiated for 3 minutes with a filtered (Schott WG345 filter) light source. Challenge sites were evaluated 15 minutes and 24, 48, and 72 h after UV exposure. Control sites were subjected to the same induction and challenge procedures, with the exception that control sites were not subject to irradiation. No phototoxic or photoallergic reactions were observed to the two nail products formulated with 30% and 25% Toluene.[194,195]

Respiratory Tract and Ocular Irritation

Two male subjects exposed to Toluene for 7–8 h developed transitory mild throat and eye irritation at 200 ppm and lacrimation at 400 ppm.[127] No complaints of respiratory tract irritation were reported by volunteers or workers exposed to Toluene concentrations of 800–1500 ppm for 8 h.[86,128,197]

Transient epithelial injury consisting of moderate conjunctival irritation and corneal damage was noted in three workers who were accidentally splashed in the eyes with Toluene. Complete recovery generally occurred within 48 h.[198]

Effects on Cardiovascular Function

Toluene has been implicated in a number of sudden deaths due to glue or solvent sniffing. In a study of 110 cases of sudden, unexpected death in solvent abusers, Bass[199] reported that the deaths were not due to suffocation secondary to intoxication but were due to a direct effect of the solvent itself. Toluene, benzene, gasoline, trichloroethane, and fluorocarbon propellants were individually implicated as causing sudden cardiovascular collapse. Severe cardiac arrhythmia resulting from light anesthesia was proposed as the most likely explanation for the cause of sudden death. Several authors have suggested that sniffing volatile hydrocarbons may cause sensitization of the myocardium to epinephrine.[199–201]

Ogata et al.[202] found an apparent decrease in the pulse rate of 23 volunteers who were exposed to 200 ppm Toluene for periods of 3 h or 7 h, but no effect was observed in those exposed to 100 ppm concentration. Systolic and diastolic blood pressure were not affected by exposure. Exposure to 100 and 200 ppm Toluene for 30 minutes did not, however, have any effect on the heart rate or electrocardiogram of 15 other subjects during either rest or light exercise.[55] In other studies, experimental exposure to Toluene at concentration of 100–700 ppm for 20 minutes[203] or 50–800 ppm for 8 h[86,128] did not produce any definite effects on heart rate or blood pressure. Suhr[204] observed that the pulse rate and blood pressure of a group of 100 printers with a 10-year history of exposure to 200–400 ppm Toluene and those of an unexposed control group of identical size were similar at the beginning and end of work shifts.

Occupational Exposure Limits

The American Conference of Governmental Industrial Hygienists[205] has adopted for toluene a "threshold limit value-time-weighted average" (TLV-TWA) and a "threshold limit value-short-term exposure limit" (TLV-STEL) of 100 ppm (375 mg/m^3) and 150 ppm (560 mg/m^3), respectively. The TLV-TWA is defined as

the airborne concentration for a normal 8-h workday and a 40-h workweek to which nearly all workers may be repeatedly exposed without adverse effect. The TLV-STEL is the maximal airborne concentration to which workers can be exposed for a period up to 15 minutes without causing irritation, chronic or irreversible tissue change, or necrosis. The threshold limit values are used as guides in the control of health hazards and are not intended to be used to differentiate between safe and unsafe concentrations.

SUMMARY

Toluene is a clear liquid that is insoluble in water. It is produced from either petroleum refining processes, as a byproduct of styrene production, or as a byproduct of coke oven operations. Commercial Toluene may contain benzene as an impurity; however, no data were available regarding the impurity content of cosmetic grade Toluene. Under experimental conditions, Toluene undergoes substitution reactions on the aliphatic side group ($-CH_3$) and on the benzene ring at the ortho and para positions. Under conditions of cosmetic use, Toluene is considered stable and unreactive.

Toluene has a wide variety of noncosmetic applications, including use as an indirect food additive, gasoline additive, solvent, and thinner (Tables 3 and 4). Cosmetic applications include use in nail products as a diluent and solvent. Cosmetic manufacturers participating in the voluntary cosmetic product registration program with the Food and Drug Administration reported that Toluene was used in 1984 in 555 nail and manicuring products. Reported concentrations of Toluene in these products ranged from >10–25% (448 products) to >25–50% (107 products) (Table 5).

The nail, the nail cuticle, and the skin surrounding the nail are the areas directly exposed to cosmetic formulations containing Toluene. Areas of the body that come in contact with the "wet" nail may also become exposed. During application of nail products, Toluene may come in contact with eyes and nasal mucosa as a result of evaporation from the formulation.

In mammals, Toluene is absorbed by the respiratory tract, gastrointestinal tract, and skin. Significant absorption may occur through the intact human skin. In one study, the rate of absorption of undiluted Toluene through the skin of the hands and forearms of humans was estimated at 14–23 mg/cm² per hour.

Although Toluene is likely metabolized to some extent in most mammalian tissues, the major site for metabolism is in the liver. Most of the absorbed Toluene (approximately 84%) undergoes sidechain oxidation to benzoic acid. Benzoic acid is subsequently conjugated with glycine and excreted in the urine as hippuric acid, although a large amount of conjugation with glucuronic acid occurs, resulting in urinary excretion of benzoylglucuronic acid. Small amounts of absorbed Toluene appear in the urine as benzylmercapturic acid and cresol derivatives. Approximately 16% of the absorbed Toluene is expired unchanged through the lungs (Fig. 1).

Toluene is lipophilic and accumulates primarily in those tissues with a high fat content. In one study, the half-life of Toluene in human adipose tissue ranged from 0.5 to 2.7 days.

Toluene was practically nontoxic when given orally to rats; reported acute

oral LD_{50} values ranged from 2.6 g/kg to 7.53 g/kg (Table 8). The acute dermal LD_{50} in rabbits was 14.1 ml/kg. A single IP injection of 0.8–1.7 g/kg was lethal to rats, mice, and guinea pigs.

A single subcutaneous injection of 1.1–1.25 g/kg and 4.3–8.7 g/kg was lethal to rats and mice, respectively. Granulopenia, followed by granulocytosis and eventual death, was noted in rabbits given Toluene as a single, subcutaneous dose of 4 ml/kg. In subchronic studies, rats given Toluene by subcutaneous injection at a dose of 1 ml/kg for 21 days had induration at the injection site, a decrease in body weight, a decrease in erythrocyte and leukocyte counts, hyperplasia of the bone marrow and spleen, focal hepatic necrosis, and nephrosis. Guinea pigs administered Toluene at a subcutaneous dose of 0.25 mg/day for 30–70 days developed polypnea, convulsions, necrosis at the injection site, as well as hemorrhagic, hyperemic, and degenerative changes in lungs, kidneys, adrenal glands, and spleen. Rabbits treated with Toluene at a subcutaneous dose of 1 ml/kg per day for 6 days developed granulopenia and granulocytosis.

Undiluted Toluene produced slight to moderate skin irritation in rabbits when tested by four different procedures. Skin necrosis was slight in one study in which Toluene was repeatedly applied to the skin of rabbits over a 2–4-week period (Table 9). Results of studies with rabbits indicate that undiluted Toluene is an ocular irritant (Table 10).

Acute inhalation LD_{50} values for Toluene in mice were 5320 ppm and 6942 ppm; the exposure period in these two studies were 6–7 h. Effects observed in mice, rats, guinea pigs, rabbits, and dogs after acute inhalation of Toluene included mucous membrane irritation, motor incoordination, prostration, changes in respiratory rate, changes in serum and blood enzyme activities, elevated blood glucose and packed cell volume, decreased body weight, and death. These effects varied according to animal studied, length of Toluene exposure, and Toluene concentration.

Progressive symptoms observed in experimental animals following subchronic inhalation to increasingly higher concentrations of Toluene included irritation of the mucous membranes, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death (Table 11).

No significant treatment-related effects were observed in four studies in which rats were given chronic oral doses or chronic inhalation exposures to Toluene. Parameters examined in those studies generally included appearance, behavior, growth, body and organ weights, blood chemistry, urinalysis, gross and microscopic lesions, and mortality.

Toluene was negative for mutagenicity in a battery of mammalian cell and whole organism test systems. Results of microbial assays also were negative for mutagenicity (Table 12). There were several reports of increased chromosome aberrations in the bone marrow of rats exposed by inhalation or by subcutaneous injection to Toluene, as well as reports of increased sister-chromatid exchanges and chromosome aberrations in the lymphocytes of workers chronically exposed to Toluene.

A 2-year inhalation study with rats and mice is being conducted by the National Toxicology Program at Research Triangle Park. Results of the investigation have not yet been published. No neoplasms were observed in mice given a 3-month subcutaneous exposure to Toluene. With the exception of one report, results of numerous skin painting studies in mice were negative for carcinogenic-

ity. The teratogenicity and embryotoxicity of Toluene were assessed in hamsters, mice, and rats by various routes of exposure (skin, oral, inhalation). Results of these studies were mixed.

Clinical data were limited to five studies involving cosmetic products. No skin irritation or sensitization was observed in subjects treated with cosmetic products containing 31–33% Toluene. No phototoxic or photoallergic reactions were noted in subjects treated with 25 or 30% Toluene.

Throat irritation, eye irritation, and/or lacrimation were noted in two subjects exposed to airborne concentrations of 200 and 400 ppm Toluene. Transient irritation of the conjunctiva and transient injury of the cornea were observed in several workers accidentally exposed in the eyes to Toluene.

Toluene has been implicated in a number of sudden deaths due to glue or solvent sniffing. Several reports also suggested that sniffing volatile hydrocarbons may cause sensitization of the myocardium to epinephrine.

DISCUSSION

No data were available to the CIR Expert Panel regarding the impurities found in cosmetic grade Toluene. One possible impurity, benzene, is a carcinogen. Therefore, cosmetic products formulated with Toluene should be benzene-free.

Two studies concluded that Toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection.[142,143] The significance of positive clastogenic effects attributed to Toluene in these studies is difficult to assess, since the purity of the test samples was not reported. More definitive carcinogenic studies were available. In eight studies, Toluene did not induce cancer. In one study, 1 of 30 mice developed skin cancer; however, there were no untreated controls for comparison.

Results of animal studies indicated that undiluted Toluene is a skin irritant. Thus, there is a potential for Toluene to cause skin irritation in humans. However, the sole cosmetic use of Toluene is in products intended to be applied directly to the nail. Therefore, human skin exposure to this ingredient will be minimal under conditions of cosmetic use.

CONCLUSION

On the basis of the available data presented in this report, the CIR Expert Panel concludes that Toluene is safe as a cosmetic ingredient in the present practices of use and concentration.

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C I R

March 15-16, 2005

Memorandum

To: CIR Expert Panel

From: Melody Chen
Scientific Analyst and Writer

Subject: Re-review of Toluene

In 1987, CIR issued a Final Report that Toluene "is safe for cosmetic use at the present practices of use and concentration" despite limited skin exposure data. Toluene reportedly was used in 555 nail care products in FDA's 1984 database; most uses were in the 10-25% range, but just over 100 were in the 25-50% range. In 2002, there were only 59 reported uses in nail products with concentrations in a 20-26% range.

A review of the recent literature uncovered numerous new references. Because of the sheer number, not all of the references were included in this re-review, but a large number from each section have been summarized to provide a sense of the import of these new data. There was abundant new information in the following areas: metabolism, studies of the brain, ototoxicity (which was not in the original report), neurotoxicity and behavioral studies, animal reproductive and developmental toxicity, analytical methods for humans samples, effects on human performance, Toluene abuse, human developmental and reproductive effects, and finally one human carcinogenicity study.

The vast majority of the studies exposed test animals or subjects to Toluene vapor.

The1987 report indicates that in eight studies, Toluene did not induce cancer. This was supported by an NTP report which has since then been published and is included in the summary of new data.

Attached is the concentration of use table as well as the original 1987 report.

The Panel should decide whether Toluene should be reopened.

Table 1. Historical and current cosmetic product uses and concentrations for Toluene

Product Category	1984 uses (Elder, 1987)	2002 uses (FDA, 2002)	1984 concentrations (Elder, 1987) (%)	2003 concentrations (CTFA, 2004) (%)
		Toluene		
Nail care products				
Basecoats and undercoats	32	21	>10-50	
Cuticle softeners				
Creams and lotions				
Extenders				
Nail polishes and enamels	501	23	>10-50	20-25
Nail polish and enamel removers		2		
Other	22	13	>10-50	26
Total uses/ ranges for Toluene	**555**	**59**	**>10-50%**	**20-26%**

TOLUENE - NEW DATA

Introduction

Substantial new studies have appeared in the literature since the original CIR Expert Panel safety assessment was completed (original safety assessment attached. As currently listed in the International Cosmetic Ingredient Dictionary, Toluene functions as an antioxidant and a solvent in cosmetics (Gottschalck and McEwen 2004).

ANIMAL DATA

Pharmacokinetics and Distribution

Sullivan and Conolly (1988) compared the blood concentrations of Toluene in rats after oral and inhalation exposures to Toluene. Sprague-Dawley rats were each given a single oral dose of 86.7, 217, 433, or 867 mg/kg Toluene by oral gavage. Other groups of rats were exposed to 200 or 1000 ppm Toluene by inhalation for 6 hours. Blood samples were collected and analyzed for Toluene content at several intervals for 24 hours from the beginning of dosing. Comparisons of the blood concentrations revealed that the relationship between the two routes of administration could be described by the equation: ln (oral mg/kg) = 1.27 x ln(inhalation ppm) - 9.22, where ln is the natural logarithm.

Takahashi et al. (1988) gave male New Zealand White rabbits a single dose of 0 or 0.5 g/kg Toluene in olive oil by oral gavage. Blood samples were collected at several post-dose time points and analyzed by GC for concentrations of Toluene. The peak blood level of Toluene (about 14 µg/g) occurred at 1 hour post-dose. This was followed by a plateau that continued to the fourth hour post-dose, and then concentrations of Toluene declined.

Gospe and Calaban (1988) described the distribution of Toluene in rat brains. Male Long-Evans rats were exposed to [^{14}C] Toluene and then measured the radioactivity in different regions of their brains. Toluene was detected in all regions of the brain with the highest concentration in the brainstem. Generally, Toluene uptake correlated with lipid-rich portions of the central nervous system.

Inoue et al. (1989) studied the differences in metabolism of Toluene in different strains of rats.

Female rats of the Donryu, Fischer, Sprague-Dawley, and Wistar strains were exposed to 0, 5, 45, 500, 2500, or 3500 ppm Toluene for 8 hours. Urine samples were collected and analyzed for the presence of hippuric acid, o-cresol, and p-cresol. Wistar and Sprague-Dawley rats had similar urinary p-cresol levels, and these were significantly higher ($p < 0.05$) than those found in Donryu and Fischer rats, which were similar. Results of hippuric acid and o-cresol analyses in this study were not reported.

Nakajima et al. (1992) studied the in vitro metabolism of Toluene by rat liver cytochrome P450 isozymes. Toluene was metabolized to benzyl alcohol by P450IIC11/6. The isozymes P450IIE1 and P450IA1/2 did not metabolize Toluene into o-cresol and benzyl alcohol, respectively. P450IIB1/2 and P450IIC11/6 were the isozymes that had the most metabolic activity on the side chain of Toluene.

Lorenzana-Jimenez and Salas (1990) compared the disposition of Toluene in young rats after chronic and acute exposure. Male Wistar rat pups were confined in a 2774 mL glass chamber with 0.1, 0.2, or 0.4 mL Toluene for 15 minutes per day on postnatal days 2 to 30. Three additional groups experienced the same set of Toluene exposures on postnatal day 30 only. Immediately after the postnatal day 30 exposure, all rats were killed, and blood, brain and liver samples were collected and analyzed by gas chromatography for Toluene content. Concentrations of Toluene found in these tissues increased with increasing exposure levels, but there were no significant differences in the concentrations of Toluene in acutely and chronically exposed rat pups' brains, livers, or blood.

Nakajima et al. (1992) studied the effects of sex, age, and pregnancy on the cytochrome P450 - mediated metabolism of Toluene in rats. Primary cell cultures were prepared from the livers of male and female young (3 weeks) and mature (18 weeks) rats and pregnant (gestation days 10 and 21) female rats. Toluene was added to the cultures at concentrations of 0.20 or 5.0 mM for 10 minutes. Livers from male rats at 18 weeks of age had higher cytochrome P450 contents than that of females of the same age and 3-week-old males. Livers from gestation day 21 females had lower cP450 levels than gestation day 10 females. Metabolism of Toluene was monitored by measuring the production of the metabolites benzyl alcohol, o-cresol, and p-cresol. Production of benzyl alcohol was dose dependently increased with Toluene concentration in all liver types. In both Toluene concentrations, mature females had lower benzyl alcohol production than mature males or immature females, and day 21 pregnant rats had lower benzyl

alcohol production than day 10 pregnant rats or mature non-pregnant females. o-Cresol and p-cresol production increased with Toluene concentration in the livers of non-pregnant rats, but not in the livers of pregnant dams.

Gospe and Al-Bayati (1994) compared the blood concentrations of Toluene in rats after oral and inhalation exposures. Male Fisher-344 rats were exposed to [^{14}C]-Toluene by oral gavage or inhalation. Oral doses were 110, 336, 741, and 911 mg/kg. Inhalation exposures were 99, 549, and 1145 ppm for 3 hours. Blood was collected and analyzed for several hours after the exposures. The blood Toluene versus time profiles for oral and inhalation exposures fit the following equation: ln (oral mg/kg) = -1.44 + 0.95 ln(3h inhalation ppm), where ln is the natural logarithm.

Nakajima et al. (1993) reported that the cytochrome P450 isozymes CYP2E1 and CYP2C11/6 are used by both rats and mice in metabolizing Toluene in rats and mice.

Tassaneeyakul et al. (1996) reported that metabolism of Toluene to benzyl alcohol is minorly mediated by cytochrome P4501A2, but the major enzyme for this transformation in human liver is cytochrome P4502E1.

Hanioka et al. (1995) investigated the metabolism of Toluene in canine liver microsomal P450 enzymes. Dog liver microsomes were prepared and exposed to Toluene with or without selective inhibitors of P4501A1/2, 2B1/2, 2C11/6, 2E1, 3A2/1, and 4A1. The metabolites benzyl alcohol, o-cresol, and p-cresol were measured. The researchers found that P4502B, 2C, and 2E isozymes in dog liver produce benzyl alcohol and p-cresol from Toluene.

Hori et al. (1999) reported that the metabolic rate of inhaled Toluene (300 ppm for 6 hours) to hippuric acid was enhanced by simultaneous co-exposure to methanol (300 ppm for 6 hours), compared to Toluene exposure alone, in male Wistar rats.

Healthy male and female Wistar rats were treated with 0.25 ml/100 g body weight (i.p.) on alternate days for 30 days. Control rats were treated with olive oil. After the scheduled treatments, urine samples were collected through metabolic cages and rats were starved overnight and killed the next morning. The liver and kidney were carefully removed and weighed. For male and female rats, liver weight in the treated group was significantly decreased compared to the controls ($p<0.05$) while kidney weights

were not statistically different from the controls. The amount of hippuric acid was also significantly increased in Toluene-treated rats ($p<0.05$) (Verma and Rana 2003).

Metabolism

Batlle et al. (1988) investigated the effect of Toluene on acidification by the urinary turtle bladder, an epithelial analogue of the mammalian collecting tubule. Bladders from fresh water turtles (*Psuedemys scripta*) (number not given) were mounted in Plexiglas chambers and short-circuited. After an equilibrium period, Toluene (1730 µg/ml) was added to the serosal and mucosal membranes of one hemibladder. Sodium transport or proton was measured continuously for 4 hr. Toluene resulted in a decrease in the rate of H^+ secretion. When mucosal pH was progressively lowered to examine H^+ secretion against an H^+ gradient, Toluene-treated bladders displayed a significant decrease in proton conductance, but the lowest mucosal pH required to nullify H^+ secretion was not different from that of control bladders. The authors concluded that Toluene does not cause H^+ back-diffusion. Decreased conductance of protons through active transport pathway is the mechanism that best explains the Toluene-induced defect in distal acidification.

Smith-Kielland et al. (1989) studied the effects of three different concentrations (about 10, 100, and 1000 µM) of Toluene on protein synthesis in the hepatocytes of fed and fasted Male Wistar rats. To measure protein synthesis, ^{14}C-valine was used as the precursor amino acid. Total valine concentration was 2 mM to ensure near-constant specific radioactivity of precursor. Toluene concentrations were measured by head-space gas chromatography. Protein synthesis was unchanged in the presence of low Toluene concentrations. Intermediate Toluene concentration decreased protein synthesis by about 20% and high Toluene concentration decreased protein synthesis by about 60%. Protein synthesis was similar in cells from fed and fasted rats.

Furman et al. (1991) studied the effect of Toluene on rat lung benzo[a]pyrene (BaP) metabolism and microsomal membrane lipids. Toluene (1 g/kg or 4 ml/kg) was diluted in soybean oil (preservative free) and administered to adult male Sprague-Dawley rats (8 in each group) via i.p. injection. This dose

has been shown to result in blood and tissue solvent levels similar to those reported following inhalation exposures of approximately 2000 ppm for 2 hours (LeBel and Schatz 1988; Benignus et al. 1981). Lungs were perfused with 0.9% saline through the right heart until tissue was light pink to white, then lungs were removed, and the microsomes were prepared according to the method of Boyd et al. (1978). BaP metabolites were measured using HPLC. Analysis of BaP metabolites showed that Toluene significantly inhibited 3-OH BaP formation in rat lung while formation of the 4,5-diol, 7,8-diol, 9,10-diol, and 9-OH BaP was unchanged. The selective inhibition of 3-OH BaP formation suggested to the authors that the balance between toxication and detoxication pathways had been altered by Toluene.

Kim and Kim (1996) studied the effects of Toluene on the metabolism of dichloromethane to carbon monoxide. Female Sprague-Dawley rats were given 0 or 2.0 ml/kg Toluene orally 16 to 24 hours prior to an intraperitoneal injection of 3 mmol/kg dichloromethane. Metabolism of dichloromethane to carbon monoxide was measured as carboxyhemoglobin levels in the blood. The researchers found that Toluene induced the activity of P4502E1 to increase the rate of metabolism of dichloromethane to carbon monoxide.

Neghab and Stacey (1997) examined the effect of in vivo treatment with Toluene on serum bile acids (SBA) and its direct in vitro effects on the transport of bile acids by isolated rat hepatocytes. In vivo treatment with Toluene (2.3 mmol/kg body weight, ip on each of 3 consecutive days) resulted in a significant rise in the serum concentrations of total and some individual bile acids. Administration of a higher dose of Toluene (9.2 mmol/kg body weight, i.p.) resulted in a further increase in total SBA levels together with a significant rise in serum activities of some liver enzymes. In vitro application of noncytotoxic doses of Toluene to hepatocytes isolated from untreated rats resulted in a significant inhibition of the initial rate of uptake of cholic acid. Similarly, accumulation of cholic acid and taurocholic acid over an extended incubation time by hepatocytes exposed to Toluene was significantly inhibited.

Age- and sex-related changes in Toluene metabolism by hepatic microsomes of male and female Sprague-Dawley rats were investigated. Benzyl alcohol, a major metabolite of Toluene, was measured by high-performance liquid chromatography. Rats were fasted, killed, and their livers removed. Toluene metabolism was determined by measuring the formation of benzyl alcohol in the liver microsomes. At low

concentrations (0.4mM) in male rats, benzyl alcohol increased dramatically with development reaching a peak at 5 weeks of age, rapidly decreasing thereafter. In female rats, benzyl alcohol increased dramatically with development at 3 to 5 weeks of age and then declined gradually to a low level. At high Toluene concentrations (5.0 mM), in male rats, the benzyl alcohol formation pattern was similar to that at the low concentration, although the rate of increase with age was slower. In female rats, a peak was obtained at 3 weeks of age, and then declined gradually to a low level. Gender differences were obtained at 5, 15, and 20 weeks of age, with benzyl alcohol products being higher in males than in females (Shimamoto et al. 1999).

Effect on Lipids/Membranes

Takahashi et al. (1988) gave male New Zealand White rabbits a single dose of 0 or 0.5 g/kg Toluene in olive oil by oral gavage. Blood samples were collected at several post-dose time points and analyzed for concentrations of plasma free fatty acids, triglycerides, cholesterol, phospholipids, and blood glucose. Free fatty acids were elevated in the Toluene group at 15 minutes post-dose ($p < 0.01$) and at 30 minutes and 2 hours post-dose ($p < 0.05$). Triglycerides were elevated in the Toluene group at 30 minutes to 8 hours post-dose ($p < 0.05$). Total cholesterol was elevated in the Toluene-dosed rabbits at 15, 30, and 60 minutes ($p < 0.02$). Phospholipids were elevated at 1, 3, and 4 hours post-dose ($p < 0.05$) in the dosed group. Blood glucose levels were increased in the Toluene group at 30 minutes ($p < 0.01$) and 4, 6, and 8 hours post-dose ($p < 0.05$).

LeBel and Schatz (1989) investigated the effects of Toluene (1 g/kg, 1 hr, i.p.) on male Sprague-Dawley rat synaptosomal phospholipid methylation (PLM) and membrane fluidity. Toluene significantly decreased basal PLM (35%) in studies using [^{3}H]methionine as the methyl donor. No effects were observed in either PLM reactions that used [^{3}H]adenosylmethionine as the methyl donor, or AdoMet synthetase, suggesting that Toluene preferentially affects PLM reactions that derive methyl groups from [^{3}H]methionine. Membrane fluidity demonstrated that in vivo administration of Toluene increased the outer synaptosomal membrane fluidity whereas in vitro administration of Toluene had no effect.

Total phospholipid content of rat lung microsomes was determined as inorganic phosphorus (P_i). Total microsomal cholesterol (CL) content was determined by the method of Rudel and Morris (1973). Toluene produced no change in either phospholipid or cholesterol content of pulmonary microsomal membranes. Speciation of membrane lipids by TLC confirmed that membrane phospholipid composition was unchanged after toluene administration (Furman et al. 1991).

Immunology

Hsieh et al. (1989) studied the immunotoxicity of Toluene in mice. Male CD-1 mice were exposed to 0, 17, 80, or 405 mg/L Toluene in their drinking water for 4 weeks, and humoral and cell-mediated immune functions were evaluated. Body weights and hematological parameters were not affected by Toluene exposure. The highest dose level caused increased liver weight, decreased thymus mass, impaired some functions of splenocytes, and adversely affected interleukin-2 synthesis.

Golubtsova et al. (2000) administered 600 mg/kg Toluene in vegetable oil to 10 random-bred albino mice. Ten control animals were given 0.2 ml vegetable oil. The spleens were collected 6 hr, 1 day, 1 and 4 weeks after administration of the toxicant. It was shown by the Falck-Hillarpp method that 6 hr after treatment, Toluene stimulated splenic mast cell populations and inhibited other amino-containing structures. The levels of catecholamines and serotonin in nervous and nonnervous structures peaked 1 week after poisoning and returned to normal after 4 weeks.

Effect on Chemicals/Proteins in the Brain

Subacute treatment with Toluene (80-1500 ppm, 6 hrs/day for 3 consecutive days) produced a dose-dependent reduction of affinity and increase in density of the β-adrenergic antagonist [^{3}H]dihydroalprenolol binding sites in the frontoparietal cortex of the male Sprague-Dawley rat, while the binding characteristics of α_1-adrenergic and α_2-adrenergic binding sites in the same region was unaffected by this treatment as evaluated in vitro (Fuxe et al. 1987).

Edelfors and Ravn-Jonsen (1987) exposed male Wistar rats to Toluene 500 ppm for 12/hrs/day for up to 80 weeks. The brains were removed and the synaptosomes prepared. Potassium stimulated and unstimulated synaptosomes were incubated with $^{45}Ca^{2+}$ for ½, 2, 4, 8, and 16 min. Toluene exposure for 4 and 12 weeks caused a significant, approximately 20%, increase in the $^{45}Ca^{2+}$ uptake into unstimulated synaptosomes. The effect was not significant after 30 and 80 weeks of exposure.

Korpela and Tähti (1988) studied the mechanism of the anaesthetic effect of Toluene on the CNS by using rat erythrocyte and synaptosome membranes as nerve cell models both in vitro and in vivo. An exposure to 2000 ppm of Toluene for 2 hr had an inhibitory effect on acetylcholinesterase, adenosine triphosphatase, and magnesium activated adenosine triphosphatase. The degree of inhibition in erythrocyte membranes in vitro and in vivo, and in synaptosome membranes in vitro were in good correlation. The synaptosome-bound enzymes in the in vivo test were significantly more inhibited by Toluene, which indicates that membranes in vivo are more vulnerable to the toxic effects of organic solvents than they are as isolated membranes in vitro.

Bjornaes and Naalsund (1988) exposed male Wistar rats to 0, 50, 250, 500, or 1000 ppm Toluene by inhalation for 8 hours per day, 5 days per week for 4 weeks or for 16 hours per day, 5 days per week, for 12 weeks. Toluene exposure of 500 ppm for 12 weeks caused a general increase in the activities of the neurotransmitter synthesizing enzymes glutamic acid decarboxylase, choline acetyltransferase, and aromatic amino-acid decarboxylase. Catecholaminergic neurons showed a 50 % reduction in the brain stem after 4 weeks of exposure to 250 or 1000 ppm Toluene. The activity of the glial enzyme glutamine synthetase increased in the cerebellar hemisphere after 4 weeks of exposure to 1000 ppm Toluene.

The effects of chronic Toluene exposure (80 ppm, 6 hr/day, 5 days/week, 3 months) were studied on neuropeptide and 5-hydroxytryptamine receptors, on protein phosphorylation levels, and on catecholamine levels in various brain regions in the male Sprague-Dawley rat. Toluene selectively reduced [^{3}H]neurotensin binding in the basal layers of the orbital cortex. Instead, Toluene increased the binding of [^{3}H]etorphine in the nucleus accumbens and of [^{125}I]vasoactive intestinal polypeptide in the area postrema and hypoglossal nucleus. The authors suggested that the regional selectivity in disturbing [^{3}H]neurotensin and [^{125}I]vasoactive intestinal polypeptide binding may be due to vulnerability of

monoamine-neuropeptide interactions to Toluene (von Euler et al. 1988a).

The effects of treatment with Toluene in vivo (80 ppm, 6 hr/day, 3 days) and in vitro (19 µmol/ml) were analyzed on the binding characteristics of [^{3}H]neurotensin in male Sprague-Dawley rat striatal membranes. Exposure to Toluene in vivo did not produce any significant effects on the binding characteristics of [^{3}H]neurotensin. However, the addition of Toluene in vitro caused a trend for a decreased B_{max} value and produced a significantly reduced K_D value of [^{3}H]neurotensin binding. The absence of effects at 80 ppm indicated that the neurotensin receptor is relatively insensitive to Toluene exposure (von Euler et al. 1988b)

Primary astroglial cell cultures from striata of newborn rats were exposed to Toluene in vitro at doses of between 4.7 and 150 µmol/ml. Calcium-induced back phosphorylation was used to detect changes in the phosphorylation state of calcium-regulated glial phosphoproteins. The 11 most back-phosphorylated protein bands were separated by gel electrophoresis. The back-phosphorylatioin of the 59-kD protein band was increased by 150% at 9.4 µmol/ml Toluene, but no further increase was observed with increasing concentrations. This increase is believed to correspond to a Toluene-induced decrease in calcium-regulated protein phosphorylation. The data indicate that the 59-kD protein in astrocytes from the rat striatum is specifically sensitive to Toluene treatment in vitro (von Euler et al. 1989).

Edelfors and Ravn-Jonsen (1989) examined the effect of Toluene on the CNS by using male Wistar rat brain synaptosomal membranes as in vitro and in vivo models. The activity of Ca^{2+}/Mg^{2+} ATPase and the membrane fluidity were determined. Short term exposure to 500 ppm Toluene had an inhibitory effect on the enzyme whereas long-term exposure to Toluene caused an increased activity. Exposures ranged from 18 hr-18 months. Exposure to Toluene had no effect at all on membrane fluidity. The in vitro experiment showed an effect of Toluene on both parameters. The alteration in enzyme activity and membrane fluidity was parallel in the exposed animals as well as those of control.

Three groups of male Wistar rats were exposed to Toluene at 300 ppm, 1000 ppm and 3000 ppm, respectively, for 8 hr/day, 6 days/week, for 2 weeks. After subacute repeated solvent exposure, both neuron-specific γ-enolase and glial marker proteins displayed an overall concentration-dependent increase tendency in separate brain regions. In the cerebrum, only the 3000 ppm group showed a

significant increase in α-enolase by 27% and creatine kinase-B by 26%. α-Enolase and γ-enolase exhibited a pronounced elevation in cerebellum relative to other brain regions, while the β-S100 protein appeared to be the most markedly altered marker in brainstem (Huang et al. 1990).

De Gandarias et al. (1993) administered 1.3 ml/kg/day Toluene by i.p. injection to male Sprague-Dawley rats on 3 consecutive days. After the last administration, rat brains were removed and dissected. Lys- and Leu-aminopeptidase activities were fluorimetrically measured in triplicate using Lys-and Leu-2-naphthylamides (Lys and Leu-NA) as substrates. Lys-aminopeptidase activity was significantly decreased in the thalamus, amygdala and medulla oblongata. Leu-animopeptidase activity was significantly decreased in the thalamus and cerebellum. The authors suggested that these aminopeptidase activities could play a part in Toluene neurotoxicity.

Stengård et al. (1993) studied the effect of toluene on extracellular levels of gamma-aminobutyric acid (GABA) in male Sprague-Dawley rats. Animals (5 in each group) had a hole (diameter 0.6) drilled through the skull bone and the dura exposed. After 2-3 days, rats were placed in chambers and exposed to 2000 ppm Toluene. Levels of GABA were then measured as described by Kehr and Ungerstedt (1988). The basal concentration of GABA for rats before treatment was 29.30±7.08 nM. There was no clear difference between the basal concentrations between the experimental and control groups. Extracellular levels of GABA in the cerebellum increased ($P<0.01$) during acute inhalation toluene for the exposed group when compared to the control group. The authors speculated that Toluene increases GABA within the cerebellum by sodium dependent mechanisms, possibly by modulating the neuronal input from the mossy fibers to the cerebellar cortex.

Stengård (1994) studied the effect of Toluene exposure on acetylcholine (ACh) release in striatum. Sprague-Dawley rats had a hole (2 mm) drilled in the skull and the dura was exposed. Microdialysis was performed as described in Stengård et al. (1994). For the exposed treatments, an average concentration of 2012±114 ppm Toluene was used. Striatal ACh release was monitored before, during, and after inhalation exposure to Toluene and compared to control animals that were exposed to air only. ACh was determined by liquid chromatography. The authors found that Toluene exposure decreases ACh release (455±32 nM before exposure in the control group (n=5) and 463±41 nM (n=5) in

the group exposed to Toluene.

Stengård (1995) studied whether behaviorally induced increase of extracellular dopamine differs from that induced by toluene in affecting striatal ACh release. Male Sprague-Dawley rats had a microdialysis probe implanted into the dorsal striatum. Rats were then placed in exposure chambers and exposed to air or air and Toluene (1957±238 ppm). Toluene exposure lasted 2 hours. Tail pinch was performed by placing a paper clip on the base of the rat's tail for 5 minutes immediately after the termination of the Toluene exposure in the Toluene exposed group. Treatment groups were as follows: control (n=4), tail pinch (n=6), and Toluene and tail pinch (n=5). ACh was measured using liquid chromatography. The pretail pinch value concentrations of ACh release in the striatum were: control (374±63 nM); tail pinch group(312±46 nM); and Toluene and tail pinch group (293±55 nM). Toluene was found not to affect the increase of ACh release caused by tail pinch.

Iizumi et al. (1995) exposed male Wistar rats to 3000 ppm Toluene for 4 hr/day for 3 weeks. Using light microscopy, the number and intensity of tyrosine hydroxylase-immunoreactive fibers and terminals were observed in most parts of the forebrain including the cerebral cortex, hippocampus, lateral septal nucleus, and hypothalamus in the Toluene-exposed rats compared with control rats. These findings suggested that chronic Toluene exposure might influence catecholaminergic neural systems.

Yamaguchi et al. (2002) exposed serum-free mouse embryo cells (a precursor to astrocytes) to Toluene concentrations of 2×10^{-16} to 20 ppm in cell culture medium for 48 hours. Induction of glial fibrillary acidic protein was measured by electrophoresis and immunoblotting techniques and found to be inhibited by Toluene exposure at concentrations above 2×10^{-4} ppm.

Cintra et al. (1996) assessed the potential neurotoxicity of Toluene to the nigrostriatal dopaminergic system in male Sprague-Dawley rats. After a unilateral injection of 6-hydroxydopamine (6-OH DA) into the substantia nigra, rats inhaled air or different concentrations of Toluene (80, 300, or 1000 ppm), 6 hr/day for 3 days. The animals were killed 2 days after the last exposure and biochemical measurements of catecholamines and 3,4-dihydroxyphenylacetic acid (DOPAC) were performed in the neostriatum and substantia nigra. Toluene at 80 and 1000 ppm significantly enhanced the depletion of striatal DOPAC levels induced by the lesion and produced at 80 and 300 ppm a trend for intensifying the

6-OH DA-induced depletion of striatal DA stores.

Male F344 rats received inhalation exposure to air or to 1000 ppm Toluene 6 hr/day, for 3 or 7 days. Rats were then killed and trunk blood collected for the corticosterone assay, using High Performance Liquid Chromatography (HPLC). Glial Fibrillary Acidic Protein (GFAP) was assayed by an Enzyme Linked ImmunoSorbent Assay (ELISA). The Toluene exposure decreased GFAP in the thalamus and elevated serum corticosterone (Little et al. 1998).

Hseih et al. (1990) exposed adult male CD-1 mice to 0, 17, 80, or 405 mg/L Toluene in drinking water for 28 days. The water bottles were shaken several times daily during the exposure period to maintain the Toluene-water mixture. Animals were killed after the treatment period, and assays were performed to measure endogenous levels of the biogenic amine neurotransmitters norepinephrine, dopamine, serotonin and their metabolites. The mice given 80 mg/L Toluene had the highest increases in the biogenic amines and their metabolites. Norepinephrine levels in the hypothalamus were increased by 51, 63, and 35 % with respective exposures of 17, 80, and 405 mg/L Toluene, compared to those of the control group. Increases in norepinephrine and its metabolites were found in the medulla oblongata, midbrain, and other brain regions. Concentrations of dopamine in the corpus striatum and hypothalamus were increased with Toluene exposure, but the dopamine metabolites were not affected. Serotonin levels were increased with Toluene exposure in all regions of the brain.

Berenguer et al. (2003) studied the neurochemical effects induced by subchronic exposure to Toluene. Male (12) and female (12) Sprague-Dawley rats were divided into 2 groups (6 of each sex), control and exposed. Exposed animals were placed in experimental chambers and Toluene was administered at a dose of 40 ppm. Rats were exposed for 16 weeks for 104 hours/week. After conducting behavioral studies (see Behavior section), rats were injected intraperitoneally with NSD_{1015} (3-hydroxybenzyl hydrazine dichloride, 50 mg/kg) in 0.9% saline to complete L-DOPA and L-amino acid decarboxylase blockade. Twenty minutes later, rats were decapitated and their brains sectioned. Discrete brain regions were punched out and homogenized. Dopamine turnover was evaluated by calculating the ratio of dopamine/3,4-dihydroxyphenylacetic acid while serotonin turnover was calculated using the ratio of serotonin/5-hydroxyindolacetic acid. Toluene exposure led to a significant increase in the dopamine/3,4-

dihydroxyphenylacetic acid ratio in the putamen of both male ($p<0.02$) and female ($p<0.05$) rats when compared to controls. No significant change in the dopamine or 3,4-dihydroxyphenylacetic acid was seen in the hippocampus, prefrontal cortex, and cerebellum. Subchronic Toluene exposure further led to a significant reduction of dopamine accumulation in the hippocampus of male rats but not of female rats, as compared to controls ($P<0.02$). This led to a significant difference in dopamine accumulation between male and female rats exposed to Toluene ($p<0.02$). No significant difference in dopamine accumulation was found between control and Toluene exposed rats in the caudate-putamen, nucleus accumbens, prefrontal cortex, and cerebellum. No significant change was found in the serotonin or 5-hydroxyindolacetic acid concentration and turnover.

Von Euler et al. (1991) investigated the effects of low concentrations of Toluene on Dopamine D-2 agonist binding. Male Sprague-Dawley rats (12 in each group) were exposed for 3 days (from 0900 h to 1500 h) by inhalation of 40 ppm or 80 ppm Toluene in inhalators. The control group was exposed to circulating air only. The rats were killed or tested for locomotor activity 18, 42, or 114 hours after the last exposure. Rats were decapitated and the brain removed. D-2 agonist binding sites were labeled by [^{3}H]NPA. Following exposure to 80 ppm for 3 days and a post-exposure delay of 18 hours, the K_D value of [^{3}H]NPA binding was increased by 50% in membrane preparations from rat neostriatum, whereas the number of binding site was not significantly affected. At 40 ppm of Toluene, the changes in the K_D value were not significant. Striatal [^{3}H]NPA binding was not modified after a post-exposure delay of 42 hours following a 80 ppm exposure to Toluene.

Stengård et al. (1994) studied how exposure to Toluene affected extracellular dopamine levels within the striatum. Male Sprague-Dawley rats (number not given) had a hole (diameter 0.6 mm) drilled through the skull bone and the dura was exposed. An intracerebral guide was inserted into the brain. Three to five days later, the animals were placed in their exposure chambers. The rats were divided into 5 groups according to exposure: (1) Toluene 500 ppm, (2) Toluene 1000 ppm, (3) Toluene 2000, (4) Isoamylacetate, or (5) control (air only). Inhalation exposure to 1000 and 2000 ppm Toluene for 2 hours was accompanied by a significant increase in extracellular dopamine levels within the striatum ($p<0.05$ and $p<0.01$ respectively when compared to controls). Inhalation exposure to Isoamylacetate did not

cause any significant changes in dopamine levels.

Reigel and French (1999) designed a study to assess the response of ventral tegmental dopamine neurons during Toluene inhalation. Adult male Sprague-Dawley rats (24) were exposed to acute (1-15.3 minute) concentrations of Toluene vapor (11,500 ppm) through a tracheal breathing tube. Toluene exposure elicited two distinctly different patterns of response in dopamine neurons. One pattern consisted of an initial stimulation of neuronal firing (+221%±6.3%: >8.5 min). The other pattern consisted of only an inhibition of firing regardless of the length of exposure. Blood samples taken at the time of the dopamine recordings revealed comparable toluene concentration (4-79 µg/ml) regardless of the patterns of response.

Beyer et al. (2001) examined how repeated exposure to inhaled Toluene affects cocaine-induced dopamine concentrations in the nucleus accumbens. Twenty-seven adult, male Wistar rats were bilaterally implanted with a guide cannulae (15 mm) directed 3 mm above the nucleus accumbens. Animals were placed in chambers and exposed to either Toluene (8000 ppm, n=15) or air (n=12) for 5 days a week for 10 weeks. Sixteen hours before microdialysis experiments began, dialysis probes were inserted into the guide cannulae. The next morning, 20 minute dialysis samples were collected. Animals then received a peripheral injection of either saline (1.0 ml/kg, i.p.) or cocaine (15 mg/kg, i.p.) And dialysis samples collected for the next 3 hours in 20 minute intervals. The amount of dopamine was determined by using HPLC on the dialysates. In all animals tested, cocaine administration enhanced dopamine concentration in the nucleus accumbens. These increases were significantly greater in rats previously exposed to Toluene ($p<0.05$).

Soulage et al. (2004) studied the effects of subchronic exposure to Toluene on the monoamine biosynthesis rate in discrete brain areas. Sixteen male and sixteen female Sprague-Dawley rats were divided into two groups. The Toluene exposed group consisted of six males and six females. The rest of the animals comprised the control group. Animals in the Toluene exposed group were placed in chambers and exposed for 4 months (104 hours per week) to 40 ppm Toluene. Control animals were placed in chambers and exposed to air. Twenty-four hours after the end of the exposure, animals were given 3-hydroxybenzylhydrazine dichloride before being decapitated. The brains were removed and the

noradrenergic cell groups A5 and A6, the catecholaminergic cell groups A1C1 and A2C2 were dissected. Tyrosine hydroxylase and tryptophane hydroxylase, the rate limiting enzymes in the biosynthesis of catecholamines and serotonin, respectively, were estimated by measuring the L-dihydroxyphenylalanine and 5-hydroxytryptophan accumulation. For the Toluene exposed animals, significant increase in tyrosine hydroxylation was selectively observed in the rostral part of A2C2 in females ($p<0.05$) while a sharp decrease was observed in A5 ($p<0.05$). No difference was found in A6 noradrenergic cell group or in A1C1 cell group. Tyrosine hydroxylase activity was not affected in monoaminergic ending areas located outside the brainstem.

An increase in tryptophan hydroxylation was observed in the rostral subset of A2C2 cell group in both genders (males, $p<0.05$; females, $p<0.025$) in the Toluene exposed group. In A1C1, Toluene elicited an increase in tryptophan hydroxylation in female rats ($p=0.025$), while males remained unaffected. No difference was found in A5 or A6 noradrenergic cell groups in both genders. Toluene significantly lowered biosynthesis rate of 5-hydroxytryptophan in the ventro-median-hypothalamus ($p<0.05$). No other brain areas were significantly affected (Soulage et al. 2004).

Effect on Brain Functions

The effects of Toluene on the electroencephalogram (EEG) and its power spectra were measured during a 2-hr exposure in an inhalation chamber. Rats were exposed to one of graded concentrations (110.6, 162.5, 432, 676, 1558, 2730 ppm) on Toluene on different days. The duration of waking was increased with a decrease in duration of rapid eye movement (REM) sleep even at 110.6 ppm. Duration of nonrapid eye movement (NREM) sleep was decreased with an increase of waking and decrease of REM sleep at 162.5 ppm. Dose-related effects were noted in higher concentrations. The power of δ frequency band was increased with a decrease of θ frequency band power at hour 1 of exposure to 676 ppm during REM sleep recorded from the visual cortex. The power of θ frequency band was also decreased at hour 2 of exposure at 432 ppm (Ghosh et al. 1989).

Ghosh et al. (1990) examined the effects of Toluene on the electroencephalogram (EEG) and its

power spectra were measured during a 2-hr exposure in a dynamic inhalation chamber in young F344 rats (30-53 days old) and compared to those in adult rats (63-77 days old). Rats were exposed to low (108-111 ppm), medium (160-163 ppm) and high (407-432 ppm) concentrations of Toluene on different days. In tests on sleep-wake cycle, in the young animals, the duration of the wake stage was increased with decreases of rapid eye movement (REM) and non-REM sleep during hour 1 and hours 2 of exposure to the low concentration. These effects were marked at the medium and high concentrations. In adult rats, at the low concentration, the increase of the wake stage and the decrease of REM were observed only at hour 1; however, at medium and high concentrations these changes of wake stage and REM sleep were marked along with a decrease of non-REM. There was a significant difference in the increase of wake stage and the decrease of non-REM sleep in young rats at hour 2 of exposure to low concentrations only compared to those in adult rats. In young rats, during REM sleep , the power of δ waves increased at the medium and high concentrations and that of θ waves decreased at the high concentration during hour 2 of exposure compared to the controls. The authors concluded that young rats are more sensitive to effects on sleep-wake cycle at low concentration caused by Toluene exposure than are adult rats.

Ladefoged et al. (1991) studied the effects of six months' exposure to Toluene (0, 500, and 1500 ppm) on the CNS of rats. After an exposure free period, neurobehavioral, morphometric, pathological, and biochemical examinations were performed. No neurobehavioral or gross pathological changes were found. Morphometric measurements did not show any loss of neurons. At 500 ppm, the mean nuclear volume and mean perikaryonal volume and the variation of the values of these parameters was increased in the exposed groups. Noradrenaline, dopamine, and 5-hydroxytryptamine levels were significantly changed in various brain regions. This experiment failed to reveal overt Toluene-induced CNS neurotoxicity; however, certain irreversible effects were found which point to the CNS-neurotoxicity of Toluene.

Mattia et al. (1991) reported that 1.0 g/kg Toluene given intraperitoneally to male CD rats did not induce cortical lipid peroxidation in the brains but did elevate formation of reactive oxygen species within cortical synaptosomes. The Toluene-induced formation of reactive oxygen species was prevented when rats were also dosed with the mixed function oxidase inhibitor metyrapone. The researchers concluded

that a metabolite of Toluene (possibly benzaldehyde) was responsible for the formation of reactive oxygen species in the rats' brains.

Huang et al. (1992) exposed male Wistar rats to 0, 100, 300, or 1000 ppm Toluene by inhalation for 8 hours per day, 6 days per week, for 16 weeks. Immunoassays were used to measure neuronal and glial marker proteins in the rats' central nervous systems. The concentration of neuronal marker proteins were not affected by Toluene exposures, but at all concentrations of Toluene tested, there was a dose dependent increase in the concentration of markers for glial cells. The authors concluded that gliosis, rather than neuronal death, was induced by chronic exposure Toluene.

A rolling bottle system containing glioma (C6) cells was used to test the toxicity of Toluene, using mitochondrial activity (MTT assay), neutral red uptake and cell growth as indicators of toxicity. Toluene was shown to have toxic effects only at gas concentrations above 12000 ppm (Ryghseter et al. 1992).

Mattia et al. (1993) gave male Sprague-Dawley rats a single intraperitoneal dose of 0 or 1.5 g/kg Toluene in corn oil. The rats were killed 1, 2, 5, or 24 hours after the dose administration. The brains were quickly removed and dissected on ice to isolate that cerebellum, hippocampus, and striatum. The liver, kidneys, and lungs were also removed. These tissues, with the selected brain regions, were prepared into tissue fractions that were analyzed for the formation of reactive oxygen species content and lipid peroxidation. Rats given the Toluene dose had elevated levels of reactive oxygen species in crude mitochondrial fractions of lung, liver and kidney tissues and in crude synaptosomal fractions from the cerebellum, hippocampus, and striatum. Toluene-induced reactive oxygen species formation reached its peak within 2 hours, which correlated directly with measured Toluene blood levels. This elevated oxidative activity was maintained throughout the next 24 hours, even though blood levels of Toluene decreased to negligible amounts. The authors concluded that exposure to Toluene resulted in broad systemic elevation in the normal rate of oxygen radical generation, with such effect persisting in the tissues despite rapid decline in Toluene blood levels.

Ikeuchi et al. (1993) investigated the effect of Toluene on neural activity by recording the postsynaptic field potential (population spike, PS) of granule cells as well as antidromic potential (AP) and presynaptic fiber potential (FP)(perforant path) using a microelectrode in guinea pig hippocampal slices.

Toluene at the concentration of 0.2 ng/ml to 20 µg/ml in the perfusion medium increased the amplitude of PS to 109-150%. Toluene also increased the amplitude of FP and AP, although the most remarkable enhancement was observed in the PS. However, Toluene at concentrations over 1000 µg/ml completely depressed the PS, whereas it increased the amplitude of AP to 130% of the original level. The authors concluded that Toluene has both excitatory and inhibitory biphasic effects on neurotransmission in the hippocampal slices to the concentration applied.

Korbo et al. (1996) exposed male Wistar rats to 1500 ppm of Toluene for 6 hr/day, 5 days/week for 6 months. This was followed by a four-month period without exposure prior to killing the animals. The total number of neurons in each of the five subdivisions of the hippocampus of six exposed and six control rats was estimated with a optical fractionator. A statistically significant neuron loss of 16% was found in regio inferior (CA3 and CA2) of the exposed rats.

Cintra et al. (1999) studied the effect of a 4-week exposure of 40 or 80 ppm Toluene on the brain in a rat model of Parkinson's disease. In the Parkinson's model, lesions of the substania nigra was induced by an injection of 6-hydroxydopamine (6-OH-DA). Toluene counteracted the 6-OH-DA -induced reductions in dopamine tissue levels. The exposure to 80 ppm Toluene led to signs of reduced dysfunction of the nigrostriatal dopaminergic system.

Euler et al. (2000) exposed Sprague-Dawley rats to 0 or 80 ppm Toluene by inhalation for 6 hours per day, five days per week, for 4 weeks. The Toluene exposure appeared to affect spatial memory in a Morris maze, increase locomotor and rearing behaviors in an open field, and reduce beam-walk performance. Magnetic resonance imaging showed a smaller area of the cerebral cortex in exposed rats. Toluene exposure did not affect dopamine agonist binding to dopamine receptors.

Gerasimov et al. (2002) exposed male Sprague-Dawley rats to 0 or 3000 ppm Toluene by inhalation for 40 minutes. Extracellular dopamine in the prefrontal cortex and nucleus accumbens was measured by microdialysis before, during, and after exposure. Extracellular dopamine in the prefrontal cortex increased steadily during the 40-minute exposure period and then declined back to baseline levels 100 minutes after the end of Toluene exposure. Extracellular dopamine concentrations in the nucleus accumbens was not affected by Toluene exposure.

Morón et al. (2004) reported that an intraperitoneal dose of 1.3 ml/kg/day for 4 days reduced food consumption and body weight gain. Immunostaining in the brain showed that Toluene treatments reduced immunostaining of NPY (a hypothalamic neuropeptide that stimulates appetite) in the paraventricular nucleus and increased NPY staining in the arcuate nucleus. Immunostaining of galanin (another endogenous appetite stimulant) was increased in the paraventricular and arcuate nuclei. The authors proposed that the anorexic effect of Toluene may have stimulated a compensatory increase in endogenous appetite stimulants.

Animal Ototoxicity Studies

Johnson et al. (1988) exposed male Sprague-Dawley rats to Toluene (1000 ppm, 16 hr/day, 5 days/week, 2 weeks), or noise (100 dB, 10 h/day, 7 days/week, 4 week) or Toluene followed by noise. Auditory function was tested by brainstem audiometry using 1/3 octave filtered sine wave stimulus at frequencies 1.6, 3.15, 6.3, 12.5 and 20.0 kHz. A high-frequency auditory impairment was observed after exposure to Toluene alone and noise alone. A slight recovery was recorded 1 and 6 months after the Toluene exposure. Toluene followed by noise resulted in a higher threshold at all frequencies. A slight recovery was recorded 6 months post-exposure.

In a similar study, Johnson et al. (1990) exposed male Sprague-Dawley rats to Toluene (1000 ppm, 16 hr/day, 5 days/week, 2 weeks) , to noise (100 dB, 10 h/day, 7 days/week, 4 week) or to noise followed by Toluene. Auditory sensitivity was tested before exposure and 1 to 4 weeks after exposure by brainstem audiometry like in the previous study. Some auditory impairment was observed after all exposures. The sensitivity loss after exposure to noise followed by Toluene was greater than that recorded after exposure to noise alone or Toluene alone, but did not exceed the summated loss caused by noise alone and Toluene alone at any frequency. This result contrasts with the previous reported effect of the same exposures in the reversed order.

Mattsson et al. (1990) exposed 6 male Fischer 344 rats to 8000 ppm Toluene (5 days a week for 13 weeks) in exposure chambers. Six other male rats served as controls. The rats were weighed prior to

initiation of the study and weekly thereafter. Necropsies were performed after completion of 13 weeks of exposure and after neurologic testing on all rats. Control rats weighed 331±29 g while Toluene-exposed rats weighed 255±6 g (mean±SD). This difference was statistically significant ($p<0.01$).

The following data were collected beginning 65 or more hours after 13 weeks of exposure: flash evoked potential (FEP), cortical flicker fusion (CFF), auditory brainstem response to clicks (ABR_c), auditory brainstem response to tone-pips at 10 kHz (ABR_{10}) and 30 kHz (ABR_{30}), somatosensory-evoked potentials (SEP), and caudal nerve action potentials to single stimuli ($CNAP_1$) and to paired stimuli ($CNAP_2$). Data sweeps (msec segments of EEG) were digitally sampled 512 times and averaged by an online computer. For the FEP test, rats, in a restrainer, were placed in an isolation cubicle that has white plastic walls. Rats faced the wall opposite the strobe. The visual system was stimulated with a low intensity flash (approximately 0.3 cd-sec/m^2) at a rate of 1.1 flashes/sec. Filters were set to pass EEG between 0.5 and 1500 Hz. One FEP sweep duration was 150 msec and the other was 750 msec. The final FEP was an average of 200 sweeps for each duration. After 13 weeks of exposure, the early components of FEPs of Toluene-exposed rats were significantly altered in shape and were very slow ($p=0.0026$). For the cortical flicker fusion test (CFF), the maximum rate of flash that elicited a synchronized cortical response was determined by increasing or decreasing the flash rate, in 2 Hz or larger steps, starting from 48 Hz. Amplifier filter settings were the same as for the FEP and a CFF response was an average of 200 sweeps. CFF was unaffected by Toluene treatment.

For the auditory brainstem response to clicks test (ABR_c), rats in restrainers were placed in a cubicle specially designed for acoustic isolation and to minimize sound reflections. Sound pressure level calibration indicated 80 dB linear scale (rat removed). The click rate was 19.1 clicks/sec. Sweep duration was 10 msec and 2000 sweeps were averaged. Bandpass filters were set at 150-3000 Hz. For the auditory brainstem response to tone pips (ABR_{10} and ABR_{30}), ABRs were tested at middle and high frequencies (10 kHz at approximately 55 dB and 30 kHz at 82 dB). Each tone-pip had a 2.25 msec rise/fall ramp and no plateau (4.5 msec total duration). Tone-pips were presented at a rate of 19.1 pips/sec. Bandpass filters were set at 150-3000 Hz. Data sweep duration was 10 msec and 4000 sweeps were averaged. ABRs were severely affected by Toluene exposure. The ABR_{10} was slowed and much reduced

in power (p=0.0211). The changes at 30 kHz (p=0.0204) and for clicks (p=0.0274) were less pronounced.

For the somatosensory-evoked potentials test (SEP), ventrolateral caudal nerves were stimulated at the base of the tail and a response was recorded at the somatosensory cortex (SEP). The stimulating electrodes were small needles set into the bottom of a plastic tray that fit the tail. A 3 mA, 50 μsec electrical pulse was presented at 1.1 pulses/sec. The amplifier bandpass settings were 1-1500 Hz. Sweep durations were made of 35 and 150 msec each. The final SEP was an average of 100 sweeps. SEPs were affected by Toluene. Most of the change occurred in the waveform shape of later components (0.96±0.02 for controls and 0.87±0.05 for Toluene-exposed, p=0.0029). For the caudal nerve action potentials test (CNAP), ventrolateral caudal nerves were stimulated near the tip of the tail and mixed nerve potentials from a single stimuli ($CNAP_1$) were recorded at the base of the tail. The stimulating and recording electrodes were mounted in a plastic tray. A single 3 mA, 20 μsec electrical pulse was presented at a rate of 10.1 pulses/sec. Subsequently, the nerves were stimulated with a pair of pulses ($CNAP_2$ with interstimulus interval of 3 msec) at 10.1 paired pulses/sec. The amplifier bandpass settings were 1-3000 Hz. Data sweep duration was 20 msec and the final CNAP was an average of 200 sweeps. Standard analyses (comparisons of individual waveforms to a template) were not conducted on CNAPs because of the small tail sizes in the Toluene-exposed rats. Instead, each animal was used as its own control (small tail size was not expected to affect a nerve's responsiveness to a second stimulus), so that each rat's CNAPs were evaluated for recovery by computing the baseline to negative peak amplitude ratio of $CNAP_2/CNAP_1$. The ratio was significantly smaller for the Toluene-treated rats (0.63±0.19 compared to 0.87±0.04, p=0.0438).

Two inbred strains of mice, CBA/Ca (with a moderate hearing loss starting late in life) and C57BL/6J (with an early onset of spontaneous auditory degeneration) were exposed to Toluene by inhalation (1000 ppm, 12 h/day, 7 days) at either 1 or 6 months of age. Thresholds of auditory brainstem response were measured 3-5 days after exposure and assessed repeatedly up to the age of 16 months (C57BL/6J) or 23 months (CBA/Ca). Both strains of mice exposed to Toluene at 1 month of age showed a mild loss of sensitivity at a high frequency (31.5 kHz) shortly after exposure. With increasing age, Toluene exposure had little effect on the aging process of the auditory system in CBA/Ca mice, but accelerated

age-related hearing loss in C57 mice. The results indicated that Toluene exposure can aggravate auditory deterioration only in mice with a strong genetic predisposition to spontaneously precocious age-related hearing loss (Li et al. 1992).

Johnson and Canlon (1994) exposed male Sprague-Dawley rats by inhalation (1400 ppm, 16 hr/day, 8 days) and killed them for morphological investigations at 3 and 5 days after the start of the exposure, and 4 days and 6 weeks after the end of the exposure. The cochleae were removed and prepared for microscopy. After 3 days of Toluene exposure, no loss of hair cells was found. A slight loss in the third row outer hair cells was observed after 5 days of exposure. Four days after the 8 day long exposure, a loss of hair cells was found in all 3 rows of outer hair cells, mainly in the middle and upper turns of the cochlea, and a 50-100% loss of outer hair cells together with some loss of inner hair cells were seen. These results indicate that the outer hair cells in the middle frequency region of the cochlea were primarily affected by Toluene exposure; however after a long post-exposure period, the damage extended basally and apically and some damage to the inner hair cells was seen.

Bushnell et al. (1994) examined the effects of Toluene inhalation on the detection of auditory signals in 12 male Long-Evans rats. For the signal detection task, rats were trained to press either the response lever for food pellets and next to discriminate between loud (77 dB) and soft (70 dB) levels of white noise. On a loud signal trial, the white noise level increased from 70 dB to 77 dB 2 sec prior to insertion of the levers and remained elevated until a lever was pressed. For 6 rats, pressing the left lever caused illumination of the food cup light and delivery of a food pellet, while pressing the right lever resulted in a 5 sec period of darkness and no food delivery. On soft trials, the noise level did not change prior to insertion of the levers. On these trials food was delivered if the right lever was pressed and withheld if the left lever was pressed. For the other 6 rats, the relationship between signal and lever position was reversed. Once rats mastered this discrimination, the signal duration was shortened in stages from 2 sec to 20 msec and a postsignal interval (between offset of the signal and insertion of the levers) was added. This interval was initially 0.5 sec and was extended to 2, 3, or 4 sec (varying randomly across trials) for the final conditions. For the final vigilance task, the background white noise level was reduced from 70 to 60 dB and signals of variable intensity (61-67 dB) were delivered. Training required 3 calendar months. Each

rat was exposed to Toluene vapor at 0 (air control), 1000, 1500, or 2000 ppm on different days. In air, the Sensitivity Index increased across the duration of the test; this within session improvement was reversed by Toluene. Responsivity Index did not change in air; it was decreased by Toluene at the beginning of each exposure session, returned to the control level during exposure to 1000 and 1500 ppm Toluene and exceeded air control after 40 min exposure to 2000 ppm Toluene. Latency increased monotonically across Toluene concentrations and time on test. Neither signal intensity nor the duration of Toluene exposure before testing altered these effects of Toluene. Toluene exposure (2000 ppm for 2 h/day for 4 consecutive days) did not affect auditory thresholds. The authors concluded that the Sensitivity Index, Response Index, and latency baselines were recovered after Toluene exposure indicating that no persistent effects of Toluene were detectable.

Long-Evans rats inhaled from 1000 ppm to 2000 ppm of Toluene for 6 hr/day, 5 days/week for 4 weeks. Auditory function was tested by recording near field potentials from the inferior colliculus. Rats were then killed and their cochleas examined histologically. There was severe damage to the cochlea, which was not reflected in the electrophysiologic results (Campo et al. 1997).

Liu and Fechter (1997) studied whether outer hair cells isolated from the guinea pig cochlea show morphological alterations consistent with a toxic response to Toluene exposure. The effect of Toluene (30-700 μM) on calcium homeostasis was monitored in both outer hair cells and ganglion cells. A dose-response relationship was observed in the extent of outer hair cell shortening produced by Toluene with a significant shortening observed at concentrations of 100 μM and higher. Fura-2 showed that Toluene enhanced free intracellular calcium levels of both outer hair cells and spiral ganglion cells within 5 min of exposure of concentration of 30 μM and higher.

Lataye et al. (1999) assessed the frequency of hearing deficits in rats exposed to Toluene. To identify the frequency range most sensitive to Toluene-induced auditory damage, the auditory function in male Long-Evans rats exposed to 1750 ppm of Toluene (6 h/day, 5 days/week, 4 weeks) was tested by recording auditory-evoked potentials directly from the round window of the cochlea. Electrocochleographic findings did not support a mid- to high-frequency loss of auditory sensitivity. However, the electrophysiologic data, obtained for auditory frequencies ranging from 2 to 32 kHz showed a hearing

deficit in the mid-frequency region (12-16 kHz) and in the mid-low-frequency region (3-4 kHz). Histological analysis was consistent with electrophysiologic data because a broad loss of outer hair cells occurred in both the mid- and mid-apical coil of the organ of Corti.

McWilliams et al. (2000) exposed pigmented guinea pigs to 250, 500, and 1000 ppm Toluene for 8 hr/ day, 5 days/week for 1 and 4 weeks. Assessment of auditory function was made using distortion product otoacoustic emissions with subsequent measurement of succinate dehydrogenase staining density in hair cells using surface preparations. Temporary disruption of auditory function was seen in all the concentrations and 500 and 1000 ppm Toluene produced greater acute dysfunction. Permanent hearing loss was observed at concentrations of 1000 ppm and greater after exposure for 6 hr/day for 5 days.

Thirty-three chinchillas were exposed to a 95 dBA 500 Hz octave band noise plus 2000 ppm Toluene for 8 or 12 hr per day for 10 days. Auditory function was estimated using the auditory brainstem response (ABR) to tones between 500 Hz and 16 Hz. There was no effect on the ABR of Toluene alone. Noise alone produced a threshold shift. There was no interaction of noise and Toluene on the ear. The results suggest that chinchillas are markedly less susceptible to the ototoxic effect of Toluene than mice and rats (Davis et al. 2002).

Effects on Visual Function

Male DA-HAN pigmented rats were exposed to Toluene (1000 ppm 21 hr/day, for 6 or 11 weeks). The function of the vestibulo- and opto-oculomotor systems was tested one month after the end of the exposure by recording of the nystagmus, induced by vestibular or optokinetic stimuli. The eye movements were recorded by a magnetic search coil technique. The optokinetic gain in the exposed animals was reduced compared to a control group. There was also a slight reduction in gain during sinusoidal oscillatory vestibular stimulation. No effect of the Toluene exposure on the gain or duration of nystagmus during acceleratory or deceleratory rotatory stimulation was demonstrated. The function of the visual system was tested 2 to 5 days after exposure by recording the electroretinogram and the visual evoked

response. The conduction velocity in the peripheral nerve was also measured. No effect of the Toluene exposure on these variables was seen (Nylén et al. 1991).

Neurotoxicity/Behavior Studies

Ghosh and Pradhan (1987) studied the effect of Toluene inhalation on an operant behavior by a fixed-ration schedule of liquid reinforcement in rats. Rats were exposed successively to 105, 214, and 382 ppm of Toluene vapor for 2 hours during 6 1/4 hour sessions. The reinforcement rate was not altered at hours 1 and 2, but was decreased at hours 3 and 6 of exposure. However, at hours 4 and 6, the reinforcement rate showed recovery from depression.

Kishi et al. (1988) tested behavioral effect of Toluene in rats. Male Wistar rats were trained to avoid a 10-second shock by pressing a lever in response to a warning light. After rats showed an 80 % success rate in avoiding the shock, they were assigned to treatment groups. The trained rats were then exposed to 0, 125, 250, 500, 1000, 2000, or 4000 ppm Toluene by inhalation for 4 hours. The rats were challenged with the shock avoidance task during, and after Toluene or control air exposures. Exposures to 1000 and 2000 ppm Toluene produced concentration-related increases in incorrect lever presses, accelerated reaction times, and decreases in effective shock avoidance response. At the 4000 ppm exposure, the response rate initially increased and then gradually decreased due to ataxia. Measurements of blood and brain concentrations of Toluene revealed that anesthetic performance decrements were observed when the blood Toluene level was 120 µg/mL and the Toluene concentration in the brain was 160 µg/g.

Arito et al. (1988) exposed Sprague-Dawley rats to 0, 900, or 2700 ppm Toluene by inhalation for 8 hours once or for 8 hours per day, 5 days per week, for 3 weeks. Indwelling electrodes measured sleep patterns. The single exposure to Toluene produced a prolonged paradoxical sleep latency and increased slow-wave sleep, and wakefulness was depressed. Repeated exposures prolonged both slow wave sleep and paradoxical sleep latencies, eliminated the increase in slow wave sleep, and increased the light-phase level of wakefulness. Blood and brain concentrations of Toluene were similar after single and repeated

exposures.

Wada et al. (1988) investigated the effects of Toluene exposure on shock avoidance learning in rats. Male Wistar rats were exposed to 1000 or 2000 ppm Toluene 4 hours per day, five days per week, for 0, 1, 3, or 6 weeks. Rats received shock avoidance training after their last dose. This learning task occurred in an apparatus consisting of two connected boxes. A warning signal was given before a mild electric current was initiated in one side of the box. The time it took the rat to move from the electrified box to the safe box was recorded as the response latency. All Toluene dosed and control rats were able to learn to avoid the shock, but Toluene treated animals took longer to learn to avoid the shock than control animals.

Wistar rats were exposed to 2000, 4000, 6000, and 8000 ppm Toluene vapor for 4 hr after they acquired shock avoidance learning. Then the effects of Toluene on avoidance performance, locomotor activity, and response latencies were simultaneously examined for 3 days. Shock avoidance responses were significantly decreased at concentrations of 4000, 6000, and 8000 ppm, but recovered 306 hr after the cessation of exposure. The 2000 ppm exposure had no effect on these responses. Locomotor activity was transiently increased at concentrations of 2000 and 4000 ppm, but recovered after 6 hr. Both 6000 ppm and 8000 ppm exposure at first decreased locomotor activity but later increased it. Response latencies were shortened at concentrations of 2000, 6000, and 8000 ppm due to hyperactivity (Hosokawa and Saito 1989).

Wood and Colotla (1990) examined changes in mouse motor activity during exposure to Toluene. Six groups of six male CRL:CD1 mice were placed in a vacuum desiccator divided into six wedge-shaped compartments. A phototransistor in each wedge measured the movement of individual mice, thus each mouse served as its own control. Six groups of six mice were exposed to each of five concentrations of Toluene (300-3000 ppm) or air in a Latin square design for 1 hr 2 days a week. Individual animals differed in their sensitivity to Toluene. The magnitude of the effect was related to concentration, the duration of exposure, and the control rate of activity. Activity increases were obvious at 560 ppm, and decreases at 300 ppm.

Ladefoged et al. (1990) studied the effect of Toluene on the preference of ethanol in rats (strain

unspecified). Toluene was administered orally by stomach tube in doses of 200, 400, or 800 mg/kg daily for 5 weeks or by inhalation at a concentration of 2000 ppm for 6 or 8 hr daily for 5 or 6 days per week for 2 weeks in rats of different age. During Toluene inhalation exposure the rats had access to either tap water or ethanol containing water (6 or 10%). After the exposure and during oral administration the rats had access to both ethanol-free and ethanol-containing water. Toluene inhibited the body weight gain in the highest oral dose group and in rats exposed to Toluene and forced to drink ethanol in the inhalation experiments. In these experiments, the intake of fluid was reduced in the exposure period in rats forced to drink ethanol-containing water and further reduced in rats exposed to both ethanol and Toluene. Exposure to Toluene alone increased the fluid consumption. The preference of ethanol as defined as consumed ethanol-containing water in per cent of the total water intake was not influenced by oral administration of Toluene. It was, however, reduced by Toluene given by inhalation to rats forced to drink ethanol-containing water during the exposure period. Toluene exposure alone or forced ethanol intake alone caused a short-lasting reduction of the ethanol preference. It was concluded that Toluene decreased the preference of ethanol in rats forced to drink ethanol during exposure to Toluene.

Knisely et al. (1990) trained 9 male Sprague-Dawley rats to discriminate Toluene (100mg/kg, IP) from saline (5 ml/kg) in a two-lever operant task. Animals were required to press one of two response levers during daily 15 min sessions for food presentation. The correct lever for each training session was dependent upon whether the animal received an injection of either Toluene or saline 20 min prior to the session. Incorrect lever responses reset the response requirement on the correct lever. Acquisition of the discrimination required a range of 85-219 training days. Injections of either methohexital (0.5-10 mg/kg) or oxazepam (0.5-20 mg/kg) produced Toluene-lever responding in a dose dependent fashion in most animals. These results provide further evidence that Toluene has stimulus properties similar to the of CNS depressant drugs.

Pryor (1990) exposed weanling rats to 0, 2000, or 2600 ppm Toluene for 8 hours per day, 7 days per week, for 7 weeks. The rats were evaluated for forelimb and hindlimb gripstrength, rotorod performance, gait analysis, and landing foot splay during and for 6 weeks after the exposure period. Grip strength was not consistently affected by Toluene exposure. In the gait analysis stride length was

consistently shorter, stride width and stride angle were consistently greater, and landing foot splay was wider in Toluene-exposed animals, compared to control animals.

Pryor (1991) conducted experiments to determine the extent to which subchronic exposure of male weanling Fischer 344 rats to Toluene might cause symptoms seen in heavy abusers of Toluene products. Rats exposed to Toluene (2000-2600 ppm for 11 weeks) developed a persisting motor syndrome characterized by shortened and widened gait and a widened foot splay. Rats exposed to Toluene were also hearing impaired. In a second experiment, rats were exposed to Toluene under three daily schedules - 2200 ppm continuously for 8 hr per day; 4400 ppm, 30 min each hr, 8 h per day; or 6200 ppm, 15 min each hr, 8 hr per day. The exposures were 7 days per week for 23 weeks. The motor syndrome and hearing impairment were replicated in all respects in all Toluene-exposed groups.

Forkman et al. (1991) studied the effect of inhalation exposure to Toluene (1000 ppm, 21 h/day, 5 days/week, for 4 weeks) on male Sprague-Dawley rats (18 rats per group, 2 groups). One of the groups was first trained in the same experimental box that was later used in the operant behavior-baseline test (Experiment 1) and the operant behavior-extinction test (Experiment 2). After training, they were exposed to toluene and then tested. The other group was first exposed, then trained and tested. Nine rats in each of the 2 groups served as controls. In Experiment 1, the rats were trained to press a lever for a reward. The test system then shifted such that no other rewards were given until the rat broke a photobeam or passed a photocell (which also resulted in an immediate reward). The authors wanted to investigate if baseline performance was affected by Toluene exposure. For rats trained first, the exposed animals had a higher lever pressing efficiency ($p<0.05$) and a lower total and unbroken persistence of lever pressing than the control animals ($p<0.05$). For the rats exposed first, the unbroken persistence of lever pressing of the exposed animals was lower than that of the control animals ($p<0.05$). In Experiment 2, the aims were to register the effect of Toluene exposure on the resistance against extinction of an operant response, and to test if the reaction to the secondary stimulus (the sound of a dipper) varied between the groups. Tests were conducted 26 days (training first group) or 35 days (exposure first group) after the end of exposure. The setup for Experiment 2 was similar to that of Experiment 1, except that after 10 minutes, the system turned off (no rewards given for pressing the lever). The rat then had the opportunity to earn rewards by

crossing photobeams only. For the rats trained first, the exposed animals made more runs ($p<0.05$) during the extinction period. For the rats exposed first, the number of runs made by exposed animals was also higher ($p<0.05$) then that of control animals.

The authors also tested motor coordination (Experiment 3), water loss and water intake after water deprivation (Experiment 4), and Holeboard activity (Experiment 5). For Experiment 3, the rats were put on a grid made of pins (diameter 1 mm) with the distance between pins 20 mm. The number of times a foot slipped through the grid during 0-5 minutes and 5-10 minutes were counted. The test was made 26 days (training first) or 35 days (exposure first) after the end of the exposure. For the rats first trained, no significant differences were found between groups. For the rats exposed first, the number of slips during the first 5 minutes made by the exposed animals was lower than that of control animals ($p<0.05$). For Experiment 4, the rats were tested for differences in water intake after 24 h of water deprivation. Rats were weighed, kept for 24 h without water, then reweighed. The rats then had access to water, and the consumption was recorded for 0-20, 20-90, and 90-180 min. The test was done 30 days (training first group) or 57 days (exposure first group) after the end of exposure. For the rats trained first, the exposed rats drank more water ($p<0.05$) than the control animals during the first 20 min following deprivation. For the exposure first group, the weight loss during water deprivation of the exposed animals was larger ($p<0.05$) than that of the control animals. For Experiment 5, a holeboard apparatus was used to monitor the exploratory response and general activity. The holeboard was 70x70 cm with 32 holes, equipped with infrared photocells. No significant differences were found in the rats trained first group. For the exposed first group, the exposed animals caused a larger number of corner and rearing counts ($p<0.05$) and they also spent a longer time rearing than the controls ($p<0.05$). The authors concluded that exposure to Toluene can cause a long-term impairment of the extinction process and a diminution in the variability in an operant behavior situation. The results indicate that Toluene exposure can cause long-term effects on complex integrative brain functions regulating behavior. There are also indications that Toluene exposure induces long-term effects on water regulation (Forkman et al. 1991).

von Euler et al. (1991) tested the locomotor activity of Sprague-Dawley rats 18, 42, or 118 hours after the last exposure to 80 ppm (6 hr/day for 3 days) Toluene. The control group was exposed to air

only. Exposure to 80 ppm Toluene and left for 18 hours without exposure did not show changes in locomotion, motility, or rearing examined in the locomotor cage, when compared with air-exposed rats (12 rats in each group). However, when rats were injected subcutaneously with 0.05 mg/kg of apomorphine, a dose which produced suppression of locomotor activity, the suppression in rearing was significantly less in the Toluene-treated group compared with the air group ($p<0.05$), and the same trend was observed for locomotion and motility. When the post-exposure delay was increased to 42 h and 114 h, the benefits of apomorphine disappeared for the Toluene-treated group.

Von Euler et al. (1993) examined the effects of subchronic inhalation exposure to Toluene (80 ppm, 6 hr/day, 5 days/week, for 4 weeks) on spatial learning (post-exposure days 3-6) and memory (post-exposure day 14) using a water maze. Spontaneous and apomorphine-induced (1 mg/kg s.c.) Locomotor activity and the binding parameters of dopamine D_2 agonist S(-)[N-propyl-^{3}H(N)]propylnorapomorphine ([^{3}H]NPA in the membrane preparations of the neostriatum of the rat were also studied. Toluene treatment caused a significant impairment in acquisition and retention of the spatial learning task. Furthermore, toluene significantly increased (2-fold) apomorphine-induced locomotion and caused a trend for a 50-60% increase in motility without any significant effect on rearing. Spontaneous locomotion, motility and rearing were not affected by Toluene. Toluene treatment produced a significant 30-40% increase in the B_{max} values of [^{3}H]NPA and a trend for a 20-30% increase in the K_D values. The results indicated that subchronic exposure to Toluene in low concentrations causes a slight but persistent deficit in spatial learning and memory, a persistent increase in dopamine-mediated locomotor activity, and an increase in the number of dopamine D_2 receptors in the rat.

To investigate how circadian variations affect acute Toluene toxicity, 12 male Wistar rats were exposed to 2000 or 4000 ppm both in the dark (the active phase) and the light (the inactive phase) for 4 hours. The decrements of rats were greater in the light phase than in the dark phase in all time zones of exposure to Toluene. In the dark phase, the performance recovered almost to that pre-exposure, whereas a significant delay of recovery was noted in the light phase. When rats were placed in Skinner boxes, the differences in the number of lever presses between exposure to 2000 ppm Toluene and control (air) exposure were also greater in the light phase than in the dark phase. Both blood and brain concentrations

in the light phase were higher than those in the dark phase at four hours after exposure to 2000 ppm or at two hours after exposure to 4000 ppm Toluene. The authors concluded that the difference in circadian susceptibility may be caused by circadian differences in pharmacokinetics of Toluene in the light and dark phases (Harabuch et al. 1993)

Male Sprague-Dawley rats were exposed to Toluene (80 ppm, 6 hr/day/ 6 days/week, 4 weeks). Rats were killed 17 days after the last exposure and the blood analyzed with radioimmunoassay for serum levels of prolactin. For the Toluene-exposed rats, there was a 67% increase in the serum level of prolactin when compared to the control group. The Toluene-exposed rats also showed a higher level of apomorphine-induced locomotion and motility but not rearing, as analyzed 17 days after the last exposure (von Euler et al. 1994).

The effects of Toluene (178-3000 ppm) were determined in 12 rats trained to nose poke on a probabilistic schedule of food delivery in a chamber that was ventilated with air or a Toluene test atmosphere. Animals served as their own controls and were exposed to Toluene twice a week for 2 hr during behavioral evaluations. Six determinations of the effects of Toluene were made at 178, 300, and 560 ppm and at least three determinations at 1000, 1780, and 3000 ppm. Rates of nose poking increased in 4 of 12 animals at 178 ppm and in 9 of 12 animals at 300 ppm; all animals were affected at 560 ppm (Wood and Cox 1995).

Miyagawa et al. (1995) exposed young Fisher 344 rats to 0 or 600 ppm Toluene by inhalation for 24 hours per day for 50 days after weaning at 3 weeks of age. The rats were trained on memory tasks using a radial arm maze. No significant effects of Toluene exposure on memory performance was observed.

The acute oral neurotoxicity of Toluene was investigated in male and female Sprague-Dawley rats. The 3 oral (gavage) doses were 3.0 ml/kg, 4.5 ml/kg, and 6.0 ml/kg. These doses were 50%, 75%, and 100% the maximum non-lethal neurotoxic dose, respectively. To characterize its neurotoxicity, a functional observational battery (FOB) and spontaneous motor activity (SMA) were measured on days 1 (2-3 hr and 4-7 hr postexposure times for FOB and SMA, respectively), 7, and 14 following Toluene administration. On day 1, when compared with saline controls, Toluene-exposed rats (males at 4.5 and

6.0 ml/kg doses and females at 6.0 ml/kg dose) exhibited significantly ($p<0.05$) greater horizontal and stereotype motor activities. However, vertical activity was significantly depressed ($p<0.01$) in both sexes at all doses (Mehta et al. 1998).

Rogers et al. (1999) studied the neurobehavioral sensitization to Toluene in Sprague Dawley rats. Two groups of 16 rats, 8 male and 8 female received whole body inhalation exposure to Toluene, either at 80 ppm for 6 hr/day for 4 weeks (Repeat group) or to 1600 ppm for 6 hr/day on one day only (Acute group). Two other groups (Trigger group and Clean group) of 16 were sham-exposed. After 17 days without Toluene exposure, the Acute, Repeat, and Trigger groups began a series of daily Toluene "trigger" exposures (10 ppm for 1 hr) followed immediately by an operant repeated-acquisitions tasks requiring learning within and across sessions. Briefly, the rats had to learn which of 3 levers produced a reward. The correct lever was then changed. The Clean group was sham-exposed prior to operant testing. Trigger or sham exposures and operant testing continued 5 days/week for 17 sessions. The progression of subjects through training did not appear to be affected by Toluene exposure. In terms of accuracy male and female rats performed in a statistically equivalent manner. Relative to the Clean (control) group, all three Toluene-exposed groups made more responses. The Acute group had the highest number of incorrect responses, followed by the Repeat group, then the Trigger group. The Acute and Repeat groups also took significantly longer to earn a reward compared to the Trigger and Clean groups.

Wada (1999) studied the effects of Toluene on temporal discrimination in Wistar rats. Thirty-five rats acquired a temporal discrimination of seven signal durations in an operant chamber with two levers. If a 2-sec or 8-sec tone signal was given, rats were required to press one lever ("short" response) or the other lever ("long" response) to be reinforced, respectively. Neither response was reinforced when five intermediate signals were presented. Percentages of a long response in each of the seven signals were calculated and the psychophysical function between signal durations and long response percentages was obtained. Intraperitoneal injections of 50 mg/kg and 100 mg/kg Toluene steepened a gradient of the psychophysical function and elevated correct responses in 2-sec and 8-sec signals. However, 400 mg/kg and 600 mg/kg Toluene resulted in a shallower gradient and reduced correct responses. Accuracy and discriminability deteriorated and, in particular, behavioral depression was observed for 600 mg/kg

Toluene. The authors speculated that temporal discrimination was sharpened by 10-20 µg/ml Toluene in blood and disrupted by 50-70 µg/ml Toluene in blood.

A conditioning paradigm of Toluene inhalation was developed in order to estimate the rewarding effect in male ICR mice. Conditioning sessions (five for toluene, five for air) were conducted twice daily for 5 days using an airtight inhalation shuttlebox which was divided into two compartments. One compartment was white with a textured floor and the other was black with a smooth floor. All conditioning sessions were 20 min in duration, with a minimum of 7 hr between sessions. Exposure to Toluene (700-3200 ppm) produced a significant conditioned place preference in mice. Mice showed preference to the compartment associated with Toluene compared to control (air-exposed) mice which showed no preference for either compartment (Funada et al. 2002).

Chen and Lee (2002) investigated whether Toluene exposure during the synaptogenesis period alters the N-methyl-D-aspartate (NMDA) and γ-aminobutyric $acid_A$ ($GABA_A$) receptor-mediated seizure susceptibility in juvenile rats. Neonatal rats were administered Toluene (1 g/kg i.p.) daily over postnatal days 4-9. Rats were administered NMDA (10 mg/ml), picrotoxin (2 mg/ml), pentylenetrzol (5 mg/ml) and 4-aminopyridine (2mg/ml) via timed tail vein infusion on postnatal days 34-36. Toluene exposure increased sensitivity to NMDA, picrotoxin, and pentylenetetrazol, but did not affect 4-aminopyridine-induced seizures in both male and female Sprague-Dawley rats. These results suggest that Toluene may pose a risk to the developing brain by inducing a long-term alteration in the function of NMDA and $GABA_A$ receptors.

Páez-Martinez et al. (2003) compared the effects of 5 solvents, including Toluene, on nociception in Swiss Webster mice. Independent groups of mice were exposed to air (control),Toluene (1000-4000 ppm), benzene (1000-4000 ppm), 1,1,1,-trichloroethane (TCE, 2000-12000 ppm), diethyl ether (10000-30000), or flurothyl (200-600 ppm). After a 30-min exposure, animals were tested in the conditioned defensive burying (CDB) test or in the hot plate test. All solvents but flurothyl produced anxiolytic-like actions with Toluene being the most potent. When tested in the hot plate test, Toluene and TCE increased nociception. The other solvents either had no effects or decreased nociception. Additional groups of mice were conditioned to recognize an adverse stimulus (electrified prod) prior to Toluene exposure and then tested in the CDB test. In unconditioned animals, Toluene increased the number of shocks that mice

received; however, when mice had previous experience in the CDB test, Toluene lacked this effect. The data suggest that acute administration of Toluene could impair learning.

Berenguer et al. (2003) assessed locomotor and rearing activities of Sprague-Dawley rats by placing animals in individual cages with 2 couples of parallel horizontal infrared beams. Beam interruptions were identified , accumulated, and recorded over 1 minute intervals. Toluene (40 ppm) did not significantly alter basal locomotor activity in male and female rats control to control males and females. However, locomotor activity was significantly higher in female rats than in male rats in both control ($p<0.01$) and toluene-exposed animals ($p<0.005$). Toluene exposure significantly decreased rearing activity in both male and female rats, as compared to control animals. The authors also measured adaptation/sensitization to narcosis by placing rats in compartments inside a cylinder which was then rotated at a rate of 1 rpm. Toluene (40 ppm) was administered into the test chambers to measure rate of righting reflex when the rat rolled over on the floor. Toluene-induced narcosis occurred at significant lower concentrations in Toluene treated rats that in control animals in both males ($p<0.02$) and females ($p<0.02$). This result may reflect a sensitization process. Males were also more prone to narcosis than females ($p<0.02$).

Chan and Chen (2003) studied the effects of Toluene on aminophylline-induced seizures in male NMRI mice. Mice were pretreated with an i.p. of corn oil or Toluene (100-500 mg/kg) followed by a timed intravenous infusion of aminophylline at various time intervals to assess the seizure thresholds and lethal doses. Toluene increased seizure susceptibility to aminophylline in a dose-and time-dependent manner. Seizures occurred as early as 30 min and persisted for at least 3 days after a single administration of Toluene (500 mg/kg).

Wiley et al. (2003) evaluated the effects of repeated or continuous exposure to Toluene. In their first experiment, ICR mice were continuously exposed to 250 ppm Toluene via inhalation for four days. They developed a mild dependence that was characterized by an increase in severity of handling induced convulsions. In the second experiment, CFW mice developed cross-sensitization to the initial locomotor stimulatory effects of Toluene following four days of injections with 10 mg/kg/day diazepam.

Genotoxicity

The mutagenic potential of Toluene was investigated with the dominant lethal mutation assay. Male Sprague-Dawley rats were injected intraperitoneally for 5 consecutive days with 346 and 692 mg per kg body weight of Toluene in corn oil. To analyze for the effect of Toluene on several germ cell stages, each male was mated with one untreated, virgin female per week for up to 7 weeks. Females were killed 14 to 17 days after insemination for analysis of their uterine contents. The total number of implantations and the number of dead and living embryos per pregnant female were determined. The dominant lethal mutation index was then calculated. There was no significant effect of Toluene on the number of implantations (total, dead, or alive) per female per week. The different stages of spermatogenesis were not affected by the action of Toluene as measured by the dominant lethal mutation assay. The dominant lethal mutation indices were small positive and negative percentages, suggesting that Toluene did not induce dominant lethal mutations (Washington et al. 1989).

Reproductive and Developmental Toxicity

Da-Silva et al. (1990) exposed pregnant Wistar rats and hamsters (*Mesocricetus auratus*) to 800 mg/m^3 for 6 hr daily from gestation days 14-20 and 6-11, respectively. Growth, neuromotor development, and performance of the offspring in behavioral tasks were assessed. In rats, Toluene exposure increased the number of litters with low birth weight pups. Male rat offspring exposed to Toluene displayed shorter latencies than male controls to choose one side of a T maze in a spontaneous alternation test. Hamsters exposed to Toluene performed worse in a rotating rod test.

da Silva et al. (1990) investigated the effects of prenatal exposure to Toluene in well nourished and malnourished rats. Sprague-Dawley rats were mated, and the presence of sperm in a vaginal smear confirmed mating on gestation day 0. Well nourished dams were fed a standard commercial rat diet ad libitum. Malnourished rats were given 50 % of the daily amount of food consumed by rats in the ad libitum

group. The restricted diet of malnourished rats began on gestation day 0 and continued throughout gestation. Subcutaneous Toluene doses of 0 or 1.2 g/kg in corn oil were given daily on gestation days 8 to 15 (n = 7 to 8 rats per treatment condition). The dams dosed on gestation days 8 to 15 were killed on gestation day 20. Caesarian sections were performed and the litters and fetuses were evaluated for visceral and skeletal evaluations. Toluene exposure alone not affect maternal weight gain, fetal body weight, or placental weight in well fed rats. Rats in the malnutrition groups had significant reductions ($p < 0.05$) in maternal weight gain, fetal body weight, and placental weight. Toluene reduced maternal body weight of well nourished and in malnourished dams during the dosing period ($p < 0.05$), and there was an interactive effect of Toluene exposure and malnutrition to further depress maternal weight gain during the dosing period. Number of corpora lutea, implants, live fetuses, and percentage of dead and resorbed fetuses were similar between all treatment groups. No malformations occurred in any treatment group. Skeletal growth was not affected by Toluene in the well nourished group. The number of ossification centra was significantly decreased ($p < 0.05$) in the malnourished-Toluene-exposed fetuses, compared to malnourished control fetuses and to well nourished fetuses exposed to Toluene. Therefore, there seems to be an interaction of malnutrition and Toluene exposure on fetal skeletal development.

In an additional experiment, well nourished and malnourished (as defined above) pregnant rats were given daily subcutaneous injections of 1.2 g/kg Toluene on gestation days 14 to 20. The litters were delivered. Three males and three females from each litter were fostered to surrogate non-treated lactating mothers. One pup per sex from each experimental litter were dissected, and their brains and liver were weighed individually. Remaining pups were killed and stained for skeletal evaluations. The fostered pups were weaned on postnatal day 25 and used for behavioral testing. On postnatal day 30, spontaneous motor activity of the pups was measured by photobeam breaks in a 40 x 25 cm motility chamber. Two-way shock avoidance (0.4 mA) was tested in an automatic shuttle-box on postnatal day 95. Food restriction reduced body maternal body weight gain ($p < 0.05$). Toluene reduced maternal body weight gain during the dosing period ($p < 0.05$), but no reduction in food consumption of well nourished dams was found with Toluene exposure. One of the malnourished and toluene-treated dams delivered all dead fetuses and another died in labor. Litter size was similar between all treatment groups. Toluene exposure and

malnutrition each reduced pup birth weights, but there was no additional reduction in pup birth weight in the toluene-treated malnourished group. Body weights at postnatal day 95 continued to be lower in the toluene-exposed groups. Toluene exposure and malnutrition each reduced brain weights, but there was no additional effect of the combination. Liver weights were affected by malnutrition but not by Toluene exposure. Skeletal development was most affected by the combination of Toluene and malnutrition. Total spontaneous motor activity over 15 minutes was similar between all treatment groups on postnatal day 30, however malnourished pups had higher activity in the first five minutes of testing. Toluene did not affect performance in the conditioned avoidance test on postnatal day 95. There was an effect from malnutrition, but no significant interaction effect with Toluene. There were no behavioral effects due to prenatal exposure to Toluene in this study (da Silva et al. 1990).

Slomianka et al. (1990) studied the effect of Toluene exposure on the development of the hippocampus in young rats. Wistar rat pups were exposed to 0, 100, or 500 ppm Toluene by inhalation for 12 hours per day on postnatal days 1 to 28. After the last exposure, the rats were killed, and their brains were removed and immediately frozen. The brains were sliced into sections and stained to define the hippocampal regions. The layers of Ammon's horn and the subiculum were not affected were not affected quantitatively or qualitatively by either Toluene exposure level. Within the dentata, the volume of the granule cell layer was 6% smaller in the 100 ppm group and 13% smaller in the 500 ppm group, compared to the control group. The volumes of the hilus, and the commissural-association zone of the dentate molecular layer (both areas rich in granule cells) were smaller in the 500 ppm group than in controls. Granule cell layers in animals of the 100 ppm group were not affected.

To determine if Toluene exposure from maternal milk would affect the developing brain in Wistar rats, three month old, lactating rats were injected with Toluene (1.2 g/kg) daily from lactation day 2 (day of delivery = day 1) to day 21. Controls were injected with vehicle (corn oil). Offspring (7 pups per litter) were evaluated for neurosomatic development and exploratory behavior before weaning and behavior in the open field. A second group of Toluene-treated rats and controls was used to evaluate behavior of the offspring in the open-field on day 35 and performance in a shuttle box in adulthood. Toluene levels in milk 4 hr after a single injection of 1.2 g/kg (sc) were 5 times higher than in blood. No effects of treatment on

offspring development or on any of the behavioral tests were observed (Da-Silva et al. 1991).

Brown et al. (1994) studied the embryotoxicity of Toluene alone and in combination with other aromatic hydrocarbons. Male and female Sprague-Dawley rats were paired, and mating was confirmed by the presence of sperm in vaginal smears on gestation day 0. On gestation day 10, approximately 10 embryos per dam were explanted into cultures. Four to six embryos were cultured in each 50-mL flask containing 6.0 mL inactivated rat serum. Cultured embryos were exposed to 0.37 to 4.06 µmol/mL Toluene (verified by GC) for 16 hours. Dimethyl sulfoxide was used to facilitate dissolution of the solvent in the serum. The embryos were washed and placed in solvent-free serum for the following 24 hours. The embryos were then examined under a dissecting microscope for the presence of a heartbeat and large functioning vessels in the visceral yolk sac. Yolk sac diameter, crown-rump length, somite number, and protein concentration were significantly reduced ($p < 0.01$) in embryos exposed to Toluene concentrations of 2.25 and 4.06 µmol/mL ($n = 8$ and 12, respectively), compared to control embryos that were not exposed ($n = 16$). These effects were not seen at Toluene exposures of 0.37 or 1.46 µmol/mL ($n = 2$ or 8, respectively). The effect was dose dependent, as the 4.06 µmol/mL exposure caused no development of large blood vessels in the yolk sac. However, embryonic survival, as determined by percentage of embryos with a heartbeat, was not affected by the Toluene exposure. The no observed effect level for embryotoxicity in this study was 1.46 µmol/mL Toluene. Additional experiments showed that xylene and benzene had similar effects at 1.08 and 1.56 µmol/mL, respectively. Exposure of the embryos to Toluene in combination with xylene or benzene produced additive effects on the measures of embryonic development without synergistic interaction.

Gospe et al. (1994) studied the effects of Toluene on embryonic development in Sprague-Dawley rats. Eleven control and 11 Toluene treated dams were used. During days 6-19 of gestation, the treated group received daily gavage doses of Toluene (520 mg/kg body weight, diluted in corn oil) while the control group received corn oil only. This dose simulates the blood toluene levels obtained after exposure to 3290 ppm, an inhalation level in the lower end of the range experienced by toluene abusers. On day 19, dams were killed. The gravid uterus and maternal liver were then removed and weighed. The uterus was then dissected and examined for live, stillborn, and resorbed fetuses. Maternal weight gain was 24% less

in the Toluene-exposed group ($p<0.002$); however, there were no maternal deaths. Toluene treatment did not affect the number of implantations or stillbirths. There were no toluene induced major congenital malformations or neuropathologic changes noted. In the Toluene-treated group, the weights of the fetuses were reduced by 9.4% ($p<0.004$) and placental weights were reduced by 10.3% ($p<0.01$). Toluene exposure also reduced fetal organ weights as follows: brain 4.6%, heart 5.9%, liver 13.2% ($p<0.02$), and kidney 13% ($p<0.05$). Organ weight/body weight ratios did not differ significantly suggesting that prenatal Toluene exposure produced a generalized growth retardation.

Gospe et al. (1996) conducted a similar experiment in which 8 pregnant Sprague-Dawley rats received a daily gavage dose of Toluene (650 mg/kg) diluted in corn oil. There were 8 control rats which received corn oil only. Rats were gavaged from gestation days 6 through 19. Dams were then killed and the gravid uterus and maternal liver removed and weighed. The uterus was then dissected and examined for live, stillborn, and resorbed fetuses. In the Toluene-exposed group, the weights of the fetuses were reduced by 21.6% ($p<0.001$), and a delay in the skeletal ossification was demonstrated. Toluene exposure significantly reduced the weight of the fetal brain by 11.9% ($p<0.001$), as well as the weights of the heart, liver, and kidney. Again, Toluene was found to produce a growth retardation in rat fetuses.

Edelfors et al. (2000) examined whether prenatal exposure to Toluene affects the response to post-natal exposure to Toluene in cultured synaptosomes. Pregnant Wistar rats were exposed to 1800 ppm Toluene or clean air for 6 hours/day on gestation days 7-20. Pups were weaned and group housed by sexes on postnatal day 21 (numbers not reported). For the in vitro study, on days 35-42, one female per litter was killed by decapitation for preparation of synaptosomes. Synaptosome suspensions were created from the entire brain except the cerebellum. The synaptosomes were then exposed to Toluene in vitro to 1800 ppm (n not reported). For the control, female rats unexposed by exposure to the dams to clean air were used (n=10). Reactive oxygen species (hydrogen peroxide) was measured by the 2,7-dichlorofluorescein technique. Malondraldehyde was determined by the LPO-586 method. Membrane leakage was measured by an adapted FURA-2 technique. Membrane fluidity was determined by fluorescence polarization using a Perkins-Elmer LS-5 luminescence spectrometer. Brain weights were reduced in Toluene exposed pups ($p<0.05$); body weight was not affected. Incubation of the

synaptosomes with 10 mmol/L Toluene for one hour showed a slight but nonsignificant difference in hydrogen peroxide production between cells from rats that had or had not been prenatally exposed to Toluene. Likewise, prenatal exposure to Toluene did not affect the malondialdehyde production in synaptosomes exposed to 10 mM Toluene for 2 hours. Membrane leakage of calcium in a Toluene-free medium was not affected by prenatal exposure to Toluene. However, addition of 10 mmol/L Toluene to the medium produced higher calcium leakage ($p<0.07$) in prenatally exposed cells than in those that were not exposed. Prenatal exposure to Toluene did not change the membrane fluidity measured in vitro in Toluene-free buffer (control rats). The membrane fluidity was significantly lower in synaptosomes from control offspring after exposure to Toluene for 2 hours compared to the level in non-exposed synaptosomes from control offspring. Prenatal exposure to Toluene induces long-lasting changes in oxidative status and membrane function.

Dalgaard et al. (2001) studied the developmental toxicity of Toluene on male offspring after in utero exposure. Pregnant Wistar rats were exposed to clean air or 1200 ppm Toluene by inhalation for 6 hours per day from gestation day 7 throughout gestation and daily after parturition to postnatal day 18. It is unclear whether the postpartum pups were also exposed to Toluene or just the mother. Sperm analysis was performed on the male offspring on postnatal day 110 (N = 10 pups per treatment group). There was no effect of Toluene on sperm motility.

In another experiment, pregnant Wistar rats were exposed to clean air or 1800 ppm Toluene by inhalation for 6 hours per day on gestation days 7 to 20. The litters were delivered and the male pups were killed on postnatal days 11, 21, or 90. Mean body weights of 11-day-old pups in the Toluene group were reduced ($p < 0.05$), compared to 11-day-old control pups. Perinatal Toluene exposure did not affect pup body weights at postnatal days 21 or 90. Absolute and relative testis weights were significantly reduced ($p < 0.05$) in Toluene exposed pups at all three ages measured. Microscopic observations of Toluene-exposed and control pups were similar. No apoptosis was detected by TUNEL assay in the hippocampus of either treatment group. However, Toluene induced a significant increase ($p < 0.05$) in the number of apoptotic cells in the cerebellar granule layer at postnatal day 21 (Dalgaard et al. 2001).

Nielsen et al. (2003) studied developmental neurotoxicity of Toluene in rats (strain not given) by

measuring the amount of L-ornithine decarboxylase in the Purkinje cells in the cerebellum. Pregnant rats were exposed from gestational day 7 to 20 to either clean air or 1800 ppm Toluene for 6 hr/day. One male from each litter (9-11 litters per group) was killed at postnatal day 11, 21, and 90. L-ornithine decarboxylase immunoreactivity in Purkinje cells were performed on sections of the cerebellum. The sections were incubated overnight with monoclonal anti-ornithine-decarboxylase diluted 1:200 in 10% normal rabbit serum/phosphate buffered saline. Tissues were rinsed and the binding detected by a final incubation with 0.8% 3-amino-9-ethyl-carbazole, then stained with Mayer's hematoxylin. The negative controls were slides with the primary antibody omitted and replacement of the primary antibody with mouse serum, while a positive control was made from rat cerebellum at gestation day 19. The level of ornithine decarboxylase immunoreactivity was graded into 4 categories (no stain, weak stain, medium stain, and distinct, clear stain). The intensity of the staining was higher at postnatal day 11 as compared to day 21 and 90, reflecting a decreasing ornithine decarboxylase immunoreactivity with age. When the treated groups were combined, there was a statistical significance when compared to the control groups ($p<0.05$) but not when each treated group was compared to its paired control.

In Vitro Toxicity

Suleiman (1987) exposed rat and rabbit pulmonary alveolar macrophages to 0.02 to 1.0 mM Toluene in cell culture medium for 0 to 9 hours. Cellular respiration and lipid peroxidation were measured as were the activities of the lysosomal enzymes cathepsin B and D. Concentration-dependent increases in the enzymes' activities were observed. The median lethal concentration (LC_{50}) for Toluene was estimated/extrapolated to be about 10 mM in the rat cells and 5.9 mM in the rabbit cells. Cellular respiration was significantly depressed in rat and rabbit cells at Toluene concentration of 0.5 and 1.0 mM. Lipid peroxidation was increased only in the rabbit cells exposed to 1 mM Toluene for 9 hours.

Hansson et al. (1988) exposed astroglial and neuronal primary cell cultures from rat striatum to 4.7 to 150 µmol/mL Toluene for 30 or 60 minutes. After 60 minutes of exposure to 40 µmol/mL Toluene, the cell bodies of astrocytes appeared contracted and their processes and nuclei were clearly visible. At

75 µmol/mL Toluene, the astrocytes were flattened and dyes indicated major cell damage. The cellular morphology of neurons did not appear to be affected at Toluene concentration below 150 µmol/mL, at which they became detached from the bottom of the culture dish.

Mollenhauer et al. (1990) grew L929 mammalian cells in a culture nutrient medium. The cells were exposed to Toluene concentrations of 50 to 500 ppm for 2 minutes to 48 hours. All Toluene exposures accelerated cell death compared to control cells. Addition of fetal bovine serum to the cultures protected the cells and delayed the toxic effects of Toluene.

Aakhus et al. (1991) studied the effects of Toluene on platelet proteins. In vitro concentrations of 1.5 to 2.8 mmol/L Toluene activated the calcium-dependent protease calpain, leading to the degradation of actin-binding protein (ABP) and release of glycocalcin from glycoprotein Ib. There was also a reduction in von Willebrand factor-induced platelet agglutination. Degradation of ABP was not seen at the lower concentrations of 0.3 to 1.4 mmol/L Toluene, but there was a temporary initial increase in agglutination.

Yelian and Dukelow (1992) reported that 8.67 µg/mL Toluene in a culture medium decreased sperm motility, inhibited in vitro fertilization, and increased preimplantation embryo degeneration.

Cruz et al. (1998) exposed Xenopus oocytes to ~9 mM Toluene in culture medium. The oocytes had previously been injected with mRNA for the N-methyl-D-aspartate (NMDA) receptor, a ligand-gated calcium channel protein. There were three subunits of the NMDA receptor, NR1/2A, NR1/2B, and NR1/2C. The Toluene exposure inhibited NMDA-mediated currents in the oocytes. The inhibition was rapid, reversible, and selective of the subunits affected. The NR1/2B subunit was most sensitive with and LC50 of 0.17 mM Toluene. The NR1/2A and NR1/2C subunits were less sensitive with respective LC50 values of 1.4 and 2.1 mM Toluene. The authors concluded that some of the effects on neuronal activity and behavior may be mediated by inhibition of NMDA receptors.

Ma et al. (2002) prepared primary cell cultures of cortical neurons from gestation day 13 rat fetuses. The acetylcholine analog carbachol binds to muscarinic receptors and causes an increase in intracellular calcium. Toluene concentrations as low as 0.15 mM in the culture medium reduced the catechol-induces rise in intracellular calcium in the cultured neurons. This effect in reducing calcium signaling was dose dependent with an IC_{50} of 0.5 mM Toluene and a maximal effect (complete blockage

of catechol-induced calcium signaling) occurred with 10 mM Toluene. The authors concluded that because muscarinic receptors mediate cell proliferation and differentiation during neuronal precursor cell development, these results suggest that depression of muscarinic signaling may play a role in Toluene's teratogenic effect on the developing nervous system.

Cécil et al. (2003) reported that in vitro Toluene exposure concentration-dependently inhibited ion currents in acetylcholine (ACh) and γ-aminobutyric acid (GABA) receptors in human IMR-32 neuroblastoma cells. The respective LC_{50} values for inhibition of ACh and GABA receptor ion currents were 276 and 39 μM Toluene. The effective concentration of Toluene for GABA receptor inhibition was comparable to brain concentrations seen in occupational exposure to the solvent.

Toxicology and Carcinogenicity

NTP (1990) described several studies on the toxicity of Toluene. F344/N rats were given 0, 312, 625, 1250, 2500, or 5000 mg/kg/day Toluene in corn oil by oral gavage 5 days per week for 13 weeks. All of the rats and mice in the 5000 mg/kg groups died within the first week of dosing. In the 2500 mg/kg groups, 8/10 male rats died before the end of the dosing period. At necropsy, rats had higher relative organ weights in the liver, kidney, and heart (female rats only). There were dose-dependent increases in necrosis of the brain and hemorrhage of the urinary bladder.

$B6C3F_1$ mice were given 0, 312, 625, 1250, 2500, or 5000 mg/kg/day Toluene in corn oil by oral gavage 5 days per week for 13 weeks. All of the mice in the 5000 mg/kg groups died within the first week of dosing. In the 2500 mg/kg groups, 40 % of mice died before the end of the dosing period. At necropsy, mice had higher relative liver weights in the higher doses, compared to the vehicle control groups.

F344/N rats were exposed to 0, 100, 625, 1250, 2500, or 3000 ppm Toluene by inhalation for 6.5 hours per day, five days per week, for 65 exposures. Eight of 10 male rats in the 3000 ppm group died during week 2 of dosing. Final body weights of rats were 14 to 25 % lower in the two highest exposure groups than in controls. Relative liver, kidney, and heart weights were increased in the 2500 and 3000 ppm groups, compared to controls. There were no effects of Toluene on sperm count or motility or on

estrous cycle.

B6C3F$_1$ mice were exposed to 0, 100, 625, 1250, 2500, or 3000 ppm Toluene by inhalation for 6.5 hours per day five days per week for 65 exposures. All of the female and 5/10 male mice of the 3000 ppm and 70 % of the females in the 2500 ppm group died in the first two weeks of dosing. Final mean body weights of all Toluene exposed mice were 7 to 13 % lower than that of controls. Relative liver weights for mice exposed to ≥625 ppm Toluene were higher than in control animals. In the 1250, 2500, and 5000 ppm groups, relative lung weights (both sexes) and relative kidney weights (females only) were higher than in the control animals. Centrilobular hypertrophy of the liver occurred in all male mice in the 2500 ppm group and in 70 % of male mice in the 3000 ppm group. There were no effects of Toluene on sperm count or motility or on estrous cycle.

F344/N rats were exposed to 0, 600, or 1200 ppm Toluene by inhalation for 6.5 hours per day five days per week for 15 months or 103 weeks. Toluene exposed rats had increased incidences and severity of non-neoplastic lesions of the nasal cavity. No Toluene-induced neoplasms were observed at 15 weeks of dosing. After two years of exposures, there were no treatment-related differences in mean body weights or survival rates. Erosion of the olfactory epithelium and degeneration of the respiratory epithelium was increased in exposed rats. Inflammation of the nasal mucosa and metaplasia of the olfactory epithelium were increased in Toluene exposed female rats. The neoplasms that occurred in rats were not considered to be associated with exposure to Toluene.

B6C3F$_1$ mice were exposed to 0, 120, 600, or 1200 ppm Toluene by inhalation for 6.5 hours per day five days per week for 15 months or 103 weeks. Hyperplasia of the bronchial epithelium occurred in 4/10 female mice after 15 weeks of exposure to 1200 ppm Toluene. No Toluene-induced neoplasms were observed at 15 weeks of dosing. After two years of exposures, there were no treatment-related differences in body weights or survival rates. There were no neoplastic or non-neoplastic lesions observed in the mice after two years of exposures.

The NTP researchers concluded that there was no evidence of carcinogenic activity for Toluene in F344/N rats and B6C3F$_1$ mice (NTP 1990).

HUMAN DATA

Pharmacokinetics and Distribution

Campbell et al. (1987) compared three methods of biological monitoring of Toluene in workers in the printing industry. Mass spectrometric analysis of breath samples taken10 to 20 minutes after a 8 hours of 100 ppm Toluene exposure revealed breath concentrations of about 600 nmol/L. Blood concentrations of Toluene after the same exposure were about 20 µmol/L. Analysis of urine samples for hippuric acid was found to be an unreliable method for estimating Toluene exposure.

Bælum et al. (1987) measured the urinary excretion of hippuric acid and o-cresol after exposure to Toluene at rest or during exercise. Healthy male volunteers were exposed to 100 to 300 ppm Toluene for 140 minutes at rest or while riding a stationary bike at 100 Watts. The exercise increased the excretion rates of hippuric acid and o-cresol by 47 and 114 %, respectively. In a second study male and female were exposed to escalating concentrations of Toluene. Of the two metabolites measured, o-cresol was more reflective of Toluene exposure than hippuric acid.

Norström et al. (1988) tested a method for measuring urine concentrations of S-benzyl-N-acetylcysteine to estimate exposure to Toluene in human subjects. The method was determined to be a poor estimator of Toluene exposure.

Nise et al. (1989) measured the elimination of Toluene from venous and adipose tissue after occupational exposure. There were three non-linear phases of Toluene elimination from the blood. The first two had estimated half times of nine minutes and two hours. The third component, with a half time of 79 hours, reflected the slower release of Toluene from the adipose tissue.

Bælum (1990) exposed 20 male and 17 female volunteers to 100 ppm Toluene by inhalation for 7 hours or to a varying exposure with the same time-weighted average but with peaks of 300 ppm every 30 minutes. Measurements of Toluene concentrations in alveolar air were made at rest and during exercise (50 to 100 Watts on a stationary bike). The average alveolar concentration of Toluene was 16.5 ± 6.8 ppm at rest and 19.5 ± 5.3 ppm with exercise, and women had higher alveolar concentrations than men. There

was no difference in alveolar Toluene concentration between the continuous and intermittent exposures. Excretion of the metabolites hippuric acid and o-cresol in urine was correlated with alveolar Toluene concentration during rest but not during exercise.

Liu et al. (1992) reported that occupational exposure to up to 548 ppm Toluene was linearly correlated with hippuric acid concentration in urine at the end of the work shift (correlation coefficient = 8.06, $p < 0.01$).

Ameno et al. (1992) reported a case of a 31-year-old man who was found dead in a room filled with Toluene vapor and a stab wound to the chest. He had a venous blood concentration of 4.61 µg/g. Concentrations of Toluene detected in all regions of the brain ranged from 7.03 µg/g in the cerebellum to 12.40 µg/g in the corpus callosum.

Jang et al. (1993) reported that 23 workers who were occupationally exposed to 38.8 ± 45.5 ppm Toluene had a mean blood Toluene concentration of 0.94 ± 1.01 mg/L and a mean urine hippuric acid concentration of 0.92 ± 0.66 g/g creatinine.

Löf et al. (1993) exposed nine male volunteers to 200 mg/m^3 $^2H_8$-Toluene by inhalation for 2 hours while exercising at a workload of 50 Watts. Total uptake of Toluene was 50 % of the amount inhaled. Four hours after the end of exposure, 65 % of the total absorbed $^2H_8$-Toluene was excreted as ^2H-hippuric acid in urine, and this hippuric acid excretion percentage rose to 78 % of absorbed Toluene at 20 hours after exposure. The excretion rate of hippuric acid in urine was highest during exposure, at 5 µmol/min.

Monster et al. (1993) monitored the Toluene in the exhaled breath and Toluene and hippuric acid in the urine of nine workers who were occupationally exposed to 27.3 to 609 mg/m^3 Toluene. At the beginning of an average work day, the concentration of Toluene in exhaled breath reached a maximum value of 4 ± 0.8 µg/L after 22.5 ± 10 minutes of exposure The correlation coefficient (R) for Toluene exposure and hippuric acid in the urine was 0.86.

Wang et al. (1993) measured blood Toluene concentrations in 232 people who were not occupationally exposed to Toluene. The sample was also considered as subgroups based on lifestyles. The mean (± standard deviation) blood Toluene level in all 232 subjects was 829 ± 1175 ng/L, in 53 smokers was 897 ± 863 ng/L, in 179 non-smokers was 809 ± 1255 ng/L, in 126 rural residents was 698 ±

954 ng/L, and in 106 urban residents was 984 ± 1383 ng/L. For comparison, the mean blood Toluene concentration in 37 chemical industry workers with Toluene exposure was 2785 ± 3756 ng/L.

Kawai et al. (1994) measured Toluene in the blood and urine and hippuric acid in the urine of workers who were occupationally exposed to an average of 2.3 ppm Toluene (max 132 ppm). Toluene measured in blood was correlated (R = .711) with Toluene exposure down to exposure concentrations as low as 1 ppm. Hippuric acid was found to correlate poorly (R = 0.61)with Toluene exposure when the exposure was below 10 ppm. The sensitivities of blood-Toluene and urine-Toluene were comparable in their power to estimate Toluene exposure.

Brugnone et al. (1995) measured the blood and urine concentrations of Toluene in 100 workers who were occupationally exposed to Toluene. At the end of an 8 hour shift of being exposed to a mean concentration of 34 ppm Toluene, workers had a mean blood Toluene level of 457 µg/L. At the beginning of the shift in the morning (after 16 hours since the previous day's exposure) the blood Toluene concentration was 38 µg/L. A control sample of people who are not occupationally exposed to Toluene had a blood Toluene concentration of 1.1µg/L.

Kawai et al. (1996) measured Toluene, hippuric acid, and o-cresol in the urine of two groups of 39 and 76 workers who were occupationally exposed to an average of 1.7 and 5.9 ppm Toluene, respectively (mean exposure for combined groups = 4 ppm Toluene). The concentration of Toluene in the urine was the best indicator of Toluene exposure, especially at lower exposures (< 10 ppm). Hippuric acid and o-cresol each had poor correlation with Toluene exposure at exposure levels less than 30 ppm.

Nakajima et al. (1997) studied the in vitro metabolism of Toluene by human liver cytochrome P450 isozymes. Toluene was metabolized to benzyl alcohol by CYP2E1, and (in descending order of activity) by CYP2B6, CYP2C8, CYP1A2, and CYP1A1. The isozymes CYP2B6 and CYP2E1 produced p-cresol fro Toluene. Metabolism of Toluene to o-cresol and p-cresol by CYP1A2.

Amorim and Alvarez-Leite (1997) used gas chromatography to measure hippuric acid and o-cresol in the urine samples of workers who are occupationally exposed to Toluene. Workers in shoe factories (n = 26) had urine concentrations of <0.2 to 2.8 µg/mL o-cresol and 0.21 to 3.02 mg/mL hippuric acid. Metal painters (n = 12) had urine concentrations of <0.2 to 1.36 µg/mL o-cresol and < 0.1 to 2.1

mg/mL hippuric acid. Print shop workers (n = 16) had urine concentrations of <0.2 to 0.51 µg/mL o-cresol and < 0.1 to 1.2 mg/mL hippuric acid.

In developing a physiologically based pharmacokinetic model for Toluene in humans, Pierce et al. (1997) exposed 33 volunteers to 50 ppm radiolabeled Toluene by inhalation for 2 hours and measured blood and exhaled breath for Toluene and its metabolites for 100 hours after exposure. The mean blood concentration was 5.9 nmol/L, and the alveolar air concentration was 310 nmol/m^3. These values fell within the predicted concentrations based on their proposed model.

Tardif et al. (1998) exposed adult non-smoking volunteers to 10, 20, 30, 50, and 100 ppm Toluene by inhalation for 7 hours. Concentrations of hippuric and o-cresol were measured in urine, and concentrations of Toluene were measured in exhaled breath at 30 minute intervals during and after exposure. There was a good correlation between Toluene exposure and urinary levels of hippuric acid (R = 0.87) and o-cresol (R = 0.81) during the exposure period. For the 17 hours after exposure ended, urinary levels of o-cresol continued to correlate with Toluene exposure concentration (R = 0.88). Hippuric acid was a less reliable indicator of Toluene exposure after the exposure ended, especially at lower exposure levels. Alveolar Toluene concentrations were strongly correlated with Toluene exposure both during the exposure period (R = 0.99) and 30 minutes after exposure ended (R= 0.97). The authors recommended that o-cresol in urine and alveolar Toluene make better indicators of Toluene exposure than hippuric acid in urine.

Pierce et al. (1999) compared the phramacokinetics of two isotope-labeled Toluene analogs. They conducted 33 controlled human exposures to a mixture of 50 ppm $^1H_8$-Toluene and 50 ppm $^2H_8$ Toluene for 2 hours. Concentrations of Toluene in the blood and breath and of metabolites in the urine were measured for 100 hours post-exposure. Compared to $^2H_8$-Toluene, the $^1H_8$-Toluene had a 6.4 % lower area under the curve, a 6.5 % higher clearance (1.46 L/h-kg $^1H_8$ versus 1.38 L/h-kg $^2H_8$) , a 17 % larger volume of distribution (66.4 vs. 57.2 L/kg), and a 9.7 % longer half-life (38 vs 34 hours). There was wide inter-individual variability, but all of these differences were statistically significant ($p < 0.05$ for all comparisons). The authors cited differences in rates of ring oxidation and reduced lipophilicity of deuterium-labeled solvents as possible reasons for the differences in the pharmacokinetics of the two

variations of Toluene.

Thrall et al. (2002) used a physiologically based pharmacokinetic (PBPK) model to evaluate the dermal absorption of Toluene in human subjects. Six volunteers were submerged to the neck in a bath of water containing 500 µg/L Toluene for 20 to 30 minutes. The subjects were provided purified breathing air to prevent inhalation of Toluene, and their exhaled breath was captured for continual analysis during the exposure and for 15 to 30 minutes after exposure. Based on the PBPK model adopted by the researchers, the average dermal permeability coefficient of Toluene was 0.012 ± 0.007 cm/h.

Chen et al. (2002) reported that gasoline service workers exposed to 60.3 to 527 ppb in their personal air space had exhaled breath concentrations of 4.3 to 41.8 ppb. The correlation coefficient between personal air concentration and exhaled breath concentration of Toluene was 0.683 ($p < 0.0001$).

Pierce et al. (2002) measured the excretion of Toluene and its metabolites from men exposed to 50 ppm $^2H_8$-Toluene for two hours at rest. Exhaled breath removed 13 ± 6.2 % of the total dose. Urinary metabolites accounted for the following proportions of Toluene excretion: 75 ± 6.4 % as hippuric acid, 0.31 ± 0.22 % as o-cresol, 0.53 ± 0.44 % as m-cresol, and 11 ± 3.8 % as p-cresol. Excretion rates of the cresols were stable for five hours after exposure, and o-cresol was considered the best urinary indicator of Toluene exposure.

Effects on Liver

Guzelian et al. (1988) studied liver structure and function in print workers exposed to Toluene. A liver biopsy was performed on 8 workers who exhibited persistently abnormal results in biochemical tests of liver function. All eight had mild elevations of serum transanimases [alanine (ALT) and aspartate aminotransferase (AST)]. However there was a marked increase in the ratio of ALT/AST (mean =1.61). In each case, the liver biopsy revealed mild, pericentral fatty change.

Metabolism

Löf et al. (1990) studied the effects of paracetamol and acetylsalicylic acid on the toxicokinetics of Toluene. Two groups of 10 healthy male volunteers were exposed to 3.25 mmol/m^3 Toluene by inhalation for four hours. One group was exposed to Toluene only or Toluene with paracetamol (500 mg/70kg, p.o.). The other group was exposed to Toluene only or acetylsalicylic acid (500 mg/70 kg, p.o.). Exhaled air and blood were collected and analyzed. Compared to the respective control groups, the paracetamol and acetylsalicylic acid increased the blood concentrations of Toluene. Paracetamol significantly reduced the apparent clearance of Toluene from the blood ($p = 0.030$). Acetylsalicylic acid did not affect Toluene clearance.

Bælum et al. (1993) examined hepatic metabolism of Toluene after gastrointestinal uptake in humans. The metabolism of Toluene and the influence of small doses of ethanol were measured in 8 male volunteers. The basal dose rates of Toluene used was equivalent to 2 mg/min continuously for 3 hr. This dose was calculated to correspond to inhalation exposure to 50 ppm in combination with light exercise. Toluene was delivered in a tube which was inserted through the nose to a depth of 110 cm. In the first study, Toluene was given at a rate of 2.3 mg/min in 4 consecutive periods, each lasting 40 min interrupted by 5 min breaks for micturition. Ethanol (equivalent to two alcoholic drinks, ~24 g) was given in the 5^{th} exposure. In the second study, the subjects were exposed to six conditions, consisting of Toluene (2.2 mg/min) administered in 3 consecutive periods, each lasting 55 min interrupted by 5 min breaks, and four levels of ethanol (0, 0.08, 0.16, and 0.32 g/kg body weight of ethanol), equivalent to 0, 0.5, 1, and 2 drinks, respectively; and exposure without Toluene (control) in combination with ethanol doses of 0 and 0.32 g/kg. The ethanol (96%) was diluted in orange juice and given orally in six fractions from 60 to 85 min after the start of the Toluene or control exposure. During Toluene exposure to 2.3 mg/min for 3 hr, the alveolar Toluene concentration was 0.07 (range 0-0.11) mg/m^3; exposure to 6 mg/min for 30 min increased the alveolar concentration to 0.9 (range 0.03-2.6) mg/m^3. Ingestion of 0.08, 0.16, and 0.32 g of ethanol per kilogram of body weight during Toluene exposure of 2.2 mg/min increased the alveolar concentration within 10 min, and maximal alveolar concentrations of 5±3, 24±11, and 39±28 mg/m^3 were reached after 30, 60, and 90 min for the three doses, respectively. Hippuric acid excretion was only decreased by an ethanol dose of 0.32 g/kg. The authors concluded very low doses of ethanol inhibit

Toluene metabolism.

Hjelm et al. (1994) studied the effects of a low carbohydrate diet and ethanol consumption on Toluene metabolism in eight healthy male volunteers. Subjects were fed a low (30 %) or high (60%) carbohydrate diet for one week. Subjects were then given 0 or 47 grams of ethanol the night before a two hour exposure to 200 mg/m^3 $^2H_8$-Toluene by inhalation while riding a stationary bicycle at a work load of 50 Watts. Each subject experienced each of the four carbohydrate-ethanol combinations, and the variety of sequences of treatments was determined by a Latin square design. The deuterium-labeled Toluene was used to differentiate the measured hippuric acid metabolite from hippuric acid from other sources. Toluene in blood was measured by GC, and hippuric acid in urine was measured by GS/MS. Ethanol consumption combined with a low carbohydrate diet enhanced the rate of metabolism of Toluene. Neither ethanol nor low carbohydrate diet alone affected the metabolism of Toluene.

Toluene metabolism was studied in 34 workers, 15 of whom were fasting during the month of Ramadan, when they had no food and water intake from approximately 6:00 a.m. to approximately 6:00 p.m. The time-weighted average (TWA) concentrations of Toluene in the breathing zone of workers were measured using passive dosimeters. Concentrations of urinary hippuric acid (HA) and o-cresol (OC) were determined by high performance liquid chromatography and gas chromatography, respectively. Good correlations were observed for environmental Toluene concentration, urinary HA, and urinary OC for normal workdays and fasting days. However, under similar exposure conditions, workers with no food and water intake excreted significantly less HA at the end of the shift than on normal workdays. In contrast, the excretion of OC was much higher during the fasting days. Thus, dietary intake may influence the metabolism of Toluene (Ong et al. 1994).

Kawamoto et al. (1994) studied the effects of the genetic polymorphism of ALDH2 (low Km aldehyde dehydrogenase) on Toluene metabolism and determined biological exposure indices of the genotypes of ALDH2. The study subjects included 45 Toluene workers and 122 nonexposed students. The genotype of ALDH2 was classified into the homozygous genotype of a normal ALDH2 gene (NN), the homozygous genotype of an inactive ALDH2 gene (DD) and the heterozygous genotype of normal and inactive ALDH2 genes (ND). The personal exposure levels to Toluene were monitored, using diffusion

type samplers and urinary hippuric acid and creatinine concentrations were determined. The urinary hippuric acid levels of the three genotypes of ALDH2 of nonexposed students did not differ. In the Toluene workers, positive correlations between the personal exposure to Toluene and urinary hippuric acid levels was observed in the NN, ND, and DD groups.

Kawamoto et al. (1995) evaluated the effects of ALDH2, CYP1A1, and CYP2E1 genetic polymorphisms and smoking and drinking habits on Toluene metabolism. The study subjects were 92 male workers who handle Toluene in a printing factory, an electrical parts factory, and a painting workplace in Japan. Their exposure levels to Toluene were monitored using a diffusion-type sampler. Benzyl alcohol concentrations in their blood and hippuric acid and creatinine concentrations in their urine at the end of a workshift were determined. The genotype of ALDH2 was classified into the homozygous genotype of a normal ALDH2 gene (NN), the homozygous genotype of an inactive ALDH2 gene (DD) and the heterozygous genotype of normal and inactive ALDH2 genes (ND). The genetic polymorphism of CY1A1 and CYP2E1 were also determined by restriction fragment length polymorphism (RFLP). A strong correlation between personal exposure and the urinary hippuric acid concentration was observed. The hippuric acid formation from Toluene was significantly ($p<0.001$) different among the genotypes of ALDH2. The slopes of the regression lines decreased from NN to ND to DD in this order. The benzyl alcohol concentration in the blood of the DD group was significantly higher than that found in the NN and ND groups. This result demonstrates that ALDH2 polymorphism affects the oxidation of benzyl alcohol to benzoic acid. The Toluene metabolism was also affected by the CYP1A1 polymorphism. A smoking habit also significantly ($p<0.05$) reduced urinary uncorrected hippuric acid concentration in both the NN and ND groups. In a multiple regression analysis, ALDH2 and the drinking habit were significantly ($p<0.01$) associated with hippuric acid excretion after Toluene exposure with and without correction for creatinine.

Ogata et al. (1999) attached personal air samplers were attached to male workers (mean age 41.1±8.9 years) wearing protective masks to determine the levels of Toluene vapor in the breathing zone. Concentrations of Toluene in exhaled air, blood and urine; and hippuric acid and o-cresol concentrations in the urine of the workers were determined. Specimens were collected at the end of the morning exposure (0-4 hr) and at the end of exposure (4-8 hr). The average values of exposure levels were used.

Toluene in exhaled air was measured by collecting exhaled air in bags and measuring with head-space gas chromatography (HS-GC). Toluene in urine and blood was also determined by HS-GC. Urinary o-cresol was extracted, and subjected to gas chromatography (GC). Urinary hippuric acid was determined by high performance liquid chromatography (HPLC). Toluene exposure of the 8 workers with masks was 18.9 ppm while that of the 8 and 16 workers without masks were 19.9 and 18.4 ppm , respectively. Using regressions, the results showed that masks removed 29-38% of the vapor.

Analytical Methods for Human Samples

De Rosa et al. (1987) collected urine samples from a group of 18 subjects working in a printing plant. High Performance Liquid Chromatography was used to determine the amount of hippuric acid and o-cresol before Toluene exposure, at the end of the work shift, and 5, 9, and 17 hours after the end of the work shift. The values were corrected for g creatinine. Toluene concentrations ranged from 51 to 221 mg/m^3. Urinary hippuric acid and o-cresol values at the end of the work shift were significantly higher than the prework shift values. Both hippuricuria and o-cresoluria end of work shift values were significantly related to the mean daily environmental concentration of Toluene, the correlation being weaker for o-cresol.

Ogata and Taguchi (1987) used high performance liquid chomatography to measure the urinary metabolites of Toluene.

Brown (1988a, 1988b) described two methods to measure Toluene in air using gas chromatography.

Pellizzari et al. (1988) described a method to measure Toluene in human breath samples using GC/MS.

Tolos (1988) described a method for measuring 1 to 600 µg/mL Toluene in blood, using headspace gas chromatography with flame ionization.

Gas chromatography analysis of postshift toluene levels in the blood of 50 assembly line workers indicates that Toluene levels in blood were correlated with the time-weighted average (TWA) exposure

levels of the same day (R=0.90). Toluene concentrations in 10 laboratory technicians not exposed to Toluene at their work gave an average level of 0.002 µg/ml of blood. Significant levels of residual toluene were found in the preshift blood of workers exposed to 57 to 146 ppm of Toluene as compared to the unexposed. The blood Toluene values determined from capillary blood taken from finger tips are higher than the reported values from venous blood (Foo et al. 1988).

Ng et al. (1990) measured the urinary excretion of retinol-binding proteins in 45 paint workers exposed to Toluene and in the same number of unexposed control subjects matched for sex and age. Urinary hippuric acid and o-cresol were also measured in the exposed subjects. A significantly higher level and increased prevalence of elevated retinol-binding protein in the urine of exposed workers was found. Urinary concentrations of retinol-binding protein was correlated ($R=0.399$, $p<0.006$) with that of o-cresol, but not hippuric acid or employment duration.

In Singapore, the relationship between exposure of workers to Toluene in the work environment and biological indicators of Toluene exposure was studied using blood and urinary hippuric acid. A total of 86 female workers exposed to Toluene and a control group which was not exposed were examined. The 8-hr time-weighted average exposure level of Toluene ranged form 1.6 ppm to 263 ppm. This study showed the expected Toluene in blood (1.4 µg/mL) and expired air (16 ppm) after an 8-hr exposure to 100 ppm of Toluene. In this study hippuric acid was not a valuable indicator of exposure (no data given) (Foo et al. 1991).

Truchon et al. (1996) used gas chromatography-flame ionization to measure human urine concentrations of the Toluene metabolite o-cresol. The detection limit was 0.36 µmol/L.

Jensen et al. (1996) used field and laboratory emission cell with photoionization detection and gas chromatography-mass spectrometry to measure the amount of Toluene emitted from printed brochures. Concentrations as high as 2145 ppm Toluene were measured within the first three minutes after printing.

Gartzke and Burck (1997) used a solid phase extraction method to analyze the Toluene metabolite hippuric acid in urine samples.

Amorim and Alvarez-Leite (1997) used gas chromatography to measure the Toluene metabolites

hippuric acid and o-cresol in urine samples. The detection limit for measuring o-cresol was 0.2 µg/mL and for hippuric was 0.1 mg/mL.

Hellman et al. (1997) exposed 11 healthy male volunteers for 15 min to 100 ppm Toluene. The dopamine decarboxylase and number of terminals in putamen were measured before and after exposure by positron emission tomography. Although there was a slight increase in the rate of dopamine synthesis in the putamen after the exposure, this difference was not statistically significant ($p=0.4$). No effect was observed with regard to the uptake of nomifensine, which was used as a tracer to estimate the number of terminals. There was no significant relationship between the dose of Toluene and rate of dopamine synthesis. The findings indicate that short term exposure to 100 ppm of Toluene does not affect the rate of dopamine synthesis or the number of presynaptic terminals.

Park et al. (1998) use GC and GC/MS to measure Toluene concentrations in human blood (0.1 to 74.7 mg/L) and in human urine (0.1 to 40.3 mg/L).

In Turkey, eighteen urine samples were collected form workers occupationally exposed to Toluene at the end of the workshift and ten urine samples were collected from non-exposed persons as the control group. High Performance Liquid Chromatography was used to measure the amount of hippuric acid while a Miran IBX portable ambient air analyzer was used to calculate the time weighted average (TWA) concentration of Toluene. It was found that urinary hippuric acid excretion is not valid to indicate low exposure of Toluene (18.51 ppm or lower) (Duydu et al. 1999).

Kim and Park (2000) used gas chromatography/mass spectrometry (GC/MS) with headspace-solid phase microextraction (HS-SPME) and GC/MS with headspace (HS) to measure concentrations of Toluene in human urine and blood. The correlation coefficients between Toluene concentrations in blood and urine were 0.96 or higher. The detection limits for measuring Toluene in blood and urine were 1.0 ng/mL with the GC/MS-HS-SPME technique and 0.01 µg/mL with the GC/MS-HS method.

El-Haj et al. (2000) developed a gas chromatography-mass spectrometry (GC-MS) method for measuring Toluene in blood. The method involves converting Toluene to benzoic acid which is then measured by GC-MS.

Immunological Responses

Little et al. (1999) measured total immunoglobin G and T-cell antigen-binding molecules against an antigen prepared by conjugation of para-aminobenzoic acid to human serum albumin in 20 patients and 16 controls. There was no significant difference in the immunoglobulin G levels to the antigen in the 2 groups, but the levels of T-cell antigen-binding molecules against the para-aminobenzoic acid conjugated to human serum albumin were elevated significantly in subjects sensitive to Toluene.

Effects on Color Vision

Zavalić et al. (1998a) investigated color vision impairment in three groups of workers, two groups occupationally exposed to Toluene and a nonexposed group. The first group, group A, comprised 41 workers (median value of Toluene in air 35.00 ppm, range 11.3-49.3 ppm) and the second exposed group, group B, comprised 32 subjects (median value of Toluene in air 156.00 ppm, range 66.0-250.0 ppm). The nonexposed group NE comprised 83 subjects. Color vision was evaluated by the Lanthony D-15 desaturated test according to Verriest's classification: type I, loss in the red-green ranges; type II, loss in the blue-yellow and red-green ranges; and type III, loss in the blue-yellow range. Subjects were classified as dyschromates if specific acquired loss was determined in at least one eye. Type III dyschromatopsia was detected in all groups examined: 26.6% of the workers in group NE, 31.7% of those in group A, and 50% of those in group B. For type II dyschromatopsia the frequencies were 1.2% of those in group NE, 4.8% of those in group A, and 15.6% of group B. Type I dyschromatopsia was not found in any of the groups studied. A statistically significant difference in the prevalence of total dyschromatopsia (type III + type II) was established among the three examined groups together ($p<0.01$), between group B and group A ($p<0.05$) and between group B and group NE ($p<0.005$).

Zavalic et al. (1998b) investigated color vision impairment in 45 male workers occupationally exposed to Toluene (mean value of Toluene in ambient air = 119.96 ppm) and in 53 controls. Color vision was evaluated by the Lanthony-D-15 desaturated test and expressed as Age and Alcohol Intake Adjusted

Color Confusion Score (AACDS) or types of dyschromatopsia. A statistically significant higher AACDS value was established in the exposed subjects compared to the controls ($p<0.0001$).

Muttray et al. (1999) examined eight male printshop workers before and after working with pure Toluene. Personal air samples revealed the Toluene concentration in the air ranged between 293 ppm and 357 ppm. Color vision was tested using the Lanthony D-15 test. Acute exposure to Toluene did not cause impairment of color vision; however, statistical power was limited due to the small number of exposed subjects.

Cavalleri et al. (2000) tested color vision in 33 rubber workers and 16 referents. Toluene exposure was estimated by measuring urinary excretion of Toluene. Color vision was tested with the Lanthony D-15 desaturated panel and the outcomes were expressed with the Color Confusion Index (CCI). Toluene-exposed workers (no Toluene concentrations given) had a subclinical reduction in color vision compared to the referents. This was seen by a larger CCI ($p<0.01$).

Schäper et al. (2004) measured color vision in employees from 14 German rotogravure printing plants. The study design was based on two factors for stratification : intensity of Toluene exposure "high" versus "low" and duration of Toluene exposure: "short" versus "long". The mean exposures were 26±21 ppm for the high exposure group and 3±4 ppm for the low exposure group. The mean durations were 23±6 years for the long exposure group and 7±2 years for the short exposure group. Color vision was measured with the Lanthony desaturated color vision test D-15d, and the color confusion index was calculated. Repeated analyses and multiple regressions did not reveal significant effects of Toluene with respect to intensity or duration of exposure.

Effects on Mental Capacity/Performance

Larsen and Leira (1988) addressed the prevalence of organic brain syndrome (OBS) among long-term exposed rotogravure workers who were still working. OBS is characterized by a reduction in higher integrative mental functions, such as impairment of memory or impaired capacity for attention. The presence of OBS was determined from a global clinical evaluation, focusing on the subjects' report of

emerging neurasthenic symptoms and on clinical testing of the individual's cognitive capacity. It was found that among 22 workers exposed to Toluene for a minimum of 12 years and 19 unexposed control subjects, matched for age and employment status, that the distribution of OBS differs significantly between the exposed and unexposed groups ($p<0.01$) with a higher number of OBS cases seen in the exposed group. There was also a significantly greater prevalence of mild chronic encephalopathy and organic affective syndrome in the Toluene-exposed group.

Symptoms of memory loss, unstable mood and some somatic complaints were experienced more frequently by women workers in an electronic factory exposed to Toluene than in unexposed female assembly workers in the same factory. Personal exposure levels of Toluene (measured by Gas Chromatography) ranged from 49 ppm to 140 ppm. Neurobehavioral symptoms were significantly more in the group exposed to higher levels as compared to the control group or the group exposed to lower levels (Foo et al. 1988).

Forty-two college students were exposed to 0, 75, and 150 ppm Toluene for seven hours over three days. Verbal and short term memory (Sternberg, digit span, Benton, pattern memory); perception (pattern recognition); psychomotor skill (simple reaction time, continuous performance, digit symbol, hand-eye coordination, finger tapping, and critical tracking): manual dexterity (one hole); mood (profile of mood scales); fatigue; and verbal ability were evaluated at 0800, 1200,, and 1600 hours. Adverse performance at 150 ppm Toluene was found at 6.0% for digit span, 12.1% for pattern recognition (latency), 5.0% for pattern memory (number correct); 6.5% for one hole, and 3.0% for critical tracking. The number of headaches and eye irritation also increased in a dose-response manner. Overall, no clear pattern of neurobehavioral effects was found consistent with the type 1 central nervous system as classified by the World Health Organization (Echverria et al. 1989).

Thirty female workers exposed to Toluene and matched controls with low occupational exposure to Toluene were administered neurobehavioral tests. The environmental air levels of Toluene was 88 ppm for the exposed workers and 13 ppm for the controls. The Toluene in blood concentrations for the exposed workers was 1.25 mg/l and for the controls 0.15 mg/l. Statistically significant differences between workers exposed to Toluene and controls in neurobehavioral tests measuring manual dexterity (grooved

peg board), visual scanning (trail making, visual reproduction, Benton visual retention, and digit symbol), and verbal memory (digit span) were observed. Further, the performance at each of these tests was related to time weighted average exposure concentrations of air Toluene (Foo et al. 1990).

Visual evoked potentials (VEPs) from stimulation by checkerboard pattern reversal were examined in 54 printers occupationally exposed to Toluene (all men, duration of exposure 22-54 years). A control group consisted of 46 subjects (23 men and 23 women). Compared to the controls, the exposed group showed more frequent responses with reduced reproducibility or absence of some waves, or both; the mean P1 wave latency was prolonged and mean amplitudes N1P1 and P1N2 were reduced. The VEPs were abnormal in 24% of the workers. The frequency of abnormal VEPs correlated positively with the duration of exposure to Toluene. No association was found between measurements of VEP and electroencephalogram (EEG) or electromyogram (EMG) examinations (Urban and Lukáš 1990).

An investigation of visual evoked potentials, as measured by EEGs, was carried out in two groups of subjects; 49 workers occupationally exposed to Toluene for 30 years and 59 non-exposed workers. The mean concentrations of Toluene in the blood was measured by gas chromatography and were 0.036 mg/l and 0.00096 mg/l for the exposed and non-exposed groups respectively. N75, P100 and N145 waves of the pattern reversal visual evoked potentials were analyzed. In the exposed group, significantly greater amplitudes were found in all waves, with significantly longer latency of the P100 wave (Vrca et al. 1995).

Six healthy adults were exposed to 100 ppm Toluene or air (control) for 6 hours with exposures separated by at least 14 days. Each subject served as his or her own control. Blood and exhaled Toluene levels were measured before, during, immediately , and 1 and 2 hr after exposure. Lung function was measured before and immediately after exposure. Three repetitions of two computerized neuropsychological tests were performed. Lung function was unchanged post-exposure. On the standard neuropsychological tests, latency, but not accuracy, proved the sensitive measure for five of the seven subtests presented (Armstrong 1996).

Brainstem auditory evoked potentials were examined in 49 workers employed in a printing press, who were occupationally exposed to Toluene for an average of 20.3 years, and in 59 subjects in a control group. In the group of exposed workers, a significant decrease was found in all wave amplitudes

examined, a significant prolongation of P1 wave latency, and an increased interval of interpeak latencies (P3-P5), indicating that the extramedullary and high medullary part of the auditory pathway are biologically most frequently affected by chronic exposure to low concentrations of Toluene (Vrca et al. 1996).

Hellman et al. (1997) exposed 11 healthy male volunteers for 15 min to 100 ppm Toluene. The dopamine decarboxylase and number of terminals in putamen were measured before and after exposure by positron emission tomography. Although there was a slight increase in the rate of dopamine synthesis in the putamen after the exposure, this difference was not statistically significant ($p=0.4$). No effect was observed with regard to the uptake of nomifensine, which was used as a tracer to estimate the number of terminals. There was no significant relationship between the dose of Toluene and rate of dopamine synthesis. The findings indicate that short term exposure to 100 ppm of Toluene does not affect the rate of dopamine synthesis or the number of presynaptic terminals.

Little et al. (1999) assessed 20 patients (11 female and 9 male, all Toluene-sensitive) neuropyschologically before and after Toluene exposure and they had impaired cognitive functioning characterized by a deterioration in short- and long-term memory and psychomotor coordination.

Eller et al. (1999) evaluated the effect of chronic exposure to Toluene on the central nervous system. Ninety-eight male workers were divided into three groups: Group 0 with no exposure to Toluene ($n=19$); Group 1 with exposure to <20 ppm Toluene for less than 13 years ($n=30$); and Group 2 with exposure for more than 12 years ($n=49$). Within Group 2, 75% had been exposed to levels exceeding 100 ppm for 10+ years. Group 2 differed significantly from the other two groups in scoring higher on a symptom index ($p=0.04$) particularly regarding concentration ability, reduced memory and fatigue. Group 2 also scored poorer on tests for visuospatial function, number learning and word recognition, while no differences regarding neurological functions were observed.

Chouanière et al. (2002) carried out a cross-sectional study in two printing plants on 129 blue collar workers exposed to low levels of Toluene. With 231 samples of ambient air, Toluene concentrations were estimated from 0-18 ppm in Plant A and from 2-27 in Plant B. Outside any period of acute exposure, the workers answered a self-administered questionnaire on neurotoxic symptoms and performed six psychometric tests. After adjustment for confounders, there was a significant relationship only between

present exposure and Digit Span Forwards performance (decrement is 1 digit for 40 ppm, p=0.04) and Digit Span Backwards (DSB) performance (decrement is 1 digit for 25 ppm, p=0.01). Neurotoxic symptoms were not significantly correlated with current exposure. No association was found between estimated cumulative exposure and either psychometric performances or neurotoxic symptoms.

Other Adverse Occupational Effects

A 60-year old man was admitted to a hospital with astenia and weight loss of 6 months duration. Since 1945 he had been working 50 hr a week manufacturing bags which involved using a glue (90% Toluene). Computer brain tomography demonstrated a moderate cortical atrophy. Electroencephalography showed light diffuse disorganization and electromyography revealed abnormalities of the distal motor innervation, and a delay in sensitivity transmission at bilateral carpal tunnel. The patient was discharged and suspension of work was recommended. One year later, he was in good health (Bosch et al. 1989).

Twenty Toluene-exposed workers had lower median plasma levels of follicle stimulating hormone (FSH) (p=0.02) and luteinizing hormone (LH) (p=0.05) and also lower serum levels of free testosterone (p=0.05), respectively, than 44 unexposed referents. The individual time-weighted Toluene levels in the air were 36 (median; range 8-111) ppm. The exposed workers' median Toluene levels in blood were 1.7 (1.0-6.6). There was a negative association between blood Toluene and plasma levels of prolactin (Svensson et al. 1992).

Wiebelt and Becker (1999) performed a historical cohort study that included 6830 German men who were occupationally exposed to Toluene from 1960 to 1992 in three work areas with different exposure levels. Overall, 466 deaths were observed which provided a standardized mortality ratio (SMR) of 91.3 for overall mortality. Mortality from cancer did not differ substantially from the expected level, but in one of the work areas, mortality from cancers of the bone and connective tissue was significantly elevated. In the entire cohort, mortality from lung cancer was increased by about 35% above total mortality and by about 95% in one work area with low Toluene exposure (not statistically confirmed).

Toluene Abuse

A 27 year old man complained of inability to walk for one day and pain in the arms and legs. He admitted to being a spray paint abuser for 10 or more years. He typically sprayed paint into a rag and placed it to his mouth and nose. Complete neurologic examination revealed extremely sluggish mentation (able to answer only the simplest of questions after a long, staring pause); 3/5 motor strength in all extremity groups except 0 to 1/5 bilateral wrist dorsiflexors. He was able to walk only with assistance and demonstrated a titubating gait (Lavoie et al. 1987).

Rosenberg et al. (1988) characterized the findings of magnetic resonance imaging of the brain in 6 chronic Toluene vapor abusers as follows: (1) diffuse cerebral, cerebellar, and brainstem atrophy; (2) loss of differentiation between the gray and white matter throughout the central nervous system; and (3) increased periventricular white matter signal intensity on T2-weighted images. Another chronic Toluene abuser (MRI not performed) died of acute Toluene overdose. The brain displayed diffuse, ill-defined myelin pallor, maximal in cerebellar, periventricular, and deep cerebral white matter.

Three patients with toluene dependency who reported visual disturbance had their electrophysiological findings recorded. In five of six eyes, the peak latency of pattern visual evoked cortical potential was prolonged as compared with that of normal subjects. The electroretinogram was investigated in one patient and showed little increase in amplitude during light stimulation, so that a lowered light peak to dark trough ratio was obtained. The findings suggested that Toluene vapors may impair any part of the visual pathway, including the distal part of the retina and retinal pigment epithelium (Toyonaga et al. 1989).

Filley et al. (1990) studied 14 chronic Toluene abusers with a comprehensive neuropsychological evaluation and cerebral magnetic resonance imaging (MRI). There were 10 men and 4 women and the mean age was 29 years. Using a blinded global assessment of neuropsychological functioning, 3 patients were found to be normal, 3 borderline and 8 impaired. Independent analyses of white matter changes on MRI revealed that the degree of white matter abnormality was strongly correlated ($p<0.01$) with neuropsychological impairment. Dementia in Toluene abuse appears to be related to severity of cerebral

white matter involvement.

Two men (aged 43 and 34), using Toluene to remove excess glue from a tile-laying activity, were overcome by the fumes and admitted to a hospital. The symptoms included stupefaction, paresis, and amnesia. The concentration of Toluene in the air was >7000 mg/m^3 (Meulenbelt et al. 1990).

In Singapore, over a period from 1983 to 1991, of a total of 19000 post-mortems, 33 were found to have at least one aromatic hydrocarbon in the blood. Of the 33 deceased, 22 had a history of toluene or petrol abuse while most of the remaining 11 were suspected to be glue sniffers. The male gender predominates (81.8%) among the 33 deceased. The blood toluene levels were found to be in the range 0.2 to 92 µg per ml blood. The causes of death are 63.6 % due to falling or suicide by jumping; 18.2% drowning; 6.1% hanging; 6.1% homicide; and 6.1% acute Toluene poisoning (Chao et al. 1993).

Three men who had a history of sniffing spray paint had MR imaging studies between November 1988 and January 1992. The CT scans showed generalized cerebral atrophy and ventricular dilation in all cases. In the two patients who underwent EEG evaluation, one was normal and the other showed diffuse slow wave activity. The abnormal MR findings were as follows: (a) generalized cerebral and/or cerebellar atrophy on both T1- and T2-weighted images (n=3), (b) atrophy of the corpus callosum (n=3); (c) loss of gray-white matter discrimination on T2-weighted images (n=3); (d) multifocal high signal intensity (above that of the background) in the cerebral white matter (n=3); and (e) symmetric hypointensity of the thalami on T2-weighted acquisitions (Xiong et al. 1993).

Eight patients with histories of Toluene abuse underwent magnetic resonance imaging (MRI). A brain autopsy was performed on one patient who died of an overdose of Toluene. MR images of the brains of patients who abused Toluene showed varying degrees of cortical cerebral, cerebellar, and brain stem atrophy, and T2-weighted MR images show periventricular hyperintensity and poor differentiation of gray and white matter. The severity of these changes appeared to depend on the duration and extent of Toluene abuse (Unger et al. 1994).

Kamijima et al. (1994) reported a case of a 22-year-old woman who exhibited weakness and unsteadiness in her lower extremities. She had a history of solvent abuse beginning at age 16, when she began by sniffing glue. Her method for inhaling Toluene was to drip paint thinner (99% Toluene) into a

plastic film bag, inflate the bag, and inhale the air in it repeatedly. She had inhaled approximately 6 L of Toluene over the month prior to admission using this method. Laboratory tests revealed metabolic acidosis (specifically distal renal tubular acidosis), proteinuria, myoglobinuria, and glucosuria. Serum aldosterone concentration was above normal. Biopsy of renal tissue showed patchy areas of tubular injury.

A 55 year old man disclosed inhaling Toluene by saturation paper towels with Toluene for "sniffing" when he was admitted to the hospital for non-Q-wave myocardial infarction (Hussain et al. 1996).

A 17 year old man with a 2 week history of auditory and visual hallucinations and decreased memory function was admitted to the hospital. Medical history was notable for chronic and frequent glue sniffing for 8 months. He showed impairment of short-term memory and mild abnormalities on tactual performance test, finger tapping test, and fingertip number writing test. An electroencephalographic mapping revealed mild cortical dysfunction of the right frontal lobe (Ryu et al. 1998).

Park et al. (1998) reported that in 36 fatal cases of Toluene intoxication, the blood Toluene concentrations ranged from 0.3 to 60.2 mg/L, with the mean at 6.9 mg/L.

A 36 year old woman saw an ophthalmologist and reported a 6 month history of oscillopsia and a feeling that her eyes were quivering. The patient had a 21 year history of sniffing glue, then paint, and eventually pure Toluene. Three years earlier, she had begun to experience seizures characterized by intermittent episodes of a dream-like state associated with unusual activity. She had no recall of anything that occurred during these periods. On examination in February 1992, the patients visual acuity was 20/30 OD and 20/25 OS. A magnetic resonance imaging scan revealed generalized cerebral and cerebellar atrophy and diffuse atrophy of the corpus callossum. In February 1994, her best corrected visual acuity was 20/100. Another magnetic resonance imaging scan showed the previous conditions as well as hyperintense lesions present on both sides of the pons (Hunnewell and Miller 1998).

Fifteen patients suffering from bilateral neuropathy caused by Toluene addiction had an electrophysiological evaluation of their visual functions. Visual acuities at the initial visit were less than 0.1 in 5 cases and 0.1-1.0 in 10 cases. Pattern visual evoked cortical potentials (PVECPs) were followed up in the 15 cases. At the first recording, PVECPs were nonrecordable in both eyes of 11 cases, the P100 peak latency was prolonged in both eyes of 3 cases, and only 1 case showed a normal P100 peak latency.

After treatment, visual acuities improved more than 2 lines in 6 cases, 3 of whom showed normal P100 peak latency in the PVECPs (Kiyokawa et al. 1999).

Aydin et al. (2002) studied the neurologic signs, symptoms, and cranial magnetic resonance findings in 41 patients who chronically abused thinner, a Toluene-containing solvent. White matter changes were classified as diffuse or restricted. Then tests were performed on the associations of the development of white matter lesions and thalamic hypointensity with patient age at onset of abuse and duration of abuse. Magnetic Resonance Images revealed white matter lesions in 46% of the patients, atrophic dilatation of ventricles and sulci in 27% and thalamic hypointensity in 20%. White matter changes were restricted in 53% and diffuse in 47%. The development of white matter changes and thalamic hypointensity were significantly associated with duration of abuse longer than 4 years ($p<0.05$ and $p<0.01$, respectively).

Dermal Toxicity

A 22-year old man, having spilled a "sealer" containing 65% Toluene on his clothing developed extensive chemical burns on approximately 71% of his total body surface area followed by acute renal failure and disseminated intravascular coagulation that led to death. Although the skin damage initially appeared mild, it was followed by blistering, extensive necrosis, and massive loss of fluid. Histological examination of the skin showed findings similar to those observed in second degree thermal burns (Shibata et al. 1994).

Genotoxicity

Hammer et al. (1998) monitored the individual Toluene burden of 42 exposed printing workers. The urinary excretion of hippuric acid was measured directly after the work shift. The results were compared with those recorded for a control group consisting of 45 blood donors. Sister chromatid

exchange (SCE) frequencies were determined from peripheral lymphocytes for both groups. The median hippuric acid excretion of the exposed and nonexposed groups amounted to 1.94 and 0.45 g/g creatinine, respectively. For both groups, different SCE rates were detected: 10.13 and 6.84 counts/lymphocyte for exposed and nonexposed persons, respectively. The results indicated a strong relationship between the individual Toluene burden and the genotoxic risk of the exposed persons.

Sister chromatid exchanges (SCEs) were studied in a group of 42 printing plant workers and a control group of 45 blood donors. At the working places, the ambient air-Toluene concentration ranged from 141 to 328 mg/m^3. SCEs were significantly elevated by three units in the exposed group, which also had considerably increased hippuric acid and o-cresol fractions (Hammer 2002).

Reproductive and Developmental Toxicity

Five pregnant women were evaluated with severe renal tubular acidosis from paint sniffing. Normal acid-base balance returned within 72 hours with cessation of Toluene abuse. Fetal heart tracings and dynamic ultrasound parameters were normal in four of five cases. Three of five infants were growth-retarded at birth; two showed anomalies and neonatal hyperchloremic acidosis (Goodwin 1988).

Hersh (1989) reported two cases of children whose mothers had inhaled Toluene regularly throughout their pregnancy. The first patient was evaluated for developmental delay. At birth, her weight was 2360 g (50th centile), length 46 cm (50th centile), and occipitofrontal circumference 30.5 cm (25th centile). There were no neonatal complications. Receptive language abilities were comparable to her mental age (same as chronological age) and she had a mild expressive language impairment and speech articulation disorder. The second patient was evaluated because of poor weight. At birth, her weight was 2550 g (slightly <10th centile) and length 48 cm (slightly >25th centile). Other than expressive language delay, early developmental milestones appeared normal. She had a short attention span.

Two mothers who sniffed paint thinner (66.5% Toluene) regularly throughout their entire pregnancies delivered infants which were dysmature (weights 2850 and 2820 g) and had dysmorphic features. Case 1 had ha short nose, thin upper lip and short broad fingers and toes, while Case 2 had

epicanthal folds and micrognathia. At birth, both infants had a hyperchloraemic acidosis and also exhibited aminoaciduria, suggesting renal tubular dysfunction (Lindemann 1991).

Wilkins-Haug and Gabow (1991) described the complications and outcomes of 30 human pregnancies in ten women who abused Toluene. Toluene-induced renal tubular acidosis occurred in over half of the women and was associated with hypokalemia, cardiac dysrhythmias and rhabdoyolysis. Outcomes of Toluene-exposed pregnancies included 16 premature deliveries, 14 perinatal deaths, and 8 incidents of growth retardation and 13 incidents of microcephaly at 1 year of age.

Pearson et al. (1994) described the complications and outcomes of 18 infants who had been exposed to Toluene in utero due mothers who abused solvents. The authors combined their data with other literature reports of Toluene abuse during pregnancy. Thirty-nine percent of Toluene-exposed infants considered were born prematurely, and 9 % died during the perinatal period. Fifty-four percent were small for their birth age, and 52 % continued to have below average growth. Postnatal microencephaly occurred in 33 % of the Toluene cases. Eighty-three percent had developmental delays, and 80 % had craniofacial features similar to those seen in fetal alcohol syndrome. The authors noted several similarities between the perinatal effects of Toluene abuse and fetal alcohol syndrome.

Arnold et al. (1994) described complications and outcomes of 35 human pregnancies that involved prenatal exposure to Toluene. There were three perinatal deaths. Of the survivors 42 % had premature births, 52 % had low birth weights, and 32 % had microcephaly. Birth weight, body length, and head circumference were reduced, compared to carefully matched control (Toluene-free) newborns. As the Toluene-exposed children developed, 46 % had growth retardation for weight and 38 % for height, 38 % had developmental delays, and 46 % had microcephaly. Six of the children were selected for further study, and all had the following features: microcephaly, narrow bifrontal diameter, short palpebral fissures, hypoplastic midface, wide nasal bridge, abnormal palmar creases, and blunt fingertips.

Carcinogenicity

A group of 1020 rotogravure printers exposed to 7-33 ppm Toluene was studied. Compared to

regional rates, total mortality did not increase during the observation period (1952-1986). There were 129 observed deaths vs. 125 expected. There was no overall excess of tumors (1958-1985). Among specific cancers, only those of the respiratory tract were significantly increased. Statistical significance was not attained, however, when only subjects with an exposure period of at least 5 years and a latency period of at least 10 years were considered. Further, there were no dose response relations with accumulated Toluene dose (ppm years). There were no significant increases of tumors at other sites, including leukemias, lymphomas, and myelomas (Svensson et al. 1990).

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STEARYL ALCOHOL, OLEYL ALCOHOL, AND OCTYLDODECANOL

A safety assessment of Stearyl Alcohol, Oleyl Alcohol, and Octyldodecanol was published in 1985 with the conclusion "safe as currently used in cosmetic products" (Elder 1985). New studies, along with the updated information in Table 25 regarding uses and used concentrations, were considered by the CIR Expert Panel. The Panel determined not to reopen this safety assessment.

Stearyl Alcohol was used in 425 cosmetic products in 1981, based on voluntary reports provided to FDA by industry with concentrations ranging from ≤0.1% to 50% (Elder 1985). In 2002, Stearyl Alcohol was reportedly used in 1063 cosmetic products (FDA 2002). Concentration of use data from an industry survey in 2003 indicated that Stearyl Alcohol was used in a range from 0.002% to 56% (CTFA 2003).

The Panel noted that the Hannuksela (1988) report reviewed the previous literature which included a report of positive patch test reactions to Stearyl Alcohol as high as 44%. Although this information raised some concern, Hannuksela (1988) did report current data with a frequency of 11 positive tests out of over 1000 patch tests performed; a low frequency consistent with current experience.

Oleyl Alcohol was used in 1018 cosmetic products in 1981, with concentrations ranging from ≤0.1% to >50% (Elder 1985). In 2002, Oleyl Alcohol was used in 343 cosmetic products (FDA 2002). Concentration of use data from a 2003 survey indicated that Oleyl Alcohol was used in a range from 0.0002% to 18% (CTFA 2003).

Although Tosti et al. (1996) reported a high proportion of 34 patients as positive to Oleyl Alcohol in a patch test, the Panel indicated that such reactions are not seen in their experience.

Octyldodecanol was used in 371 cosmetic products in 1981, with concentrations ranging from ≤0.1% to >50% (Elder 1985). In 2002, Octyldodecanol was used in 814 cosmetic products (FDA 2002). Concentration use data from 2003 indicted that Octyldodecanol was used in a range from 0.006% to 85% (CTFA 2003).

Table 25 presents the available use information for Stearyl Alcohol, Oleyl Alcohol, and Octyldodecanol. The most current information now represents the present practices of use.

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TOLUENE

A safety assessment of Toluene was published in 1987 with the conclusion that Toluene "is safe for cosmetic use at the present practices of use and concentration" despite limited skin exposure data (Elder 1987). Since then a large number of studies

TABLE 25

Historical and current cosmetic product uses and concentrations for Stearyl Alcohol, Oleyl Alcohol, and Octyldodecanol *(Continued)*

Product category	1981 uses (Elder 1985)	2002 uses (FDA 2002)	1981 concentrations (Elder 1985) %	2003 concentrations (CTFA 2003) %
Shaving products				
Aftershave lotions	—	2	—	0.03–0.07
Preshave lotions	1	3	>0.1–1	—
Shaving cream	1	1	>0.1–1	0.4
Other	—	3	—	—
Skin care				
Cleansing creams, lotions, etc.	9	22	≤0.1, >1–10	0.03–17
Face and neck skin care	23*	19	>0.1–50*	0.03–85
Body and hand skin care		59		0.006–6
Moisturizers	14	35	≤0.1–25	2–3
Night skin care	3	15	>1–5, >10–25	1
Paste masks/mud packs	—	7	—	
Other skin care	7	24	>1–25	0.03–14
Wrinkle smoothers**	1	NA**	>1–5	NA**
Skin lighteners**	4	NA**	>0.1–5	NA**
Suntan				
Suntan gels, creams, liquids, and sprays	3	9	>5–25	3–59
Other suntan	1	4	>1–5	
Total uses/ranges for Octyldodecanol	**371**	**814**	**≤ 0.1–>50**	**0.006–85**

*This category was combined when the original safety assessment was performed and is now two separate categories.

**No longer included as a cosmetic product category.

have appeared in the scientific literature. These studies, along with updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. Based on its consideration of the available data, the Panel decided to not reopen this safety assessment.

Toluene was used in 555 cosmetic products in 1981, based on voluntary reports provided to FDA by industry with concentrations ranging from >10%–50% (Elder 1987). In 2002, toluene was reportedly used in 59 cosmetic products (FDA 2002). Concentration of use data from an industry survey in 2003 indicated that Toluene was used in a range from 20% to 26% (CTFA 2004).

Table 26 provides the available data on usage and use concentration as a function of cosmetic product category. The most current information now represents the present practices of use.

Many of the newly available studies reported findings consistent with the data in the original safety assessment.

New findings of adverse effects included the following effects: Toluene was ototoxic for guinea pigs; interferes with performance and learning in neurotoxicity and behavior studies in animals; increased numbers of litters with low birth weights pups and adversely affected brain development; in cultured embryos exposed to Toluene, yolk sac diameter, crown-rump length, somite number, and protein concentration were significantly

TABLE 26

Historical and current cosmetic product uses and concentrations for Toluene

Product category	1984 uses (Elder 1987)	2002 uses (FDA 2002)	1984 concentrations (Elder 1987) %	2003 concentrations (CTFA 2004) %
Nail care				
Basecoats and undercoats	32	21	>10–50	—
Polishes and enamels	501	23	>10-50	20–25
Polish and enamel removers	—	2	—	—
Other nail care	22	13	>10–50	26
Total uses/ranges for Toluene	**555**	**59**	**>10–50**	**20–26**

reduced. A National Toxicology Program study concluded that there was no evidence of carcinogenic activity for Toluene in F344/N rats and $B6C3F_1$ mice.

The new adverse effects noted above appeared only at high exposures. They were found only when animals were exposed to Toluene vapor at a level of 10^2 to 10^3 ppm. Such exposures, however, were not attainable in an exposure study of human subjects using nail polish—those values ranged from 1–4 ppm.

The Panel recognized that other data indicate adverse effects in the brain of Toluene abusers and in children born to mothers who inhaled Toluene during pregnancy. Again, the nature of these studies suggests high exposures and are not relevant to the use of Toluene in cosmetic products.

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~~TOLUENESULFONAMIDE/FORMALDEHYDE RESIN~~

~~A safety assessment of Toluenesulfonamide/Formaldehyde Resin (including Toluenesulfonamide/Formaldehyde Resin-80) was published in 1986 with the conclusion that these ingredients were safe as cosmetic ingredients in the present practices of use and concentration (Elder 1986). Studies available since that time, along with updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. Based on its consideration of the available data, the Panel decided to not reopen this safety assessment.~~

~~The terminology for this ingredient in the *International Cosmetic Ingredient Dictionary and Handbook* has changed—Tosylamide/Formaldehyde Resin is the current terminology (Gottschalck and McEwen 2004).~~