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# Amended Safety Assessment of Hydroquinone as Used in Cosmetics

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The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

**MEMORANDUM**

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.  
Scientific Analyst and Writer

Date: February 21, 2014

Subject: Hydroquinone As Used In in Cosmetics

In December 2013, the Panel tabled the Draft Amended Report of hydroquinone and *p*-hydroxyanisole as used in nail products for the purpose of collecting more data on the use of UV nail lamps. The papers that were requested have been sent to you by email and are summarized in the appropriate section of the report. A paper that Dr. Liebler asked to review (Hansch et al. 2000) is also included in the email. The previous safety assessments on these ingredients are also included in case more information than what is provided in the summaries is needed.

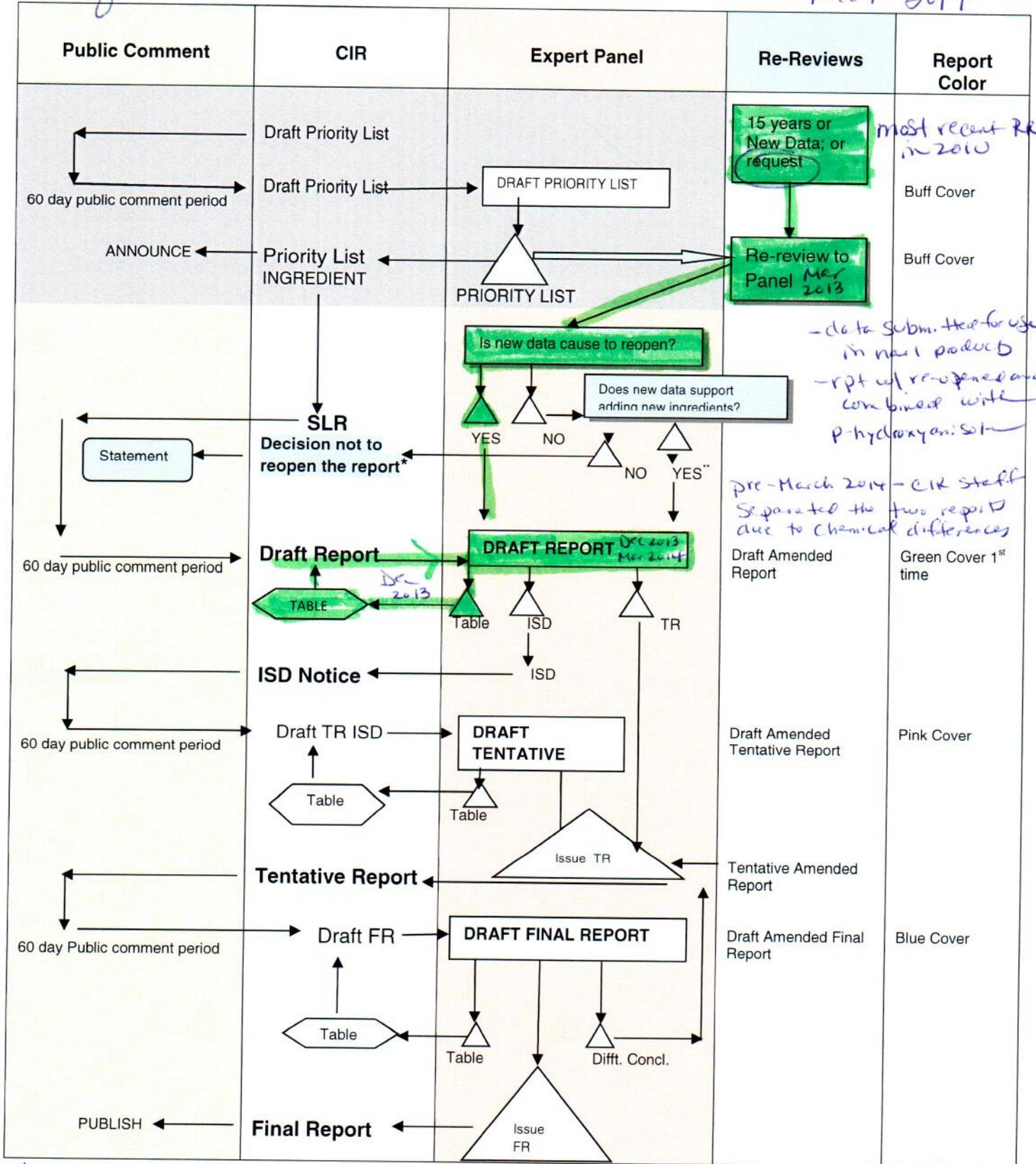
The initial reviews of hydroquinone and *p*-hydroxyanisole were done as individual ingredients. While these two ingredients are used for the same purpose in the same types of nail products, after further examination the CIR staff decided that they chemically do not belong together in a single report. These ingredients appear to be similar, but the small difference in the chain length makes for the possibility of very different reactions. Data on the individual ingredients have been pulled apart. Common data (i.e., information on nail gel application and UV lamp data) is included in both reports.

The Panel is to review the presented data and decide if there is sufficient information to come to a conclusion on the use of hydroquinone in nail products that require UV curing. If so, then the Panel is to provide the new conclusion with any qualifications for this use (as well as confirming or changing the previous conclusion). If not, then an insufficient data conclusion for these types of products is to be issued. The Panel is to develop the basis for the Discussion and Abstract. A Tentative Amended Report is to be issued.

Distributed for Comment Only -- Do Not Cite or Quote  
**SAFETY ASSESSMENT FLOW CHART**

*Hydroquinone*

*Mar 2014*

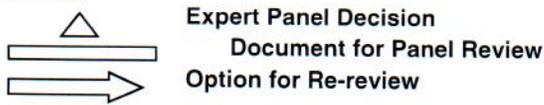


*- data subm. here for use in next product  
 - rpt w/ re-opened and combined with p-hydroxyan. soln*

*pre-March 2014 - CIR staff separated the two reports due to chemical differences*

\*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

\*\*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.



## History of Hydroquinone and p-Hydroxyanisole

- 1985** – Safety assessment of p-Hydroxyanisole published with an unsafe for use as a cosmetic ingredient.” This conclusion was based primarily on depigmentation of black guinea pig skin in studies in which concentrations as low as 0.25% or less (0.1% in some animals) were applied to the skin daily for 1 or more months (which were close to use concentrations up to 0.1% to 1.0% at the time of the FDA survey in 1981).
- 1986** - A safety assessment of hydroquinone and pyrocatechol was published with the conclusion that these two ingredients were safe for use in cosmetics at concentrations up to 1.0% in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair.
- 1994** - An amended safety assessment of hydroquinone alone was published with the conclusion that hydroquinone was safe at concentrations of 1.0% or less for aqueous cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone was not safe for use in leave-on, non-drug cosmetic products.
- 2010** – Safety assessment of hydroquinone published with a safe at concentrations  $\leq 1\%$  in hair dyes” and “safe for use in nail adhesives in the practices of use and concentration described in this safety assessment,” although it “should not be used in other leave-on cosmetics.” The Panel noted that, while absorption through the skin could be appreciable in leave-on products, hydroquinone in nail adhesives “is oxidized during use and is no longer present in the preparation and minimal dermal exposure and absorption is expected to occur from this application.” The use concentration was reported to be 0.5% in nail adhesives at the time of the survey (2008). The Panel’s discussion does not explicitly include a warning to avoid skin contact or specify that nail adhesives containing this ingredient should be for professional use only. The cosmetic use section of the CIR safety assessment noted that the EU banned the use of this ingredient in hair dyes in 2008 and approved its use in professional-use-only artificial nail systems up to a maximum of 0.02% after mixing with methacrylate monomers (hydroquinone at 0.02% in methacrylate monomer preparations was undetectable in the finished product).
- March, 2013** - Data were submitted to the Panel with the request to reopen these two safety assessments with the purpose of changing the conclusion with regard to hydroquinone and p-hydroxyanisole’s use in nail products using UV for polymerization and drying.
- December, 2013** – The Panel tabled the report without a conclusion to have further information collected on UV nail lamps.
- March, 2014** – The CIR staff split the report for these two ingredients due to their chemical differences. The Panel is to examine each report separately and come to independent conclusions.

New data on UV lamps and photo effects have been added.

The Panel is to examine the newly presented data and come to a conclusion on each ingredient.

### **Search Strategy – Hydroquinone & *p*-Hydroxyanisole**

**SciFinder** – Searched by CAS No. Refined by date, publication type, and toxicity terms. 10 papers ordered.

**ECHA** – Data for hydroquinone.

**Web Search** – by CAS Nos. and ingredient names. Located FDA drug application documents; SCCNFP opinion; and NAILS Magazine.

### **UV Lamps**

**SciFinder** – “UV nail lamp” – 4 hits.

**Web Search** – found [www.hooked-on-nails.com](http://www.hooked-on-nails.com) and incorporated useful information.

## Hydroquinone and *p*-Hydroxyanisole December 2013 Transcripts

### Dr. Marks' Team

DR. MARKS: So next is hydroquinone and para-hydroxyanisole. Is David here? Good, because this is interesting, hydroquinone. Let me bring that up.

So this is a draft report that Lillian put together as used in nail products. It's interesting. So let me go back here. In March there was a request to amend the 2010 conclusion to include the use of nail polishes that require UV curing with these ingredients. As you remember we reopened it to evaluate the safety of these ingredients in UV nail adhesives.

Hydroquinone was found to be safe in 2010 was the most recent report. Para-hydroxyanisole was found to be unsafe in 1985 because of its de-pigmenting toxicity of the skin. Interestingly, the most recent wave, Wave 2, there are no reported uses for these ingredients, so the question is do we reopen it.

I actually had reviewed it and had the irritation sensitization UV ultraviolet light was okay was nail use. That was on page 22, 25. I felt we could amend it with a "safe." The hydroquinone conclusion would be the same, but the para-hydroxyanisole in the conclusion would be "safe only in nail products." And then I had some questions a little bit, Lillian, about the format.

But at any rate, if it's being used, then do we need to reopen? And Ron Shank felt that Wave 2 states no uses, so don't reopen. So, David, do you have data that the PCPC doesn't have --

DR. ANSELL: It's being used extensively --

SPEAKER: Didn't we agree to reopen it at the last meeting?

DR. BERGFELD: Yeah, I think so.

DR. MARKS: Well, we agreed to reopen it, but if you remember, the reopening is, and we can always shut it again so we don't have to reopen it if we find as we go through the reopening and analyzing, we could go back and say, and remember it should be a non-brainer if we reopen on.

DR. ANSELL: Well, I think the question on the table then would be do we close it since we have reopened it.

DR. MARKS: Yes, okay. Okay, thank you, Jay. You're being very, how do I want to say, precise in the terminology. So thank you.

So the question is do we close it. So, Lillian, you were going to say something.

MS. BECKER: I will point out that there are uses in the VCRP.

DR. MARKS: Pardon?

MS. BECKER: There are uses in the VCRP, involuntary reporting to the FDA.

DR. MARKS: Oh, there are uses?

MS. BECKER: Yes. And on page 19 -- nobody reported any to the Council, but they did report to the FDA that there are uses.

DR. MARKS: Okay, so there are uses.

MS. BECKER: Seven nail extenders and 11 skin preparations. No uses were reported for the hydroxyanisole.

DR. MARKS: None for the para-hydroxyanisole.

MS. BECKER: Right.

DR. MARKS: So I'm glad I read the report before I saw Wave 2 because I would've said why are we spending more time.

So with that in mind that there are uses, so there are uses for the hydroquinone, the para-hydroxyanisole. At least in the database we don't have uses, but, David, you feel they are being used in nail adhesives?

DR. STEINBERG: Not nail adhesives. Well, they could be used in nail adhesives. The main thing is the nail polish.

DR. MARKS: Okay.

DR. STEINBERG: MEHQ, which is how (inaudible) is the preferred polymer inhibitor industrially, and you could go back to your '85 conclusions. It's cited that its big use in industry where they're taking acrylic acid or anything thereof, and inhibiting polymerization until you get polymerization.

We have in, with maybe the only exception being the adhesive use, up until a few years ago, we never sold monomers to consumers. So it was never considered an ingredient, and what's happened is technology has changed, and the most important thing that has happened is the use of the gel nail polishes which are cured by light.

And these are safe and are being used by consumers. So the issue is now we have an inhibitor that's in the raw material. We do not put it in. This how the raw material is purchased from your large chemical companies, and it has to be there.

And the polymerization process destroyed the inhibitor. That's how polymerization takes place. And so the question came back that we had an unsafe report from 1985 and then now we're using it safely in nail polishes right now, the gel nail polishes.

DR. MARKS: Right. Okay.

DR. STEINBERG: So that's why we requested it to be reopened for that specific use.

DR. MARKS: Tom, did you hear all that?

DR. SLAGA: Yeah, and I agree with David. This is completely different, and I'd just leave it in as "safe as used."

DR. MARKS: So the way I had it we would issue -- it's open, as Jay pointed out. Now hearing this, I have a feeling Ron Shank wouldn't say do not reopen, or he would say do not close. And I would propose -- let's see, who's presenting tomorrow? Oh, I am. That we issue an amended report, so this would be what, Lillian, a tentative amended report with hydroquinone, the same conclusion because that was found to be safe. And the amended would be para-hydroxyanisole as "safe only in nail products," as used.

Tom, do you have any problems with that?

DR. SLAGA: I do not.

DR. MARKS: Okay.

DR. BERGFELD: I don't either. That's what I put.

DR. MARKS: Good. Thanks, Wilma. And we'll get Ron Hill and Ron Shank's input, but I have a feeling. Now, and I think one of the things that's reassuring to me, David, is, as you said, these gels. I have not seen any case reports, and you didn't find any, Lillian, in the literature of periungual depigmentation of using this in these gels. So that's reassuring, plus I suspect it is all used up very quickly once the polymerization begins.

DR. STEINBERG: Right. And the other thing is the instructions, and these are the critical safety issues as far as I was concerned was what happens if the woman accidentally puts the gel on her skin.

Well, the gels are very (inaudible). It falls off. It's removed. And you don't want to have nail polish on your skin. So it's quickly removed.

The other thing is what happens is it gets on the cuticle, and the instructions are very clear that you can't have it on your cuticle because when it cures, what will happen is the cuticle will cause the gel to (inaudible) like this instead of like this, which is not very satisfactory with nail polish. It'll just come off. So there are common sense reasons why it's just avoid skin contact. And one of the reports says is what happened with the cuticle and the nail --

DR. BERGFELD: I think that should be included in the discussion.

DR. MARKS: Exactly, Wilma. So could you summarize that in a couple of sentences, David, and give it to Lillian so it can appear in the discussion? I think that's --

DR. STEINBERG: Sure. Yeah, I believe that we have in my report, but I'll be glad to --

DR. MARKS: And then, Doug, are you from the --

DR. STEINBERG: I'd like to introduce some people who -- and Doug [Schoon] is with the (inaudible). Sunil is with OPI, one of the largest producers. Larry [Steffier], who just came in, is with Keystone Laboratories. They are one of the largest manufacturers of these gels for the industry. And Kevin works for Larry. So there are all experts in the world in this type of technology here to answer any of your questions.

DR. MARKS: Yeah, I recognize Doug from the phthalate discussions in the past.

DR. BERGFELD: And before.

DR. MARKS: So any comments that you have? I want to be sure that we capture this, David. It sounds like you've summarized it very well. You've been quiet, Doug, or your colleagues there.

DOUG SCHOON: I think the only thing I would add is these products are educated for use by professionals, and they're educated to avoid skin contact. So they understand it's important to avoid skin contact for one reason -- for the reasons Dave has pointed out. But the product will lift and come up if they do touch the skin. It'll separate from the nail because oils can go underneath the coating. So the skin contact is avoided.

DR. MARKS: Good. Tom and Wilma?

DR. SLAGA: Yes?

DR. MARKS: How did you like the format of this? If we're going to send an amended report, Lillian did a lot of, I guess, summary sections. Wilma, how do you -- I mean, Lillian, how do you want to -- let me see. I'll go on page 15, and it says "summaries of the hydroquinone safety assessments." Do you like the -- first, there's '86, then '94, and then 2010. And it's kind of interesting, you know, when you normally think of a final report it has section and not a whole bunch of summaries in there. So I just wanted to bring that up and make sure that was fine for an amended report.

DR. BERGFELD: Well, as long as you refer back to the references, and I just went back to look at them and it's under reference two, three, and four, and actually five, and six. So you do have those references. But why do references two and three have no authors?

MS. BECKER: Because that's the way they originally published them, Allen as the editor.

DR. BERGFELD: That wasn't the original. That was the second wave of change.

MS. BECKER: Yeah.

DR. BERGFELD: Is there a reason? I mean, that looks sort of funny --

MS. BECKER: He's technically the editor. We have put him as the author. We can go either way.

DR. BERGFELD: Yeah, I think you ought to have somebody there. You've got Andersen (inaudible) if that was the case.

DR. MARKS: Yeah. Anything else, Wilma, that you --

DR. BERGFELD: I thought the summaries were fine. As long as these references were there, that's why I went back to check them, and we asked that question.

DR. MARKS: Tom, were you fine with them?

DR. SLAGA: I thought the summaries were good. It really brought me up to speed.

DR. MARKS: And then if an individual wants to go back to the original report, they can.

MS. BECKER: Correct.

DR. MARKS: And what you added, Lillian, was just essentially updates when you went in the irritation sensitization and such that weren't in the originals.

MS. BECKER: Correct.

DR. MARKS: So, good. I just wanted to be sure that we were okay with the formatting.

DR. BERGFELD: I think when you present it, you should that, too, because that may be (inaudible) of the group. I mean, when we're introducing it, somewhere you just say something about the format being --

DR. MARKS: Okay.

DR. BERGFELD: That would be good.

MS. BECKER: When I originally put them in, it was mostly for your context.

DR. BERGFELD: But everybody else's, too, that reads it. But the references are key to match up with it.

DR. MARKS: Okay.

DR. ANSELL: We do have a comment that within the discussion of dermal penetration, dibutyl phthalate is selected as a surrogate. It's not exactly clear why. And we'd like to see at least some discussion as to why we think that's an appropriate surrogate for dermal penetration.

DR. SLAGA: Yeah, I had the question. I couldn't come up with why it should be a surrogate. Hello?

DR. MARKS: Oh, we hear you. I was waiting for David or Doug to comment or his other two colleagues about that.

DR. SLAGA: Okay.

SPEAKER: It's very difficult to get anything to penetrate the nail. If we could get things to penetrate the nail, there are a lot of diseases or nail conditions that we could treat, which we just do by oral ingestion that (inaudible) satisfactory. And one of the studies that was done, which was in terms of looking at the safety of dibutyl phthalate, which was a plasticizer for normal nail polishes, showed how difficult it was to even get something like dibutyl phthalate to penetrate the nail. So that was published in a paper and just shows, you know, we just can't get things through the nail.

DOUG SCHOON: Even when penetration answers (phonetic) are mixed in with the ingredients it's difficult to get penetration. So without them, it's insignificant.

DR. BERGFELD: Is that documented somewhere?

DOUG SCHOON: I'm sorry?

DR. BERGFELD: Documented somewhere? Is there a reference we could have for that?

DOUG SCHOON: The paper?

DR. BERGFELD: Yeah, or the absorption which you just said. I mean, it's an understanding that you have because you've tried, but has anyone written --

SPEAKER: The penetration paper is published.

DOUG SCHOON: Well, and there are also published reports from dermatologists who have developed antifungal compounds for the nail plate to get these antifungal ingredients into the nail plate to use penetration enhancers. And even when they do that, there's like an eight percent efficacy rate. It's so low. And they cite the lack of penetration of the nail plate as the reason.

DR. BERGFELD: But no one officially has done it. I mean, these are clinical studies? Do they not have any basic science about their absorption through the nail plate like they do with skin?

DOUG SCHOON: Yes, there are. There are studies. I can't cite them right now, but they do exist.

MS. BECKER: If you can send me one, I'd be glad to stick it in.

DR. BERGFELD: That would be -- we think it's good to have a document and source.

DR. MARKS: Lillian, where exactly in this document does it have the previous conclusion for hydroquinone as "safe?" Can you find that for me? I was looking for it because tomorrow if I say, hey, our conclusion is "safe." Were there any caveats to that safety with hydroquinone or was it just "safe as used?" Oh, hydroquinone was safe at a concentration of less than one percent for cosmetic formulations, designed for discontinuous brief use followed by rinsing from the skin and hair. Hydroquinone is safe for use in nail adhesives in the private practice. Hydroquinone should not be used in other leave-on cosmetic products. So that was the conclusion in 2010. That's page 15, so I'll reference that. It's page 15 right above the summaries of the hydroquinone safety assessments. Do you see that, Wilma and Jay?

DR. BERGFELD: I picked it up out of the article.

DR. MARKS: Yeah.

DOUG SCHOON: The other thing to consider, if I might add, is that once these materials polymerize on the nail plate and solidify, now you're looking at just the fusion from a solid coating into the nail plate, which even further slows the penetration.

DR. MARKS: Okay. Page 18, okay. Tom, any other --

DR. BERGFELD: Do you have any data on the UV damage to the nail bed after this polymerization?

DOUG SCHOON: You're talking about the safety of the UV nail lamps. Yeah, there's actually three studies that have been performed on the safety of these (inaudible). But I can send you that information.

SPEAKER: There's one in here --

DOUG SCHOON: There's a Brown University study. And there was also a study by Dr. Robert Sayer studying these lamps through using RP-27 ANSI Standard, and all the conclusions that they're safe, that there's very little risk. And David is going to get you that information.

DR. BERGFELD: Okay.

DOUG SCHOON: So we think there's pretty strong evidence that these lamps are safe.

DR. MARKS: Yeah. That was documented in there. Okay. Tom, any other comments?

DR. SLAGA: That's all I have.

DR. MARKS: So tomorrow I'll recommend or I will move that a tentative amended report with the 2010 conclusion of hydroquinone be reaffirmed, and that we change para-hydroxyanisole from unsafe to safe in nail use only. Does that sound good?

DR. SLAGA: Sounds good.

DR. MARKS: Okay.

MS. BECKER: Question. In the discussion, you won't mention the nail lamps at all.

DR. BERGFELD: I think so. What do you think? I think just to clarify that because every dermatologist or someone in clinical medicine will ask that.

DR. MARKS: Okay. Any other comments?

(No response.)

### **Dr. Belsito's Team and Combined Session**

DR. BELSITO: So now we've got to look at hydroquinones and para-hydroxyanisole. So we've looked at this before and we said para-hydroxyanisole was unsafe because of skin de-pigmentation. And hydroquinone we looked at to re-add it for use as a nail adhesive. And how we're being asked to relook at it because of a potential use as an antioxidant. And then we get data back from the council saying that they're not seeing any reported uses or not getting any reported uses for it.

So the question is, you know, where do we go from here? So we've been told by industry that it is used as an antioxidant in UV-cured acrylics or for nails. And do we proceed now that we're told that there are no reported uses? Do we not proceed?

And, in addition, I would just like to point out that while the Marcova and Weinstock reference about risk of skin cancer associated with the use of UV (inaudible) was quoted in our document that the online journal of the American Academy of Dermatology for December, acrylic nail curing, UV lamps, high-intensity exposure warrants further research of skin cancer risk that would not have been captured. But they reference two articles -- MacFarlane and Alonso -- occurrence of non-melanoma skin cancers on the hands after UV light exposure.

And an article by Runger, et al. in *Photochemistry, Photobiology*, comparison of DNA damage responses following equimutagenic doses of UVA and UVB. So this is beginning to become a controversy in dermatology, whether the use of UV-cured nail acrylics increases the risk of cancer of the hands and periungual area. So I would suggest that, at this point, this be tabled and that everyone get a chance to read the article that is online and the two referenced articles that bring up that possibility; that we ask industry if they're really interested in us reviewing this that we get a sense of what the levels are.

But I'm a little bit -- while I think if they are, in fact, used in UV-cured nail acrylics, their use is not dangerous, but then that raises a whole, for me, ethical conundrum if that practice of doing UV-cured nail acrylics is not a good practice. And I don't know that it is or it isn't. All I'm saying is that this is a -- there's some literature out there that the Panel has not been made aware of by virtue of this document, but I think everyone should see before we further discuss this.

DR. BERGFELD: Is that a motion?

DR. BELSITO: Motion to table, yes.

DR. BERGFELD: Is there a second?

DR. MARKS: I second that motion.

DR. BERGFELD: There's no discussion on that table (inaudible).

DR. BELSITO: And Lillian --

DR. BERGFELD: All those in favor of tabling --

DR. BELSITO: -- this is the online report with the references.

DR. BERGFELD: -- raise your hand. Dan, are you raising your hand? Let's see it. Thank you.

DR. MARKS: Is Tom on?

DR. SLAGA: Yes, I'm on.

DR. BERGFELD: Oh, you are.

DR. SLAGA: (inaudible).

DR. MARKS: Yes, so, Tom, obviously for our team, I'll be interested in your take of these articles.

DR. BERGFELD: Well, to remind you and your team, we did ask David about that and about the absorption, and he was going to supply some additional references, basically search references, about the nail thickness and penetration, et cetera. That does not have to do, though, with the skin of the nail.

DR. MARKS: So what our team -- and, Ron, just chime in since we didn't discuss all the ones that Ron, Tom, and I, Ron Shank and I, without knowing what you just presented, Don, we -- just to kind of give you a sense where we were going, we thought we could go to a tentative amend and not with what you said, but with the hydroquinone safe using the same language as previously; and para-hydroxyanisole, also safe for nail use only. So that's the way we were thinking, but I really like the idea you want to clarify this issue, is there enough ultraviolet to be carcinogenic?

DR. BELSITO: Yeah, I mean, I would really like for Tom and Ron and members of my panel to look at these reports, you know, because it is -- it obviously is becoming somewhat controversial, at least in the derm literature.

DR. MARKS: Yeah. The other thing to clarify with the wave 2 where it said it wasn't being used, David Steinberg and the other individuals from the Nail Council assured us that, indeed, this is being used as a polymerization inhibitor because of these ingredients.

MS. BECKER: Right, and its use was captured in the VCRP, by FDA, their Volunteer Report Program.

DR. BELSITO: And Lillian, I think it would be helpful because at least one of the articles I couldn't even get on Columbia online, is probably to provide copies of those three articles in the documents you send, not just a summary. And then the paper, the current paper, that you reference them, our (inaudible) paper, it's reference 42. If you could include a copy -- the entire copy of that from the *Journal of Investigative Dermatology*. Those authors are arguing that the UV exposure from UV-cured nail acrylics does not enhance the risk. So I think we need to be fair and balanced, and also maybe do some additional online searches, make sure that there are not additional articles out there that should be referenced.

MS. BECKER: Okay, just want to be clear for next time. You want these two articles you just handed me.

DR. BELSITO: No, there are three altogether. There's one I could not get, the article on the --

MS. BECKER: Right, the number 5, I was getting to that.

DR. BELSITO: Yeah.

MS. BECKER: The two you handed me, the one you marked --

DR. BELSITO: Right.

MS. BECKER: -- and the number 42 --

DR. BELSITO: Right.

MS. BECKER: -- you want all those included in the package next time.

DR. BELSITO: Right.

MS. BECKER: Not a problem.

DR. GILL: But I thought I heard in any additional ones we'll look.

DR. BELSITO: In so many additional ones. And then I guess clarification, it's my understanding that the lights that are used are UVA/UVB, but they're UVA only.

DR. STEINBERG: It's principally visible.

DR. BELSITO: Principally visible.

DR. STEINBERG: Yes, the cure generally takes place around 400 nanometers, between 400 and 410, 420, which is the visible. You just can't get bulbs that sensitive and economically. So typically, the bulbs that are used are giving you radiation of 380 to 420.

DR. BELSITO: But I think when you --

MR. STEINBERG: But the cured takes place principally in the visible range.

DR. BELSITO: We need all of that specific information. It's also my understanding that different manufacturers (inaudible) recommends one bulb, that there are different bulbs out there that can be used. Correct?

DR. LIEBLER: There are different consumer-use machines with different bulbs. Yes, that's correct.

DR. BELSITO: Right. So we need to gather as much information on that as we can.

DR. BERGFELD: Excellent.

DR. HILL: Actually there was a fair amount in terms of positioning and wavelength and all of that, that was in our extra data that we got already. Because what's perpendicular, what's parallel? Talking 3-D here. Anyway, but I sorted it out.

DR. BELSITO: Right, I understand, but just making sure that we capture --

DR. HILL: We know what we're talking about.

DR. BELSITO: -- everything.

DR. HILL: The other question I had is on the dermal sensitization, I'm not sure this is even relevant at this point, but the concentrations for para-hydroxyanisole were not provided, so to me that renders the piece of information we got on that pretty well useless. I mean, again, the idea is the stuff is sequestered in the polymer anyway and it doesn't matter, but under dermal sensitization the only information I had on para-hydroxyanisole there was no concentration given. So I just wanted to make sure everybody noted that.

DR. BERGFELD: Any other discussion? Paul?

DR. SNYDER: I had just one comment. So in the document it clearly stated that the removal of these, you know, (inaudible) excluded from the report, but then doesn't that fall under "as used?" I mean, is there anything we should worry about upon the removal? Is there a solvent used that then makes these -- I mean, I just -- I wondered why because of the as-used designation that we used, that we're excluding one of the components in the removal, so I just wanted to throw out there for other opinion.

DR. HILL: That crossed my mind, too. I don't know if that's within our purview. That was what also crossed my mind.

DR. BERGFELD: Well, we can get clarification on that. David?

DR. STEINBERG: Several different things because there are about three -- four or five different topics that they're going through here. The first is the question of the use of para-hydroxyanisole. The industry does not put this into a product. Monomers are sold with polymerization inhibitors and the favorite one right now is para-hydroxyanisole. That's how we apply it. We can't get it without it or else you wouldn't have the monomers that are used. It's used sometimes in combination with hydroquinone, sometimes, depend on the monomer and who was manufacturing it, you might just have it inhibited with hydroquinone, which is why they sort of go together.

Until recently, we did not sell monomers to consumers. They were not part of cosmetics. They weren't considered cosmetics with the exception of the nail adhesives which the Panel has already reviewed. Recently, and that's just been the past three years, I guess, we have developed originally for professional use only the now-polished gels. And these are shear-thinning gels which consist of monomers and oligomers, which have a jelly-like consistency, which are applied to the fingernail where it becomes shear (inaudible). And then it is put under the lamp for a very short period of time and is cured.

The resulting nail polish lasts around two weeks to three weeks. It doesn't chip, it doesn't break. It is removed -- you asked the question -- the way it's removed is you. It's basically you guy Band-Aid --

the way I would describe it is Band-Aids, in which the pad is soap with acetone. And you wrap it around the fingernail for a certain period of time and that softens the polymer, so it peels off.

The questions that were always of interest to us and of concern to the industry was what about the exposure of the gel to skin or the cuticle? And these are very hydrophobic type gels. When they are put on the skin, they pull up just from the natural moisture of it, and they're removed. The instructions say remove it. Who wants nail polish on their skin while they're trying to polish their nails? So the consumer removes it whether we tell them to or not.

The second question is what about the cuticles? And all of the instructions, which we incorporated in our presentation, say that you cannot have the gel on the cuticle. You have to have the gel off the cuticle because when you cure it will not adhere to the cuticle. So instead of getting a cure like this, if you have gel on the cuticle, it would be like this and it will peel. It would be a very unsatisfactory nail polish.

I think we have more information. Doug, do we have more information on the lamps? Because you're more familiar with the lamp work than I am.

MR. SCHOON: Yes. Doug Schoon speaking.

You, I think, included -- David included in the package that he submitted several studies that have been performed. There has been a study performed by Dr. Robert Sayre, a photobiologist of great renown who used the RP-27 ANSI standard, which is an international standard, to test all of the different - five or six different lamps, covering all the categories used in their industry. And he's come to the conclusion that they are very safe and we can -- we have submitted that information to you.

But even more interestingly, there was a Brown University study that compared exposure of these lamps to the types of UV exposure that dermatologists frequently use for psoriasis. And the Brown University study looked at these exposures and said you'd have to have 250 years of weekly manicures to equal one course of what dermatologists consider safe exposure for psoriasis studies, which I thought was a pretty convincing study as well. So then three studies -- one done by the Nail Manufacturers Council, one done by Dr. Robert Sayer, and one by Brown University -- all point to the fact that the amount of UV exposure is extraordinarily low, in fact, surprisingly low. And we can provide all this data to you, again, tested to international ANSI standards.

DR. BELSITO: The Marcova-Weinstock article is the Brown article I'm referring to. I'm just saying that we need to see fair and balanced data, and we're not seeing any data that questions that it's unsafe. And that data needs to be brought before the Panel and we need to consider it.

MR. SCHOON: And nor do I disagree. We could do that.

DR. BELSITO: So I'm not saying that they're unsafe, Doug. I'm simply saying that there's data out there, another side of data out there, with another opinion that this Panel has not seen.

MR. SCHOON: Sure. No, I certainly understand that.

DR. BELSITO: So I'm going to table it to see it.

MR. SCHOON: I certainly understand that and I know the question was raised by Dr. Bergfeld at the last meeting, which is why we included it in our information now. And it's to our best interests that you all understand it, so thank you for raising the question. I appreciate it.

DR. BERGFELD: All right. Thank you so much. We're going to move on now to the next ingredient.

# Amended Safety Assessment of Hydroquinone as Used in Cosmetics

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## INTRODUCTION

This is an amended safety assessment of hydroquinone. A new use in nail gels and adhesives that require UV curing has been identified and new data are being considered to evaluate the safety of this use. This assessment was initiated in response to a request from industry to review both hydroquinone and *p*-hydroxyanisole, which are used interchangeably or in combination as polymerization inhibitors in nail gels sold separately to consumers for home use. *p*-Hydroxyanisole is the focus of a separate amended safety assessment addressing this new use.

Hydroquinone (CAS No, 123-31-9) is defined as “the aromatic organic compound that conforms to the formula in Figure 1.” It is currently reported to function as an antioxidant, fragrance ingredient, hair colorant, reducing agent, and skin bleaching agent.<sup>1</sup> Hydroquinone is the common name for 1,4-dihydroxybenzene.

In 1986, The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) published a safety assessment of hydroquinone and pyrocatechol with the conclusion that these two ingredients were “...safe for use in cosmetics at concentrations up to 1.0% in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair.”<sup>2</sup> In 1994, an amended safety assessment of hydroquinone was published with the conclusion “...safe at concentrations of 1.0% or less for aqueous cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair.”<sup>3</sup> Hydroquinone was not safe for use in leave-on, non-drug cosmetic products. In 2010, the Panel concluded that hydroquinone was “...safe at concentrations of  $\leq 1\%$  for cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair.”<sup>4</sup> Hydroquinone is safe for use in nail adhesives in the practices of use and concentration described in this safety assessment. Hydroquinone should not be used in other leave-on cosmetic products.” The summaries of these reports are provided below.

This report presents new data pertinent to the new use in nail products, as well as new toxicity data that have become available since the last review of this ingredient.

### Summaries of Hydroquinone Safety Assessments

#### 1986

[Note: References to data exclusively on pyrocatechol in this safety assessment summary have been removed.]

*Hydroquinone and pyrocatechol are two benzenediol isomers, 1,4-benzenediol and 1,2-benzenediol. Both ingredients are used in cosmetics as couplers in oxidative hair dyes at concentrations of less than 1.0%. Hydroquinone, a known skin-depigmenting agent, is also used in cleansing preparations at concentrations between 1% and 5%.*

*Both Hydroquinone and pyrocatechol inhibit bacterial growth.*

*Both compounds are absorbed from the gastrointestinal tract. Small amounts of nonmetabolized hydroquinone are excreted in the urine of rabbits; however, most of the compound is excreted as hydroquinone ethereal monosulfate and as the monoglucuronide.*

*The results of acute oral studies in animals indicate that hydroquinone is practically nontoxic to moderately toxic; the data from subchronic feeding studies of hydroquinone indicated that it was not toxic at 1%, slightly toxic at 2%, and toxic at 5%.*

*No adverse local systemic effects were produced in rabbits when 2.0% hydroquinone was applied to intact and abraded skin (3.9 - 9.4 mL/kg). The results of subchronic and chronic dermal studies of hydroquinone in animals for time intervals up to 6 months indicated that the ingredient was a weak depigmenter at 1.0%. Other animal studies indicated that the time required for depigmentation was dependent upon both the concentration and the dispersion medium used. When 2.0% hydroquinone was tested in rabbits using a single-insult patch test, a [primary irritation index] PII of 1.22 (scale 0 - 4) was reported. Guinea pigs were sensitized to hydroquinone when injected at concentrations above 2.0%. The severity of the sensitivity reaction induced by 10% hydroquinone was not increased when exposed to UVA light.*

*In a rabbit eye irritation test, an undiluted product formulation containing 2.0% hydroquinone produced mild conjunctivitis in 3 of 6 animals evaluated at 24 h. The conjunctivitis had subsided on the second day.*

*When hydroquinone (0.003% - 0.3%) was included in the diet of two groups of 10 pregnant female rats, no differences were found between the test and control groups relative to gestation length, mean litter size, viability, and lactation index. In a second study 0.5 g of hydroquinone included in the diets of a group of 10 mated female rats produced no significant difference in resorptions when compared to control groups. Hydroquinone was evaluated in a teratology study in which daily dermal exposure of pregnant rats (20 animals/group) was up to 810 mg/kg; no remarkable difference was found between the control and test groups.*

*The results of mutagenesis assays of hydroquinone have varied with the assay system used. In four Salmonella typhimurium strains, both with and without activation, the mutagenesis assay was negative. One strain tested was positive, with activation using one medium, but not with a second medium. Hydroquinone did not increase antibiotic resistance in Staphylococcus aureus. Hydroquinone was mutagenic in the Escherichia coli DNA polymerase and Saccharomyces cerevisiae mitotic recombination assays. Hydroquinone produced positive results both with and without activation in the HeLa DNA synthesis test but was not considered mutagenic in assays using Chinese hamster cells. Hydroquinone induced Sister Chromatid Exchanges (SCE) and delayed cell turnover time in human lymphocyte studies. Oral doses of hydroquinone did not inhibit testicular DNA synthesis in male mice and was nonmutagenic in the mouse sperm-head abnormality test. Hydroquinone is considered a mitotic poison.*

*In multigeneration rat studies of topically applied hair dyes containing 0.2[%], hydroquinone, no effect on reproduction was observed and embryotoxicity and teratogenesis were not produced. The F<sub>1A</sub> animals were used for carcinogenic assay of the hair dyes. The results were negative. Hydroquinone, when applied topically, was neither a tumor promoter nor a cocarcinogen in Swiss mice. Harding-Passey melanoma transplants were decreased when hydroquinone was administered after implantation.*

*Hydroquinone studies in humans at doses of 500 mg and 300 mg to males and females, respectively, for 5 months produced no signs of toxicity.*

*Positive sensitization reactions to hydroquinone were reported in 8.9% of 536 dermatologic patients challenged with a 5.0% solution. At higher concentrations (10% and 30%) dermatitis was produced in 2 of 5 black subjects. A cosmetic formulation containing 2% hydroquinone produced one or more mild irritation reactions in 69 of 90 subjects in the induction phase of a sensitization test. In this latter study, 22 subjects had a mild reaction when challenged by the same formulation and scored at 24 h. Only 3 of the 22 subjects had either mild or barely perceptible reactions at 48 h. The use of ointments containing 2, 3, and 5% hydroquinone in 94 white and 43 black men with normal skin produced at least minimal depigmentation in white but not black subjects. Two of 38 patients treated with an ointment containing 5.4% hydroquinone became sensitized. Other studies on dark-skinned subjects have confirmed these sensitization results.*

*Ocular lesions but no other systemic effects have been found in workers involved in the manufacture of hydroquinone. Recommended limits for occupational exposure of hydroquinone have been set at 2[%].*

#### **1994**

*This addendum to the final report on hydroquinone was prepared in response to the release of a National Toxicology Program (NTP; 1989)<sup>5</sup> report of an oral carcinogenicity study. In the original CIR report, it was concluded that hydroquinone was safe for cosmetic use at ~1% in formulations designed for discontinuous, brief use followed by rinsing from skin and hair. This conclusion applied primarily to the use of hydroquinone in hair dye formulations. The use of hydroquinone to lighten the skin was not addressed because such use is regarded by the Food and Drug Administration (FDA) as a drug use.*

*In 1993, hydroquinone was reported to be used in 206 formulations, 185 hair dyes, two lipsticks, one skin freshener, and 18 other skin care preparations.*

*Hydroquinone in an alcoholic vehicle was absorbed through the skin of the forehead of male subjects; absorption of hydroquinone from a solution that also contained Escalol 507 (a sunscreen) and Azone (a penetration enhancer) was 35 ± 17%, from a solution containing Azone was 66 ± 13%, from a solution containing Escalol 507 was 26 ± 14%, and from a solution containing only hydroquinone was 57 ± 11%. The average percutaneous absorption rate of hydroquinone using 48-h excretion data from dermal and i.v. absorption studies using dogs was estimated to be ~0.15 nmol/cm<sup>2</sup>/min (1.1 kg/cm<sup>2</sup>/h). Hydroquinone was rapidly absorbed and excreted by male and female Fischer rats following oral administration; overall recovery was ≥ 96% from females after 24 h and from males after 48 h. In a study using urinary excretion data, dermal absorption was estimated to be 10.5% for male rats using 72-h data and 11.5% for female rats using cumulative 48-h data.*

*Hydroquinone was found to have some immunologic effects; it especially had effects on bone marrow. In a functional-observation battery (FOB), hydroquinone was not found to cause central or peripheral nervous system lesions. Hydroquinone was nephrotoxic in male F344 rats. Hydroquinone also showed cytotoxic properties.*

*According to the terminology of Hodge and Sterner (1949)<sup>6</sup>, hydroquinone is slightly toxic, with an oral LD<sub>50</sub> of 743 and 627 mg/kg for male and female rats, respectively.*

*Administration of hydroquinone to rats in drinking water (2,500 - 10,000 ppm) for 8 weeks resulted in significant increases in liver and kidney weights. Hydroquinone administered orally to rats (63 - 1000 mg/kg) and mice (31 - 500 mg/kg) for 14 days resulted in tremors and deaths in the high-dose groups. Dermal administration to rats (240-3840 mg/kg) and mice (300 - 4800 mg/kg) for 14 days caused neither death nor any significant adverse effects. For mice given i.p. injections of 10 mg/kg hydroquinone for 6 weeks, it was concluded that hydroquinone may cause hematologic injury.*

*Rats given 1000 - 4000 ppm hydroquinone in drinking water for 15 weeks had significantly increased liver and kidney weights. Oral administration of 25 - 400 mg/kg hydroquinone to rats and mice for 13 weeks resulted in mortality in the high-dose groups for both rats and mice. Other adverse signs, such as lethargy, tremors, and changes in relative liver to body weight ratios, were observed.*

*Dermal application of 25 or 150 mg/kg hydroquinone to rats produced slight to severe erythema.*

*In a Magnusson-Kligman guinea pig maximization test, hydroquinone was classified as an extreme sensitizer. Hydroquinone was positive for sensitization in an LLNA.*

*Oral administration of hydroquinone did not produce embryotoxic, fetotoxic, or teratogenic effects in rats, nor did it produce significant adverse reproductive effects in a two-generation study. Using rabbits, various teratogenic/reproductive treatment-related effects were observed at doses of 200-500 mg/kg. All dams dosed with 30 to 500 mg/kg hydroquinone died. Some maternal toxicity was observed at a number of dose concentrations.*

*Hydroquinone induced SCEs, chromosomal aberrations, and mitotic division aberrations increased the frequency of mitotic crossovers, caused c-mitotic effects, and induced chromosome loss. It was clastogenic for male mouse germ cells and*

for mouse bone marrow cells. Hydroquinone induced DNA strand breaks and inhibited DNA, nuclear DNA, and mtDNA synthesis in rabbit bone marrow mitochondria. It also inhibited mtDNA transcription synthesis and RNA synthesis. Hydroquinone caused the formation of hydrogen peroxide and 8-hydroxydeoxyguanosine (8-OHdG) in calf thymus DNA and produced DNA adducts in HL-60 and other cells. Forward mutation assays with and without metabolic activation were positive, as were numerous micronucleus assays. Results of the Ames test and a mouse spot test for somatic gene mutations were negative.

In an NTP study, hydroquinone was given to rats orally by gavage five times per week for up to 103 weeks at doses of 25 or 50 mg/kg. The higher dose induced a significant incidence of renal adenomas in males and both doses caused a significant incidence of renal adenomas in males and both doses caused a significant increase in the incidence of mononuclear cell leukemia in females. Mice were dosed with 50 or 100 mg/kg hydroquinone following the same schedule as that used for the rats. The incidence of hepatocellular adenoma was significantly increased in female mice.

NTP concluded that Hydroquinone produced “some evidence of carcinogenic activity” for male and female F344/N rats and female B6C3F<sub>1</sub> mice but “no evidence of carcinogenic activity” for male B6C3F<sub>1</sub> mice in an oral carcinogenicity study.

Shibata et al. (1991)<sup>7</sup> conducted a study in which rats and mice were fed diet containing 0.8% hydroquinone for 104 and 96 weeks, respectively, and concluded that “the study strongly suggested that since hydroquinone has apparent carcinogenic potential for rodents, there is a possibility that it may play a role in human cancer development.” Hydroquinone did not induce a significant number of neoplasms in either the glandular or nonglandular stomach of hamsters fed 0.5% hydroquinone in the diet for 20 weeks or rats fed 0.8% hydroquinone in the diet for 51, 49, or 8 weeks.

When hydroquinone was fed to rats after pretreatment with methyl-N-amyl nitrosamine (MNAN), hydroquinone was marginally effective in enhancing esophageal carcinogenesis and had marginal activity in the promotion of upper digestive tract carcinogenesis. Other studies did not prove hydroquinone to be a tumor promoter.

No reaction to hydroquinone was observed when patients positive to at least one hapten of the para group of the International Contact Dermatitis Research Group (ICDRG) standard series were tested using the Al test. Hydroquinone contact has caused dermatitis and hydroquinone exposure can result in ocular effects. Hydroquinone has caused hypomelanosis hyperpigmentation of the skin and depigmentation of black skin. Ingestion of 1 g hydroquinone by humans can produce severe toxicity; ingestion of 5-10 g can be fatal.

## 2010

Hydroquinone is reportedly used in hair dye preparations, skin care products, nail products, and as recently as 2007 in lipstick. Information provided to the FDA through the Voluntary Cosmetic Registration Program (VCRP) indicates that the use of hydroquinone has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009. Hydroquinone is a component of artificial nail products because it is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon polymerization of the acrylic monomers, hydroquinone is oxidized and is no longer detectable in the final polymer using analytical techniques for identifying trace amounts in a solid matrix. Any residual hydroquinone is trapped in the polymer and is therefore unavailable and not likely to be absorbed.

While an earlier *in vitro* study suggested that hydroquinone would be considered a “slow permeant,” a more recent *in vivo* study demonstrated that hydroquinone is in fact rapidly absorbed through the skin from an aqueous preparation. Hydroquinone is metabolized to the sulfate and glucuronide conjugates, with oxidation to 1,4-benzoquinone, resulting in a reactive metabolite that forms mono- or polyglutathione conjugates. The glutathione conjugates are believed to be responsible for the nephrotoxicity observed in rats. In addition to nephrotoxicity, hydroquinone has some immunotoxic effects and has been positive in many mammalian cell assays *in vitro* and *in vivo* including micronuclei formation, SCE, and chromosomal aberrations despite being mostly negative in *in vitro* bacterial mutagenicity assays. The induction of renal cell tubule tumors in male F344 rats has raised concern regarding the nephrocarcinogenicity of hydroquinone and has led to several mechanistic studies which suggest that the male F344 rat is more susceptible to the glutathione conjugates of hydroquinone due to the spontaneous occurrence of chronic progressive nephropathy (CPN) which nearly all rats develop as they age. There is no human disease that shares all of the features of rodent CPN, however, there are histopathological similarities between human chronic renal disease and CPN that do not allow the proposed mode of action (MOA) to be ruled out entirely on a qualitative basis. Quantitatively, the use of hydroquinone containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

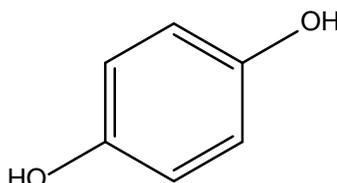
Hydroquinone has been reported to cause exogenous ochronosis in several ethnic populations following prolonged use (>6 months) of at least a 1% to 2% cream. These effects along with the NTP cancer study findings have led the FDA to reconsider the generally recognized as safe and effective (GRASE) label for hydroquinone in leave-on drug products.

The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the available hair dye epidemiology data is available at <http://www.cir-safety.org/findings.shtml>.

## CHEMISTRY

### Definition and Structure

Hydroxyquinone is a substituted phenol (Figure 1).



Hydroquinone

**Figure 1.** Hydroquinone.

## USE

### Cosmetic

Data on ingredient use are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP).<sup>8</sup> The VCRP reports that hydroquinone is used in 7 nail extenders and 11 skin care preparations.

A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for these ingredients.<sup>9</sup> There were no reported uses for this ingredient.

Hydroquinone is listed in Annex III of the European Council Directive with the following restrictions: only for use in artificial nail system, maximum concentration of 200 ppm after mixing, for professional use only, avoid skin contact, read use directions carefully.<sup>10</sup> Hydroquinone is also listed under Annex II and may not be used in cosmetic products with the exception of the use listed in Annex III.<sup>11</sup>

### *Use In Nail Products*

Hydroquinone, alone or in combination with *p*-hydroxyanisole, is used as a stabilizer that inhibits the polymerization in the liquid component of two-component methacrylate artificial nail systems.<sup>10</sup> The maximum concentration of hydroquinone alone, or in combination with *p*-hydroxyanisole, is reported to be 200 ppm (0.02%). After mixing 2 parts liquid to 1 part powder in preparation for use, the final concentration of hydroquinone, or hydroquinone and *p*-hydroxyanisole combined is approximately 133 ppm (0.0133%).

When used as a nail adhesive, a brush is wetted in the liquid component which contains the stabilizer(s) and acrylate monomers. The wetted brush is then dipped into the powder which contains the initiator to produce an 'aspirin sized' bead. The liquid:powder ratio is approximately 2:1. The two components are mixed into a 'slurry bead', which is applied to the center of the nail plate and then shaped. The polymerization is complete in 5 - 15 min. Contact is to the keratin of the nail plate and not to the skin or cuticle.<sup>10</sup>

Hydroquinone is added to the monomer and oligomer (i.e., dimer, trimer, tetramer) preparations during manufacturing to prevent polymerization.<sup>12</sup> This preserves the integrity of the monomers or oligomers until they are used to produce polymers or other derivatives. For polymerization to occur, the inhibitors must either be destroyed or inactivated. Hydroquinone (and *p*-hydroxyanisole) is destroyed during polymerization (using light) and any residual inhibitor is fully enclosed in the hardened polymer.[Dr. David Steinberg, pers. comm.]

In a guide to using UV gel enhancements, the manicurist is instructed to carefully prepare the nail bed by removing the cuticle from the area of the nail where the product is to be applied.<sup>13</sup> If the cuticles are not cleared away from the nail bed, natural oils and moisture under the nail gel or the enhancement adhesive prevents the product from adhering to the nail and the product will peel off, creating an unsatisfactory result.[Dr. David Steinberg, pers. comm.]

The direct sale to consumers of these products, which contain hydroquinone and/or *p*-hydroxyanisole are being offered for "at home" use. The direct sale to consumers of such products, which contain one or both of these stabilizers, constitutes the new use considered in this safety assessment.<sup>12</sup>

The nail gels and adhesives are removed by the application of a solvent (that is provided on a presoaked pad) for 15 to 30 min.<sup>14,15</sup>

### Non-Cosmetic

The re-evaluation of hydroquinone's GRASE label in leave-on drug products by the FDA, noted in the 2010 summary above, has not been completed.<sup>16</sup>

## **TOXICOKINETICS**

### **Absorption, Distribution, Metabolism, and Excretion**

#### ***Nail Penetration***

Dibutyl phthalate (100%), a plasticizer in nail products, did not penetrate human cadaver fingernails to any significant extent (n = 12; three donors; thumb and little finger nails not used) in tests using modified Franz cells.<sup>17</sup> Because dibutyl phthalate (and, by analogy, any residual hydroquinone) would be bound within a polymerized nail product after application and curing, no significant exposure to the test material through the nails would be expected. The receptor cell was sampled every 24 h for 17 days and analyzed for dibutyl phthalate. On day 7, the residual material in the donor cell was removed and the surfaces of the nails were washed with isopropanol. On day 17, the nails were removed from the cells, cut into quarters, and analyzed for dibutyl phthalate by high-performance liquid chromatography/ultraviolet light. On day 7, penetration rate was approximately 3 µg/cm<sup>2</sup>/h. An average of 8.5 ± 1.3% of the applied test material penetrated the nails; an additional 0.3% of the test material remained on the nails on day 17.

#### **Cytotoxicity**

Hydroquinone (0, 10, 20, 30, 40 µM) was not cytotoxic to human L-02 liver cells but was cytotoxic to the same cell line with silenced DNA polymerase eta (Pol η) after 24 h of incubation.<sup>18</sup> Cell survival was determined using the MTT assay.

Hydroquinone (500, 750 µM) was cytotoxic, in a concentration-dependent manner, to F344 rat hepatocytes when incubated for 2 h.<sup>19</sup>

Hydroquinone was cytotoxic to human lymphocytes at 270 µM, but not at 180 µM, when incubated for 3, 24, or 48 h with metabolic activation and 3 h without metabolic activation.<sup>20</sup>

## **TOXICOLOGICAL STUDIES**

### **Repeated Dose Toxicity**

#### ***Dermal – Non-Human***

Hydroquinone (2% in a topical cream) caused liver and kidney damage when administered to rabbits (n = 6) for 6 weeks.<sup>21</sup> The test substance was administered daily to one or both ears of the rabbits or to the shaved abdomen; the rabbits were killed and necropsied. Findings in the liver included hydropic degeneration, bile duct hyperplasia, and glycogen depletion. Hydropic degeneration, hyaline casts, congestion, perivascular edema, and fibrosis were observed in the kidneys. For both the kidneys and livers, the effects were greater in the groups in which the test substance was administered to the ears. Dermal effects included hyperkeratosis, lymphocytic and eosinophilic infiltration, and congestion of dermal blood vessels.

Dermal depigmentation was observed when hydroquinone (5% in propylene glycol/ethanol, 50:50) or *p*-hydroxyanisole (5% in propylene glycol/ethanol, 50:50) was dermally administered to multiple sites of the backs of Yucatan miniature pigs (n = 2) twice/day, 7 days/week for 90 days.<sup>22</sup> Microscopic examination of biopsies from the test area showed decreased pigment and melanocytes.

## **GENOTOXICITY**

### **In Vitro**

#### **HYDROQUINONE**

Hydroquinone (0, 10, 20, 30, 40 µM) did not induce DNA damage to human L-02 liver cells but was genotoxic to the same cell line with silenced DNA polymerase eta (η) after 24 h of incubation.<sup>23</sup> DNA damage was determined by means of the Comet assay, apoptosis and cell cycle distribution were determined using flow cytometry, the mRNA expression levels of Polη were determined by real-time PCR, the protein expression levels of Polη and γ-H2AX were determined by Western blot, and γ-H2AX foci were visualized by confocal laser scanning fluorescence microscopy after cells were exposed to hydroquinone. The down-regulation of Polη led to a decrease in cell proliferation and an enhanced susceptibility to hydroquinone-induced cytotoxicity. Polη-deficient cells were 2-fold more sensitive to hydroquinone when compared with nonspecific siRNA control cells. Also, treated Polη-silenced L-02 cells displayed increased levels of DNA double-strand breaks as measured by olive tail moment, and an elevated DNA damage response, as indicated by the induction of γ-H2AX. In addition, knockdown of Polη resulted in more enhanced apoptosis and more pronounced S phase arrest following hydroquinone treatment. The authors concluded that Polη plays an important role in the response of L-02 cells to hydroquinone-induced DNA damage.

Hydroquinone (45-900 µM; 50 µL) was not clastogenic in cultured human lymphocytes with or without metabolic activation.<sup>20</sup> The lymphocytes were treated in accordance with the Organization for Economic Co-Operation and Development (OECD), European Economic Community (EEC), and the Environmental Protection Agency (EPA) guidelines for mutagenicity testing. The lymphocytes were incubated with hydroquinone (18 – 73 µM) for 17 h prior to the addition of hydrogen peroxide (12 mM). Pre-incubation with hydroquinone reduced the number of chromosomal aberrations compared to negative controls.

**IRRITATION AND SENSITIZATION*****Dermal – Non-Human*****HYDROQUINONE**

In a local lymph node assay (LLNA; n = 5) repeated in four different laboratories, hydroquinone (0, 0.10%, 0.25%, 0.50%, 1.00%, 2.50% in acetone:olive oil 4:1; > 99.5% pure) was predicted to be a dose-dependent sensitizer.<sup>24</sup> The EC<sub>3</sub> values were 0.07%, 0.03%, 0.08%, and 0.07% for the four laboratories.

When hydroquinone (5% in propylene glycol/ethanol, 50:50) was dermally administered to multiple sites of the backs of Yucatan miniature pigs (n = 2), the test sites exhibited severe erythema, scaling and crusting.<sup>22</sup> The test substance was administered twice/day, 7 days/week for 90 days. Microscopic examination of biopsies of the test area showed reduction in pigment and number of melanocytes.

***Dermal – Human***

In multiple human repeated insult patch tests (HRIPT) of nail gel products, there were no signs of potential cuticle irritation or allergic contact sensitization (Table 1).<sup>25-36</sup> The test materials were administered to a fingernail of the subjects and removed by wiping with a proprietary remover solution after 10 minutes three times per week for nine applications. Two weeks later, the test material was administered to the same fingernail in the same manner. The amounts of hydroquinone or *p*-hydroxyanisole were not provided.

**UV NAIL LAMPS**

UV lamps are used to cure nail gels, acrylic nails, and nail fill-ins, and to dry traditional nail polish and UV top sealers/topcoats.<sup>37</sup>

The UV nail lamps produce light mostly in the UVA-1 range with little UVA-2, and there is virtually no UVB or UVC radiation emitted.<sup>38</sup> UVA-1 is the least erythemic and photocarcinogenic range in the UV spectrum. The bulbs in UV nail lamps have internal filters to eliminate UVB and are reported to emit exclusively in the 265 – 370 nm range.<sup>39</sup>

In 2010-2011, over 87% of professional nail salons reported using UV nail lamps.<sup>40</sup> Typical client usage is 1 – 4 times/month for 2 min or less per visit.<sup>39</sup>

An instructional pamphlet for the application of nail polish directs, that in the course of applying a base coat, color coat, and top coat, the polish is to be cured for 30 sec for each coat using the proprietary UV light (for a total of 90 sec) or for 1min, 2 min, and 3 min, respectively for a total of 6 min using another UV light.<sup>41</sup>

Nail gels shrink with curing under UV lamps. Thus, it has been recommended that three or four separate thin coats of nail gel be applied and cured for 3 min each coat to achieve the desired results.<sup>37,42</sup>

In a study of two UV nail lamps (each from a different nail product company) cumulative exposure measured as minimal erythema doses (MED) were low. However, measured in J/m<sup>2</sup>, cumulative exposures were equivalent, in less than 10 min, to the recommended limit of 30 J/m<sup>2</sup> for 8 hours of outdoor work and recreation by the International Commission on Non-Ionizing Radiation Protection.<sup>43</sup> Dosimeters that measure DNA damage caused by UV irradiation of viable spores were used to make these measurements. Manufacturer's instructions for curing acrylic nails using UV light were followed. It was assumed that the nails would be refinished every 3 weeks, or 17 times/year; the dosimeters were exposed for the equivalent of the cumulative dose that would be expected over 1 year of using such lamps. The UV lights yielded 0.6 MED/h for phototype II skin. The curing time recommended by the manufacturers yielded from 0.06 to 0.09 MED per treatment and yearly cumulative exposures estimated between 1.1 and 1.5 MEDs. Total exposures were estimated to be 285 and 386 J/m<sup>2</sup>/y from 15 and 22.5 J/m<sup>2</sup> per nail session, respectively (Table 2).

In the same study, a spectrometer calibrated to measure absolute UV irradiance was used to compare solar radiation with radiation emitted from the lamps. The spectra indicated that the lamps emitted 4.2 times more energy (μW/cm<sup>2</sup>/nm) than the sun (UV Index = 6) in the 355 to 385 nm range. The authors recommended the use of full spectrum sun block to the hands 30 minutes before exposure.<sup>43</sup>

In an evaluation of six UV nail lamps, the authors concluded that total UV exposure following programmed times and steps, analogous to nail polish application, accumulate to only a small fraction of the recommended practice (RP)-27 permissible daily occupational exposure of UV.<sup>44</sup> The UV nail lamps used were representative of major US manufacturers and evaluated for radiant hazards as defined in the American National Standards Institute/Illuminating Engineering Society of North America Recommended Practice - 27 (ANSI/IESNA RP-27), the Recommended Practice for Photobiological Safety. Lamps were evaluated at three positions: 1 cm above the inner surface, which approximated exposure to the hand; 20 cm directly in front of the box opening; and 20 cm outside the box and 45° above the hand opening.

Three of the devices were fluorescent UV nail lamp systems with 2, 3 or 4 small 9 W lamps. Lamps were of two base types with tubes oriented either perpendicular (in the case of the two-lamp device) or parallel to the fingers of a hand undergoing a procedure. The tubes in the three- and four-lamp units were arrayed in an arc-like configuration to irradiate from above and from the sides of the hand while the perpendicular-oriented tubes of the two-lamp unit were in a planar configuration above the fingertips. The other three devices were light-emitting diode (LED)-based with arrays of 6 or 32 LEDs or, in the case of a single finger unit, one LED. These LED arrays were mounted in planar configurations oriented generally perpendicular to the fingers in approximately equidistant arcs above the fingertips. The 32 LED devices had four

of its LEDs oriented in two lateral pairs positioned on either side. The entrance aperture of the spectroradiometer was positioned to receive the full intensity expected at each of the three different measurement positions chosen to approximate expected intensities to which a user's skin or eyes might be exposed.

Hazard to skin at intended-use distance enabled classification of these devices into Risk Group 1 or 2 (Low to Moderate) with the  $S(\lambda)$  (i.e., distance between the source and the object) weighted Actinic UV range of 1.2–1.7  $\mu\text{W}/\text{cm}^2$  and 29.8 - 276.25 min permissible daily exposure. At 20 cm on center and at 45° from center, UV risk to skin and eyes were within the Exempt classification. Actinic UV ranged 0.001–0.078  $\mu\text{W}/\text{cm}^2$  and unweighted near UV (320 - 400 nm) range was 0.001–0.483  $\text{mW}/\text{cm}^2$ . The retinal photochemical blue light hazard and retinal thermal and cornea/lens IR were also Exempt. One device was found to be an aphakic eye hazard slightly rising into Risk Group 1 (low hazard). There were no other photobiological risks to normal individuals. The potential risks estimated in this study are likely to be substantial overestimates of any actual risks in realistic non-occupational use scenarios because such exposures to these lamps would unlikely be a daily occurrence.<sup>44</sup>

The carcinogenic-effective irradiance from three different UV nail lamps used 10 min/week was estimated to be over 250 years.<sup>45</sup>

### **Risk Analysis**

In a risk analysis, it was concluded that 72 709 women using UV nail lamps to cure their nail gels 8 min/application, every 3 weeks, for 20 years would increase the chance that one more woman might develop squamous cell carcinoma on the back of the hand compared to women who were never exposed to UV nail lamps (Table 3).<sup>46</sup> The model UV nail lamp used in this analysis had an unweighted UV irradiance of 115  $\text{W}/\text{m}^2$  with an erythemally weighted output of 1.58 SED/h. The authors stated that the estimated risk of squamous cell carcinoma could be reduced to virtually zero by wearing fingerless gloves when the hands are being exposed to UV radiation from such lamps.

### **Light Penetration of Nails**

UVB light did not penetrate the finger nails of a cadaver ( $n = 10$ ).<sup>47</sup> An average of 1.65% of UVA light penetrated the nails in this study.

### **Case Reports**

Nonmelanoma skin cancers were observed on the dorsum of the hands of two women who reported exposure to UV nail lamps.<sup>37</sup> The first woman was 55 years old, in good health, and was not taking immunosuppressive medication. She had an indoor occupation and participated in little outdoor recreation. Her family had no history of skin cancer. She had been exposed to a UV nail light twice monthly for 15 years. She presented with an erythematous plaque on the dorsomedial aspect of her right index finger. Biopsy revealed a squamous cell carcinoma.

The second woman was 48 years old, in good health, and not taking immunosuppressive medication. She had an indoor occupation with moderate outdoor recreational exposure to UV. She had no personal or family history of skin cancer except for a previous squamous cell cancer that had been removed from the dorsum the left finger 3 years earlier. She presented with a scaly papule on the dorsum of her right hand. Biopsy revealed a squamous cell cancer. Over the next 4 years, two further squamous cell cancers on the dorsum of both hands were treated. She had had exposure to UV nail lights eight times within a year several years before her the first appearance of the skin cancer.<sup>37</sup>

### **SUMMARY**

This is an amended safety assessment of hydroquinone prepared to address a new use in nail gels and adhesives that require UV curing. The CIR Expert Panel concluded in 2010 that hydroquinone is safe for use in nail adhesives but should not be used in other leave-on cosmetic products.

The VCRP reports that hydroquinone is used in 7 nail extenders and 11 skin care preparations.

Hydroquinone was reported to be used in the liquid component of two-component artificial nail systems at a maximum concentration of 200 ppm, which decreases to approximately 133 ppm after mixing with the solid component just before application. Polymerization was reported to take 5 – 15 min in a nail adhesive product. Hydroxyquinone is used interchangeably and in combination with *p*-hydroxyanisole to control polymerization in nail gels and nail adhesives.

Dibutyl phthalate at 100%, used as a surrogate for substances like hydroquinone and *p*-hydroxyanisole, penetrated human fingernails at approximately 3  $\mu\text{g}/\text{cm}^2/\text{h}$ . Hydroquinone was not cytotoxic to human liver cells up to 40  $\mu\text{M}$  but was cytotoxic to rat hepatocytes at 500 and 750  $\mu\text{M}$ . It was cytotoxic to human lymphocytes at 270  $\mu\text{M}$  but not at 180  $\mu\text{M}$ .

Six weeks of dermal administration of hydroquinone at 2% in a topical cream caused liver and kidney damage in rabbits.

Hydroquinone up to 40  $\mu\text{M}$  did not induce DNA damage in human liver cells but was genotoxic in the same cell line with silenced DNA polymerase eta (Pol  $\eta$ ). Hydroquinone up to 900  $\mu\text{M}$  was not clastogenic in cultured human lymphocytes with or without metabolic activation.

Hydroquinone caused severe erythema, scaling and crusting in miniature pigs.

Hydroquinone at 0.10% to 2.50% was predicted to be a sensitizer in an LLNA.

In multiple HRIPTs of nail products, there were no signs of cuticle irritation or allergic contact sensitization when products containing hydroquinone and/or *p*-hydroxyanisole were administered to the fingernails.

UV lamps are used to cure nail gels, to cure acrylic nails and nail fill-ins, and to dry traditional nail polish and UV top sealers/topcoats.

In a study of UV exposure from different UV nail lamps using two different measurement methods, the cumulative minimal erythema doses (MED) were low. However, in less than 10 minutes, the exposure measured in J/m<sup>2</sup> was equivalent to the day-long recommended limit for outdoor work and recreation.

In tests of multiple types of UV nail lamps used as intended, the estimated UV exposure was below levels associated with potential carcinogenicity.

A risk analysis of the use of UV nail lamps concluded that tens of thousands women would have to use UV nail lamps to dry their nail gels 8 min/manicure, every 3 weeks, for 20 years to increase the chance that one more woman would develop squamous cell carcinoma on the back of the hand, compared to women who were not exposed to UV nail lamps.

UVB light did not penetrate finger nails; very little UVA light penetrated fingernails.

There were two case reports of nonmelanoma skin cancers on the dorsum of the hands of two women who used UV nail lamps were reported.

It was recommended that fingerless gloves or full-spectrum sun block be used when UV nail lamps are to be used.

### **DISCUSSION**

*This Discussion will be reviewed and further developed at the March, 2014 CIR Expert Panel Meeting.*

Hydroquinone causes depigmentation to the skin starting at 1% and was found to be safe at that concentration or less in rinse-off products and nail adhesives in 2010. This conclusion did not contemplate nail applications as currently used.

The Panel noted that there is no dermal exposure of hydroquinone when properly used in nail products. Any accidental application to the surrounding skin is promptly removed for best visual results and adherence. Therefore, there is no risk of more than momentary exposure that should not result in skin pigmentation. However, the Panel does stress that contact with the skin is to be prevented and that professionals be properly trained in the application of these products. The Panel also notes that this ingredient is either consumed during the curing or trapped within the polymerized matrix, so post application exposure is not an issue.

However, there are "home kits" now available to the consumer. [*The Panel will further develop this discussion*]

The Panel noted that the sensitization studies conducted by applying the nail gel to the fingernails did not provide the concentration of hydroquinone/*p*-hydroxyanisole. While the study does not demonstrate the sensitization potential of these products, it does demonstrate how unlikely it is for sensitization to develop when these products are used properly.

### **CONCLUSION**

*The Conclusion will be developed at the March, 2014 CIR Expert Panel Meeting.*

**TABLES AND FIGURES****Table 1.** HRIPTs of nail products containing hydroquinone and/or *p*-hydroxyanisole administered to the fingernails. The amount of hydroquinone and/or *p*-hydroxyanisole in the products was not provided.

Product	n	Result	Reference
UV gel top coat nail polish	51	No signs of potential cuticle irritation or allergic contact sensitization	30
UV gel top coat nail polish	51	No signs of potential cuticle irritation or allergic contact sensitization	29
Builder gel	51	No signs of potential cuticle irritation or allergic contact sensitization	28
Clear overlay gel	51	No signs of potential cuticle irritation or allergic contact sensitization	27
Soak-off sealer	51	No signs of potential cuticle irritation or allergic contact sensitization	26
Soak-off gel lacquer	51	No signs of potential cuticle irritation or allergic contact sensitization	25
Gel system-thick gel sealer	50	No signs of potential cuticle irritation or allergic contact sensitization	31
Base gel	51	No signs of potential cuticle irritation or allergic contact sensitization	32
No-cleanse overlay gel	51	No signs of potential cuticle irritation or allergic contact sensitization	33
Soft white sculpting gel	51	No signs of potential cuticle irritation or allergic contact sensitization	34
Pink builder gel	51	No signs of potential cuticle irritation or allergic contact sensitization	35
Luminous white overlay gel	51	No signs of potential cuticle irritation or allergic contact sensitization	36

**Table 2.** Ultraviolet nail lamp measurements.<sup>43</sup>

Lamp	Exposure time (min)	Total MED/yr	Total J/m <sup>2</sup>	MED/h	Total MED/manicure	Total J/m <sup>2</sup> /manicure
OPI lamp	150	1.5	386	0.62	0.09	22.5
CND lamp	108	1.1	285	0.63	0.06	15.0

**Table 3.** The number of women who would need to be exposed to ultraviolet A (UVA) nail lamps<sup>a</sup> for one woman to develop squamous cell carcinoma who would not have done so otherwise.<sup>46</sup>

Age when UVA nail lamp use begins	Number of years of use			
	5	10	20	40
20	218 604	125 629	72 709	44 254
30	271 521	155 688	89 435	52 952
40	332 747	189 670	107 287	60 863
50	395 768	223 255	123 290	-

<sup>a</sup> Assumes a typical level of exposure of 8 min per hand, once every 3 weeks.

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4

# Final Report on the Safety Assessment of Hydroquinone and Pyrocatechol

Hydroquinone and Pyrocatechol, two benzenediol isomers, are used as couplers in oxidative hair dyes at concentrations of less than 1.0%. Both compounds are absorbed from the gastrointestinal tract; Pyrocatechol is also readily absorbed through the skin. Both compounds are excreted in the urine, mainly as the ethereal sulfate. In acute oral studies Hydroquinone is practically nontoxic to moderately toxic; the data from subchronic feeding studies of Hydroquinone indicated that it was not toxic at 1% but was at higher concentrations. Pyrocatechol was moderately toxic in acute studies. Subchronic oral studies of Pyrocatechol at 0.25% produced hepatic cell hyperplasia in rats. Hydroquinone was a weak depigmenter but not an irritant when tested at 1.0%. The ingredient was a sensitizer when injected at 2.0%. The acute dermal LD<sub>50</sub> of Pyrocatechol was 0.8 g/kg. Pyrocatechol did not depigment rabbit skin at 1.0% but did at 3.0%; skin irritation was observed at 5.0%. Guinea pigs were sensitized when Pyrocatechol was injected at concentrations above 0.2 μM. Undiluted product formulation containing 2.0% Hydroquinone produced mild conjunctivitis in 3 of 6 animals; undiluted Pyrocatechol is an extreme ocular irritant.

Hydroquinone was not teratogenic in three separate studies. The results of mutagenesis assays of Hydroquinone varies with the assay system used. In four *Salmonella typhimurium* strains, both with and without activation, the mutagenesis assay was negative. Hydroquinone produced positive results both with and without activation in the HeLa DNA synthesis test but was not considered mutagenic in assays using Chinese hamster cells. Hydroquinone induced SCE and delayed cell-turnover time in human lymphocyte studies. Oral doses of Hydroquinone did not inhibit testicular DNA synthesis in male mice, and was nonmutagenic in the mouse sperm-head abnormality test. In multi-generation studies with rats, topically applied hair dyes containing 0.2% Hydroquinone had no effect on reproduction; the dye was neither embryotoxic or teratogenic. Dermally applied hair dyes containing Hydroquinone were not carcinogenic. Hydroquinone when applied topically was neither a tumor promoter nor a cocarcinogen in mice. The mutagenicity of Pyrocatechol also varies with the test system used. In most studies, Pyrocatechol was nonmutagenic, both with and without metabolic activation, in the Ames' assay. The compound was negative in the *Escherichia coli* DNA polymerase assay, but

was positive in the yeast, *Saccharomyces cerevisiae*. Pyrocatechol was negative in the HeLa DNA synthesis test and with Chinese Hamster V79 cells. The compound increased the numbers of chromatid breaks and exchanges in Chinese hamster ovary cells and induced SCE and delayed cell turnover time in human lymphocyte cultures. The compound given by intraperitoneal injection to mice was negative in the sperm-head abnormality test but was positive in the bone marrow assay.

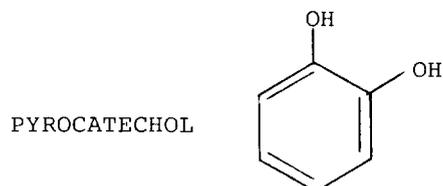
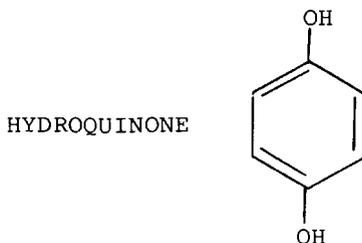
In three studies in mice, topically applied Pyrocatechol was not a tumor promotor. However, topically applied Pyrocatechol was a cocarcinogen for mouse skin in two other studies. Positive sensitization reactions to Hydroquinone were reported in 8.9% of 536 dermatologic patients. Two of 38 patients treated with an ointment containing 5.4% Hydroquinone became sensitized. A cosmetic formulation containing 2% Hydroquinone produced one or more mild irritation reactions in 69 of 90 subjects in the induction phase of a sensitization test; 22 of the 69 subjects were mildly sensitized when challenged. The use of ointments containing 2, 3, and 5% Hydroquinone produced at least minimal depigmentation in white but not black subjects. It is concluded that Hydroquinone and Pyrocatechol are safe for cosmetic use at concentrations of  $\leq 1.0\%$  in formulations that are designed for discontinuous, brief use followed by rinsing from the skin and hair.

## INTRODUCTION

Hydroquinone and Pyrocatechol are used in cosmetic products as couplers in oxidative hair dyes and colors. This presentation and evaluation of the published and unpublished safety data is directed toward the cosmetic product use of these two ingredients.

## CHEMICAL AND PHYSICAL PROPERTIES

Hydroquinone (CAS No. 123-31-9) and Pyrocatechol (CAS No. 120-80-9) are two benzenediol isomers, 1,4-benzenediol and 1,2-benzenediol. Throughout this report the ingredient name Pyrocatechol will be used instead of the current preferred name of catechol. Pyrocatechol is the recognized name to be used for ingredient labeling on cosmetic products for catechol. The chemical structures of these compounds are as follows<sup>(1)</sup>:



Hydroquinone is composed of colorless crystals that sublime. Solutions turn brown in air due to oxidation; this occurs rapidly in alkali. Hydroquinone is soluble in water, alcohol, ether, acetone, and carbon tetrachloride and is slightly soluble in benzene at room temperature.

Pyrocatechol is a colorless to white crystalline solid with a phenolic odor and taste. The crystals discolor in air and light. They sublime and are volatile with steam. Pyrocatechol is soluble in water, alcohol, ether, acetone, aqueous alkalis, carbon tetrachloride, benzene, and chloroform. Aqueous solutions soon turn brown.<sup>(2-5)</sup> The melting range of Hydroquinone is 169 to 171°C; upon ignition a maximum residue of 0.07% remains. Pyrocatechol is listed in two grades. The assay for the CP Grade is 99.1% and for the Resublimed Grade at least 99.7%. The freezing points are 103.6°C and 103.9°C, respectively, for the two grades. Each has a maximum residue on ignition of 0.05%.<sup>(6)</sup> A summary of the physical properties of the two benzenediols is presented in Table 1.

Hydroquinone and Pyrocatechol undergo chemical reactions typical of phenols and may give rise to ethers and monoesters and diesters. Ring substitutions may be made by halogenation, sulfonation, alkylation, nitration, Kolbe and Reimer-Tiemann reactions, Friedal-Crafts acylation, and condensation with aldehydes, esters, or ketones. The benzenediols may take part in reactions leading to multiring systems.<sup>(2,7)</sup>

Pyrocatechol has the highest redox potential of the three benzenediols. Hydroquinone is a widely used organic reducing agent.<sup>(2)</sup> Quinones result from the oxidation of Hydroquinone and catechol.<sup>(8)</sup> Both benzenediols can act as antioxidants.<sup>(2)</sup>

In aqueous solution and in the presence of oxygen, Hydroquinone undergoes autoxidation at a rate depending on the solution pH; autoxidation increases with increasing pH. The initial products are 1,2-quinone and hydrogen peroxide; the quinone has a positive catalytic effect upon the reaction. Continued oxidation gives condensation products with quinoid structures that account for the dark color of old solutions of Hydroquinone.<sup>(2)</sup> At pHs of 7-9, Hydroquinone readily autoxidizes; the autoxidation of Pyrocatechol occurs only to a limited extent.<sup>(9)</sup>

Several studies have explored the effects of the benzenediols on amine N-nitrosation. Hydroquinone and Pyrocatechol at concentrations of 0.06 M were added to an aqueous solution (pH of 4.05 and at a temperature of 37°C) of diethylamine and sodium nitrite at concentrations of 0.5 M and 0.016 M, respectively. Hydroquinone and Pyrocatechol inhibited the formation of N-nitrosodiethylamine by the consumption of nitrite in their oxidation to quinone. Under alkaline conditions (pH 11.0) N-nitrosodiethylamine was formed in the presence of Hydroquinone and Pyrocatechol.<sup>(10,11)</sup> In an acidic solution (pH 3-4) at 37°C containing concentrations of 0.5 M diethylamine and 0.2 M sodium nitrite, Pyrocatechol at concentrations of 0.005-0.050 M inhibited the nitrosation reaction; smaller concentrations of catechol had no effect on the reaction.<sup>(12)</sup> In a solution at a pH of 3.0 and at a temperature of 37°C that contained 0.010 M sodium nitrite and 0.001 M dimethylamine, diethylamine, pyrrolidine, and piperidine, a 0.001 M concentration of Pyrocatechol inhibited the formation of the respective nitrosamines.<sup>(13,14)</sup>

Qualitative and quantitative determinations of Hydroquinone and catechol

TABLE 1. Physical Properties

	<i>Hydroquinone</i>	<i>Pyrocatechol</i>	<i>Reference</i>
Formula	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	4
Molecular weight	110.11	110.11	4
Specific gravity at:			
15/4°C		1.371	2
—	1.324–1.328		2
15°C/		1.341	3
20/4°C	1.358		3
—			
15°C/	1.328		4
21°C/		1.1493	4
—		1.344	5
/15°C	1.332		5
Melting point (°C)	166	104	2
	170.5	105	3
Stable			
Unstable			
	173–174	105	4
	170–171	105	5
Boiling point (°C) at:			
—		245	2
—	286.2	246	3
760 mm Hg	285–287	245.5	5
750 mm Hg		245	4
730 mm Hg	285		2
730 mm Hg	285		4
400 mm Hg		221.5	5
200 mm Hg		197.7	5
100 mm Hg		176	5
60 mm Hg		161.7	5
40 mm Hg		150.6	5
20 mm Hg		134	5
10 mm Hg		118.3	5
5 mm Hg		104	5
Vapor pressure (mm Hg) at:			
118.3°C		10	3
132.4°C	1		3

are made by colorimetric methods, gravimetric procedures,<sup>(2,15)</sup> differential calorimetry,<sup>(16)</sup> spectrophotometric methods including mixed color photometry,<sup>(2,17,18)</sup> atomic absorption spectroscopy,<sup>(19)</sup> ultraviolet spectroscopy,<sup>(20)</sup> a ring-oven technique,<sup>(21)</sup> colorimetric analysis,<sup>(22)</sup> titrimetric procedures including oxidimetry, iodometry, and potentiometry, paper chromatography,<sup>(2,17)</sup> thin-layer chromatography,<sup>(17,23)</sup> gel chromatography,<sup>(2,20,24)</sup> high-pressure liquid chromatography,<sup>(25,26)</sup> gas chromatography,<sup>(2,20,27,28)</sup> gas-liquid chromatography,<sup>(29)</sup> and mass spectrometry,<sup>(15,20,30)</sup> and a field-ionization mass-spectrometric method.<sup>(15)</sup>

Positive identification of the benzenediols can be made by comparison with

published infrared and ultraviolet absorption spectra, nuclear magnetic resonance, mass and Raman spectra, and x-ray diffraction patterns.<sup>(2,4,17)</sup>

Hydroquinone has been found in cigarette smoke (up to 80  $\mu\text{g}/\text{cigarette}$ )<sup>(2,20,24)</sup> and in effluents resulting from the production of coal tar chemicals.<sup>(17)</sup>

Pyrocatechol has been found in cigarette smoke (80–300  $\mu\text{g}/\text{cigarette}$ ),<sup>(2,20,24,27,30)</sup> in onions, in crude beet sugar, in crude wood tar, in waters from bituminous shale, in coal,<sup>(2)</sup> in effluents resulting from the production of coal tar chemicals,<sup>(17)</sup> and in lignin, wood, and other plant materials.<sup>(2,31)</sup>

Although a variety of methods had been developed for the production of the benzenediols, only a few methods are commonly used in their commercial manufacture. Hydroquinone is produced by the oxidation of aniline with manganese dioxide and sulfuric acid, followed by reduction with iron dust and water. Also, it may be produced by the alkylation of benzene with propylene to produce a mixture of di-isopropylbenzene isomers, followed by the isolation of the *p*-isomer which is oxidized with oxygen to produce the corresponding dihydroperoxide and treated with acid to produce acetone and Hydroquinone. Pyrocatechol is produced by the alkaline fusion of *o*-chlorophenol, by recovery from the lignin-containing wastes of wood pulping operations, or by the oxidation of benzene with hydrogen peroxide.<sup>(2,17)</sup>

The Cosmetic, Toiletry and Fragrance Association (CTFA) Standards<sup>(6)</sup> for Pyrocatechol require it to assay a minimum of 99% and to ash a maximum of 0.05%. The Japan Cosmetic Industry Association standards require a minimum of 98% Pyrocatechol.<sup>(32)</sup>

## USE

### Cosmetic

Hydroquinone and Pyrocatechol are used in cosmetics largely as couplers in oxidative hair dyeing where the colored material is produced inside the hair fiber by oxidation of colorless intermediates.<sup>(33)</sup> Hydroquinone may also be used in some skin care products.<sup>(34)</sup>

Product types and the number of formulations containing Hydroquinone and Pyrocatechol reported voluntarily to the Food and Drug Administration in 1981 are presented in Table 2. Voluntary filing of this information by cosmetic manufacturers, packagers, and distributors conforms to the prescribed format of preset concentration ranges and product types as described in the Code of Federal Regulations (21 CFR 720.4). Some cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, and, therefore, the value reported by the cosmetic formulator or manufacturer may not necessarily reflect the true concentration of the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the 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 2- to 10-fold overestimation in the assumed ingredient concentration. In 1981, Hydroquinone was an ingredient of 147 hair dyes

TABLE 2. Product Formulation Data<sup>(34)</sup>

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)			
			Unreported concentration	>1-5	>0.1-1	≤0.1
<i>Hydroquinone</i>						
Hair dyes and colors	811	147	—	—	73	74
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	1	—	1	—	—
Skin lighteners	44	21 <sup>a</sup>	—	19	2	—
Other skin care preparations	349	1	—	1	—	—
1981 TOTALS		170	—	21	75	74
<i>Pyrocatechol</i>						
Hair dyes and colors	811	36	—	—	16	20
Hair tints	15	4	—	—	—	4
1981 TOTALS		40	—	—	16	24

<sup>a</sup>FDA has issued a Notice of Proposed Rule-making for the use of Hydroquinone as a skin bleacher in drugs at concentrations between 1.5 and 2.0%.<sup>(36)</sup>

and color preparations and 23 skin care products, including products intended for medical use as skin lighteners. The concentration of use ranges from ≤0.1% to between 1 and 5%. Pyrocatechol was reported to be an ingredient of 40 cosmetic formulations at concentrations ranging from ≤0.1% to 1%.<sup>(34)</sup>

Hydroquinone and Pyrocatechol are primarily ingredients in hair dyes and colors, although they are also used in other hair and skin products. Cosmetic formulations containing the benzenediols may be applied to the hair and may come in contact with the skin, eyes, hair, and nails (Table 2).<sup>(34)</sup>

Hydroquinone has been evaluated in the FDA over-the-counter (OTC) drug review program. FDA has issued a Notice of Proposed Rule making for the safe and effective use of Hydroquinone as a skin bleacher in drug products with concentrations between 1.5 and 2.0%.<sup>(36)</sup>

### Medical Use

Hydroquinone has been used by dermatologists as a depigmenting agent since 1961. Previously, it had been discovered that a sunscreen containing Hydroquinone was being purchased and used as a bleaching agent rather than as a sunscreen.<sup>(37)</sup> Hydroquinone is used in products designed to lighten small areas of hyperpigmented skin; it is used in the treatment of melasma (chloasma), freckles, senile lentigines, and postinflammatory hyperpigmentation. In addition, products containing Hydroquinone are sometimes used to lighten facial skin. Avoidance of sunlight exposure is necessary for successful skin lightening.<sup>(38-40)</sup>

Pyrocatechol has been used in antiseptic solutions and ointments for application to wounds and burns and in other medicines<sup>(2)</sup> but is no longer used for these purposes.<sup>(8)</sup>

### Industrial Use

Hydroquinone is listed in the Code of Federal Regulations as an indirect food additive. There are no concentration limits for its use in adhesives used as components of articles intended for use in packaging, transporting, or holding food or for its use as an optional adjuvant substance/inhibitor in cross-linked polyester resins used as articles or components of articles intended for repeated use in contact with food. Hydroquinone, at no specific concentration limits, may be used as an inhibitor for monomers in the coated or uncoated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packaging, processing, preparing, treating, packing, transporting, or holding aqueous and fatty foods.<sup>(35)</sup>

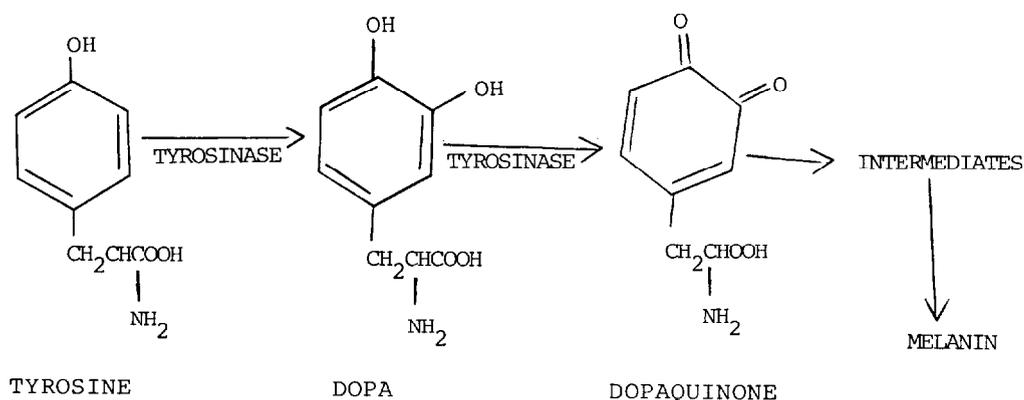
Hydroquinone is used as a laboratory reagent, as a chemical intermediate, particularly in rubber processing chemicals, as a photographic developer, as an antioxidant and polymerization inhibitor, and in pharmaceuticals and dyes.<sup>(2,5,17)</sup>

Pyrocatechol is used as an analytical agent, as an antioxidant, in polymerization inhibitors, in tanning, in photography, in the synthesis of pharmaceuticals and pesticides, in dyes, and in the rubber and metal plating industries.<sup>(2,5,17)</sup>

## GENERAL BIOLOGY

### Hydroquinone

Hydroquinone affects melanogenesis in vitro and in vivo. A particular reaction of interest is the conversion of tyrosine to melanin as follows<sup>(41)</sup>:



Denton et al.<sup>(42)</sup> used tyrosinase from mouse melanomas and determined that Hydroquinone, at 12 times the molar concentration of tyrosine (exact concentrations unspecified), completely inhibited the reaction of conversion of tyrosine to

DOPA. No inhibition was observed when DOPA was the substrate. Iijima and Watanabe<sup>(43)</sup> reported that  $1.7 \times 10^{-7}$  to  $1.7 \times 10^{-4}$  M Hydroquinone slightly inhibited and  $1.7 \times 10^{-3}$  M Hydroquinone completely inhibited the DOPA reaction in human epidermis that had been surgically removed from the axillary region for the treatment of osmidrosis.

Two tyrosinases were obtained from human melanoma cells. A concentration of  $1 \times 10^{-3}$  M Hydroquinone inhibited the tyrosinases, but that concentration was toxic to melanocytes in tissue culture.<sup>(44)</sup> Hydroquinone, at a concentration of 0.625  $\mu\text{g}/\text{ml}$ , inhibited the growth of B16 mouse melanoma cells. Pigmented cells were particularly sensitive, and treated cultures contained only a few pigmented cells (in contrast to the controls). Growth was completely inhibited at concentrations of 1.25–2.5  $\mu\text{g}/\text{ml}$ . A concentration of 1  $\mu\text{g}/\text{ml}$  Hydroquinone had no effect on melanin granule movement; 2.5  $\mu\text{g}/\text{ml}$  induced cessation of granule movement, vacuolization of the cytoplasm, and clumping of melanin granules. Concentrations of 20–40  $\mu\text{g}/\text{ml}$  Hydroquinone abruptly stopped granule movement in the same way as a fixative.<sup>(45)</sup> Pawelek et al.<sup>(46)</sup> investigated the effect of Hydroquinone (concentration unspecified) on five cell lines: nonpigmented Vero green monkey kidney cells, an amelanotic mouse melanoma (Cloudman S-91), two melanotic mouse melanomas (Cloudman S-91), and a melanotic hamster melanoma (Greene). Hydroquinone was highly toxic to all the cell lines and was not selectively toxic to melanotic cells. The *in vitro* actions of Hydroquinone alone or in combination with beta-mercaptoethanolamine on B-16, Cloudman S-91, and Harding-Passey murine melanomas grown *in vivo* have been investigated. The cells were incubated with the chemicals for 5 to 240 or 480 minutes at 37°C. Hydroquinone, at a concentration of 100  $\mu\text{g}/\text{ml}$ , inhibited B-16 and stimulated S-91 tyrosinase activity. In Harding-Passey cells, Hydroquinone at first stimulated and then inhibited tyrosinase activity. Except for S-91 in which the cGMP content was not unaltered, cAMP and cGMP values in the other cell lines were elevated after exposure to Hydroquinone. Hydroquinone did not affect peroxidase but inhibited DNA and RNA synthesis in all three cell lines and inhibited protein synthesis in the S-91 and Harding-Passey cells. Hydroquinone in combination with beta-mercaptoethanolamine did not generally affect the cells in the same way that Hydroquinone alone did.<sup>(47–50)</sup> Recent studies have suggested that the depigmenting effect of Hydroquinone is the result of a selective action on melanocyte metabolism instead of a specific effect on melanin synthesis.<sup>(51)</sup>

Protein biosynthesis by organ cultures of pig skin was measured by the uptake of arginine and tyrosine; the skin degenerated after 3 days. A 10-mM concentration of Hydroquinone caused marked degeneration of the skin after 1 day and almost complete inhibition of amino acid incorporation into black and white pig skin.<sup>(52)</sup>

Hydroquinone produced depigmentation in the skin of the guinea pig, man, mouse, rat, cat, and goldfish.<sup>(53)</sup> In one study guinea pigs received topical application of creams containing 2% and 5% Hydroquinone in an oil-in-water base 6 days a week for 3 weeks or subcutaneous injection of 2 ml of a 1% Hydroquinone in normal saline solution daily for 8 days. It was observed that Hydroquinone affected the nonfollicular and follicular melanocyte system and that it had no effect on keratinocytes. In areas depigmented by Hydroquinone, the changes

were decreased formation, marked alteration of internal structure, and increased degradation of melanosomes. The membranous organelles in the melanocytes were destroyed. Hydroquinone eventually caused necrosis of melanocytes.<sup>(54)</sup>

Hydroquinone ointments of 2, 3, and 5% were applied to the senile lentiginos of a white man. In depigmented areas, the observations included perivascular infiltrates with less pigment in areas of inflammation, less melanin present in supranuclear caps, greater dispersion of melanin, some alterations in melanin granules, approximately the same number of melanocytes, fewer melanin granules, and melanocytic melanin production halved.<sup>(55)</sup>

Kligman and Willis<sup>(56)</sup> tested an ointment containing 5.0% Hydroquinone, 0.1% tretinoin, and 0.1% dexamethasone on black human skin. In depigmented areas irritant reactions consisted of an increase in perivascular monocyctic cells; dopa-positive melanocytes were increased and were larger and more active enzymically; epidermal melanin granules were fewer and supranuclear caps were absent.

This formulation did not affect melanin granules already in the dermis but affected the synthesis and transfer of melanin. Sun exposure after treatment resulted in rapid repigmentation, with pigmentation even greater than before.<sup>(57)</sup> Findlay<sup>(58)</sup> described several cases in which melanocytes overcame the bleaching effect of Hydroquinone. The prolonged use and thorough inunction of Hydroquinone bleaching creams on black facial skin followed by sun exposure can eventually lead to ochronosis and colloid milium production; the colloid milium were mostly darker than normal skin.

After exposure to Hydroquinone (0.5 to 3 mM) human erythrocytes were depleted of reduced glutathione, and methemoglobin was produced. No Heinz bodies were produced and hemolysis was not observed.<sup>(59,60)</sup> It has been reported that Hydroquinone forms Heinz bodies in animals<sup>(61)</sup> and that Hydroquinone can cause methemoglobinemia in animals.<sup>(62)</sup>

### Pyrocatechol

Pyrocatechol has antiseptic properties; its phenol coefficient (the ratio of the dilution of Pyrocatechol to the dilution of phenol required to kill a particular strain of a definite organism) is 0.87 for *Salmonella typhosa* and 0.58 for *Staphylococcus aureus*.<sup>(8)</sup>

## ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

### Hydroquinone

Hydroquinone is absorbed through the gastrointestinal tract and possibly through skin and excreted in the urine as free Hydroquinone and quinone or conjugated with glucuronic, sulfuric, and hexuronic acids.<sup>(62)</sup>

Hydroquinone was "weakly" absorbed by hairless rat skin in vitro and in vivo and by healthy human skin in vitro.<sup>(63)</sup>

Radioactive Hydroquinone, at doses of 1.3–14 mg/kg, was injected into the lateral tail vein of rats and the rats were killed 2 h later. Radioactivity was found

in bone marrow, in the white pulp of the spleen, and in the thymus. Some radioactivity was also distributed in subcutaneous tissues, in sebaceous glands, in brown fat, and in the white matter of the brain and spinal cord.<sup>(64)</sup> In another experiment, the lateral tail vein of rats was injected with 14 mg/kg of radioactive Hydroquinone, and some of the rats were killed at 2 h and the others were killed at 24 h. The liver, thymus, and bone marrow were homogenized and examined for radioactivity. The concentration of acid-soluble radioactivity decreased in the liver and thymus and remained approximately the same in the bone marrow between 2 and 24 h. The concentration of covalently bound radioactivity in the liver, thymus, and bone marrow increased between 2 and 24 h; the greatest increase was in the bone marrow. The researchers suggested that the autoxidation of Hydroquinone would result in the formation of semiquinone oxidation products and superoxide radicals, which may be cytotoxic.<sup>(65)</sup>

Rabbits were given 100–230 mg/kg Hydroquinone orally and the 24-h urine was collected. The urine contained (as a percentage of dose) 30% Hydroquinone ethereal monosulfate, 43% Hydroquinone monoglucuronide, and trace amounts of free Hydroquinone (0.065%). There was no evidence of the oxidation of Hydroquinone to any trihydroxybenzenes.<sup>(66)</sup> In rabbits given Hydroquinone orally, the rate of glucuronide conjugation was proportional to the body concentration of Hydroquinone. The rate of organic sulfate formation remained approximately constant until almost the whole dose was excreted.<sup>(67)</sup> Hydroquinone administered orally to rats at a dose of 0.09 g/kg increased the excretion of organic sulfates but, contrary to other reports, had no effect on glucuronic acid excretion.<sup>(68)</sup>

Radioactive Hydroquinone was administered iv at a dose of 20 mg/kg to cats. The components in the urine were approximately 10% unchanged Hydroquinone, approximately 87% Hydroquinone sulfate, and approximately 3% glucuronide. The researchers suggested that some Hydroquinone might be metabolized to quinone.<sup>(69)</sup>

A volunteer received 200 mg (3 mg/kg) Hydroquinone orally. The composite 24-h urine contained no free Hydroquinone, but Hydroquinone was excreted as the ethereal sulfate and as the glucuronate.<sup>(26)</sup>

Hydroquinone was detoxified by the liver.<sup>(70)</sup> The results of a study with microsomes from rat liver indicated that *p*-benzosemiquinone and/or *p*-benzoquinone was formed from benzene via Hydroquinone; these compounds bind covalently to macromolecules.<sup>(71)</sup>

### Pyrocatechol

Pyrocatechol was absorbed readily from the gastrointestinal tract and through the intact skin of mice. After absorption, some of the Pyrocatechol was oxidized by polyphenol oxidase with the formation of *o*-benzoquinone. Some Pyrocatechol conjugated with hexuronic, sulfuric, and glucuronic acids and the conjugates were excreted in the urine. A small amount of free Pyrocatechol was also excreted in the urine. Pyrocatechol may also be methylated by catechol-*o*-methyltransferase.<sup>(8,62)</sup>

Radioactive Pyrocatechol, at doses of 1.2–12 mg/kg, was injected into the lateral tail vein of rats, and the rats were killed 2 h postinjection. Radioactivity

was found in the bone marrow, the spleen, and the thymus. Some radioactivity was also distributed in subcutaneous tissue, in sebaceous glands, in brown fat, and in the white matter of the brain and spinal cord.<sup>(64)</sup> In another experiment, 14 mg/kg of radioactive Pyrocatechol was injected via the lateral tail vein into rats. Some of the rats were killed at 2 h, and those remaining were killed at 24 h. The liver, thymus, and bone marrow were homogenized and examined for radioactivity. The concentration of acid-soluble radioactivity decreased in the liver and thymus and remained the same in the bone marrow between 2 and 24 h postinjection. The concentration of covalently bound radioactivity in the liver and bone marrow was increased and in the thymus remained approximately the same between 2 and 24 h; the greatest increase was in the bone marrow. The researchers suggested that Pyrocatechol may be metabolized to cytotoxic products.<sup>(65)</sup>

Mice were exposed for 10 minutes to diluted smoke of cigarettes containing radioactive Pyrocatechol. The deposition and distribution of inhaled Pyrocatechol was determined in certain internal tissues, urine, and feces for up to 2 h after exposure. Immediately after exposure, 56% of the radioactivity (in the total body) was in the blood, 14% was in the kidneys, 13% was in the liver, and 8% was in the lungs. The blood contained the greatest percentage of radioactivity at all times (measured up to 2 h after exposure). The radioactivity decreased over time in all tissues; 2 h after exposure, approximately 11% of the radioactivity remained in the body. In another experiment, urine and feces were collected; 91.2% of the radioactivity (in the total body) was excreted in the urine, and 1.5% of the radioactivity was excreted in the feces within 2 h after exposure. Less than 1% of the radioactivity remained in the lungs, turbinate, liver, or kidneys 2 h after exposure.<sup>(72)</sup>

Rabbits were given Pyrocatechol orally, and the urine was collected over 24 h. The urine contained (as a percentage of dose) 18% catechol ethereal sulfate, 70% catechol monoglucuronide, and 2% free Pyrocatechol. The urine also contained traces of hydroxyquinol as an ethereal sulfate; apparently, there was further *in vivo* oxidation of Pyrocatechol.<sup>(66)</sup> Pyrocatechol was administered orally to rats in a dose of 0.06 g/kg, and urine was collected over the next 3 days. Pyrocatechol had little or no effect on the excretion of glucuronic acid or organic sulfate.<sup>(68)</sup>

Radioactive Pyrocatechol was infused at a rate of  $5 \times 10^{-8}$  mol/min into the renal artery of dogs, and ureteral urine collections were made during infusion. The sulfate fraction of the urine contained 60–70% of the radioactivity; only trace amounts of glucuronide and free Pyrocatechol were detected.<sup>(73)</sup>

Hirosawa et al.<sup>(74)</sup> used a color test to examine the urine of workers exposed to workroom air in which the concentration of Pyrocatechol varied with time and the specific environment. Twenty-four-hour urine samples were examined after 7–9 h of exposure to air polluted with catechol and phenol. Twenty-four-hour urine collections taken from the same workers during a 1-month period in unpolluted air served as controls. Average Pyrocatechol values for 24-h urine samples of 6 workers were 24.2 mg upon catechol exposure and 19.2 mg upon exposure to the unpolluted control environment. The differences were insignificant; however, exposure of an individual to air polluted with Pyrocatechol and phenol caused a temporary increase of the urinary excretion by Pyrocatechol;

the urinary Pyrocatechol was measured several times over 24 h. The calculated biological half-life of inhaled Pyrocatechol in humans was 3–7 h. Pyrocatechol inhibited catechol-O-methyltransferase but did not affect catecholamine metabolism; urinary excretion of catecholamines and their metabolites (epinephrine, norepinephrine, metanephrine, and vanilylmandelic acid) was within the normal range (for adult, Japanese males) except for a slight decrease in norepinephrine. The 24-h urine of nonsmokers and of cigarette smokers who were on the same restricted diet, was analyzed for Pyrocatechol. The nonsmokers excreted  $4.4 \pm 1.2$  mg and the cigarette smokers  $6.8 \pm 3.0$  mg of Pyrocatechol. This finding indicates that diet was a major factor in determining urinary catechol concentrations.<sup>(75)</sup>

Catechol-O-methyltransferase (COMT) catalyzed the transfer of a methyl group from S-adenosylmethionine to a Pyrocatechol substrate with the formation of O-methylated products. COMT activity has been found in many mammalian tissues; it is involved in the extraneuronal inactivation of endogenous catecholamines, in the further metabolism of oxidized catecholamine metabolites, and in the detoxification of Pyrocatechol drugs.<sup>(76)</sup> Pyrocatechol was a substrate for rat liver COMT, and it competitively inhibited the enzyme's activity with other substrates.<sup>(77)</sup>

## ANIMAL TOXICOLOGY

### Oral Studies

#### Acute Toxicity

The acute oral toxicity of Hydroquinone has been studied in rats,<sup>(78–82)</sup> mice, guinea pigs,<sup>(81,82)</sup> rabbits,<sup>(62)</sup> dogs,<sup>(79,81,82)</sup> cats,<sup>(62,79,81,82)</sup> and swine<sup>(83)</sup> (Table 3). The LD<sub>50</sub> values for rats ranged from 0.1 to 1.3 g/kg; LD<sub>50</sub>s were lower in fasted rats. In the Hodge and Sterner<sup>(84)</sup> classification of single-dose oral toxicity for rats, Hydroquinone would be classified as practically nontoxic to moderately toxic.

The acute oral toxicity of Pyrocatechol has been studied in rats,<sup>(7,82)</sup> mice, guinea pigs,<sup>(82)</sup> dogs,<sup>(2,62)</sup> rabbits, cats, and pigs.<sup>(62)</sup> The LD<sub>50</sub> values for rats were 0.3 g/kg in one experiment and 0.26 g/kg in another experiment. In the Hodge and Sterner<sup>(84)</sup> classification of single-dose oral toxicity for rats, Pyrocatechol would be classified as moderately toxic.

#### Subchronic and Chronic Toxicity

The subchronic and chronic toxicity of Hydroquinone has been studied in rats,<sup>(79,82,85)</sup> mice,<sup>(42,86)</sup> guinea pigs,<sup>(42)</sup> and dogs<sup>(79)</sup> (Table 4). Concentrations of up to 1.0% Hydroquinone in the diet of rats for 2 years were not toxic. At 5% in the diet for 9 weeks, signs of toxicity were seen. An oral dose of 500 mg/kg given 101 times within 151 days resulted in the death of half of the test rats within 2 months; 1750 mg/kg given orally 9 times within 12 days resulted in 71% mortality within 24 h of the last exposure. A dose of 262 mg/kg per day Hydroquinone in the drinking water or a concentration of 100 mg/kg in the diet of mice resulted in some fur depigmentation (amount not stated). An oral dose of 88 mg/kg per

day to guinea pigs was not toxic. Dogs were fed up to 40 mg/kg per day Hydroquinone in their feed for 50 weeks, 16 mg/kg per day for 80 weeks, or 100 mg/kg per day for 26 weeks; no toxicity was observed.

The chronic toxicity of Pyrocatechol has been studied in rats<sup>(8)</sup> and in mice.<sup>(87)</sup> The results of these studies as well as those for Hydroquinone are summarized in Table 4. Rats fed up to 1.0% Pyrocatechol in their diets for 2 years had the "beginning" of hepatic cell hyperplasia. Mortality rates were similar to the control rats. Mice that received up to 0.4% Hydroquinone in their drinking water for 20 weeks had reduced body weight but no other signs of toxicity.

## Dermal Studies

### Acute Toxicity

Hydroquinone, as a 2% solution in dimethyl phthalate, was applied to the intact and abraded skin of rabbits in doses of 0.08 to 0.19 mg/kg. It was held in contact with the skin for 24 h with a rubber sleeve. No adverse local or systemic effects were observed.<sup>(88,89)</sup>

Pyrocatechol, in doses of 0.25, 0.50, 1.0, and 2.0 g/kg, was applied to the abraded and intact skin of 4 rabbits for each dose group for 24 h; the rabbits were observed for an additional 14 days. Deaths occurred in the three highest dose animals (1/4, 2/4, and 4/4). The acute dermal LD<sub>50</sub> of Pyrocatechol was 0.8 g/kg. Dead rabbits had subdermal hyperemia and edema. Pyrocatechol produced moderate erythema and slight edema in all surviving rabbits; necrosis was associated with compound application at abraded skin sites. Slight epidermal flaking persisted, and incrustations of dead tissue were sloughing at the end of the 14-day observation period. The body weight gains of survivors were less than the weight gains of the control rabbits. No lesions were observed at necropsy of the survivors.<sup>(7)</sup>

### Subchronic and Chronic Toxicity

The subchronic and chronic dermal toxicity of Hydroquinone has been studied in rabbits<sup>(88,89)</sup> and guinea pigs<sup>(54,90-92)</sup> (Table 5). Hydroquinone, at a concentration of 2% in dimethyl phthalate, was applied dermally daily for up to 90 days in doses of up to 0.08 mg/kg to rabbits; skin lesions and a slight thyroid hyperplasia were observed but no other toxic effects. Ultraviolet radiation for up to 21 days of this regimen increased the severity of the skin lesions.<sup>(88)</sup> Hydroquinone, at concentrations in ointments and creams from 1-10%, applied daily to black guinea pigs for 1 month produced weak depigmentation at 1% and moderate at 3%. It was an irritant above 3%.<sup>(90)</sup> The minimal time interval for Hydroquinone and Pyrocatechol to produce uniform depigmentation was dependent upon both concentration and suspending medium.<sup>(91)</sup> In one study, ointments containing 5-20% Hydroquinone were applied over 90% of the bodies of guinea pigs daily for 50 days; body weight gains were subnormal and the adrenals were enlarged.

A 13-week dermal toxicity study was conducted in rats with a cosmetic formulation containing 2% Hydroquinone.<sup>(93)</sup> The product was applied to 15 animals at a dose of 886 mg/kg, once daily, at a dorsal-anterior, shaved skin site.

TABLE 3. Acute Oral Toxicity

Material, concentration and vehicle tested	No. and species of animal	Method	LD <sub>50</sub> (g/kg)	Comments	Reference
Hydroquinone	Rats	—	0.78	LD <sub>100</sub> was 1.125 g/kg	78
Hydroquinone, in glycerin	6–40 per trial Priestly rats	Given by stomach tube Not fasted	1.30	LD <sub>50</sub> value was lower in fasted rats	79
glycerin	Priestly rats	Not fasted	1.00	LD <sub>50</sub> value was lower in fasted rats	79
propylene glycol	Sprague-Dawley rats	Not fasted	1.09	LD <sub>50</sub> value was lower in fasted rats	79
distilled water	Sprague-Dawley rats	Not fasted	1.18	LD <sub>50</sub> value was lower in fasted rats	79
glycerin	Sprague-Dawley rats	Not fasted	1.08	LD <sub>50</sub> value was lower in fasted rats	79
propylene glycol	Sprague-Dawley rats	Fasted (for 18 h prior to hydroquinone administration)	0.32	LD <sub>50</sub> value was lower in fasted rats	79
propylene glycol	Wistar rats	Not fasted	0.73	LD <sub>50</sub> value was lower in fasted rats	79
propylene glycol	Wistar rats	Fasted	0.30	LD <sub>50</sub> value was lower in fasted rats	79
Hydroquinone, in sugar-coated tablets	Dogs Cats	Given with small amount of meat	0.30 between 0.04 and 0.09	—	79
Hydroquinone, in water	Rabbits Cats	—	0.2 0.08	—	62
Hydroquinone	Rats	—	0.37–0.39	—	80
Hydroquinone	Rats Mice Guinea pigs Dogs Cats	—	0.32 0.4 0.55 0.2 0.07	Hyperexcitability, tremors, convulsions, salivation in dogs and cats, and emesis and incoordination of the hind legs in dogs occurred within 30–90 minutes; deaths occurred within a few hours; 0.1 g/kg in dogs and 0.07 g/kg in cats caused mild to severe swelling of the area around the eye, of the nictitating membrane, and of the upper lip; routine blood counts in dogs and cats indicated increased activity of the “cell-forming tissues”	81, 82

Hydroquinone, in saline	Young swine (feeder pigs)	Two groups of 2 given 0.18 and 0.35 g/kg by gavage; killed and necropsied 48 to 72 h later	–	No clinically adverse symptoms or lesions noted in the 0.175 g/kg pigs; both 0.35 g/kg pigs died; shaking, nausea, erythema, severe convulsions, and deaths occurred within 30 minutes of dosing; they were markedly hyperglycemic when comatose; isocitric dehydrogenase and serum glutamic oxaloacetic transaminase were slightly elevated; no gross or microscopic changes or bacterial isolates found	83
Pyrocatechol	Rabbits Dogs Cats Pigs	–	0.2 0.3 0.1 0.2	–	62
Pyrocatechol, crystalline	Rats	Four groups of 5 rats given 0.158–1.26 g/kg by stomach intubation; observed for 14 days	0.3	At doses of 0.63 and 1.26 g/kg, 5/5 died within 1 day and 1 h, respectively; dead rats had hyperemia of the stomach and intestines; survivor rats had no gross lesions and all but 1 had body weight gains similar to controls	7
Pyrocatechol	Rats Mice Guinea pigs	–	0.3 0.3 0.2	–	82
Pyrocatechol	Dogs	Given 0.03 and 0.05 g/kg	–	0.03 g/kg caused anemia and "other complications" followed by death in several weeks; 0.05 g/kg caused death within 48 h	2

**TABLE 4.** Subchronic and Chronic Oral Toxicity

<i>Material tested</i>	<i>Dose and vehicle</i>	<i>Length of study</i>	<i>No. and species of animal</i>	<i>Results</i>	<i>Reference</i>
Hydroquinone	0.003–0.3% in diet	10 days prior to insemination and 32–33 days after	Female rats	No maternal mortality	85
Hydroquinone	0–1.0% in diet	103 weeks	10 male and 10 female Sprague-Dawley rats of weaning age at each of 4 doses	Final body weights of treated rats were similar to controls; in first month, growth rate was slower in the 0.5 and 1.0% groups; hematological analyses and histopathology normal	
Hydroquinone, heated with lard to 190°C for 30 min	0–0.5% in diet	103 weeks	16–23 male and 16–23 female rats at each of 4 doses	Final body weights of treated rats were similar to controls; hematological analyses and histopathology normal	79
Hydroquinone	0–1.0% in diet, with 0.1% citric acid	103 weeks	20 male and 20 female rats at each of 4 doses	Final body weights of female rats and 0.5% male rats were similar to controls; slightly lower final weights observed in 0.1 and 1.0% male rats; in first month, growth rate was slower in the 0.5 and 1.0% groups; hematological analyses and histopathology normal	79
Hydroquinone	5% in diet	9 weeks	14 adult rats (14 controls)	Treated rats had a 46% loss in weight; aplastic anemia, average of 66% decrease in bone marrow cellularity with marked atrophy of the hematopoietic elements, atrophy of liver cord cells, splenic lymphoid tissue, adipose tissue, and striated muscle, and superficial ulceration and hemorrhage of stomach mucosa	79

Hydroquinone	500-1750 mg/kg by stomach tube	9 administrations in 12 days	20-48 rats at each of 6 doses	71% of total mortality rate occurred within 24 h of first dose; for next 11 days, average mortality rate was less than 5%/day	79
Hydroquinone	500 mg/kg by stomach tube	101 administrations in 151 days	16 rats	More than half died in first 2 months (no data given); autopsy findings were negative; survivors grew at same rate as controls	79
Hydroquinone, in sugar-coated tablets	16 mg/kg per day in feed	80 weeks	14-month-old dog [2 controls]	Treated dogs grew normally; negative necropsy findings, urinalyses, hematological analyses, pathology; hemosiderosis was usually more marked in spleens, livers, and marrow of controls	79
Hydroquinone, in sugar-coated tablets	1.6 and 40 mg/kg per day in feed	First dose for 31 weeks, second dose for 49 weeks	2 4-month-old dogs		
	100 mg/kg per day in feed	26 weeks	5 adult dogs	Dogs maintained their weight; negative necropsy findings, urinalyses, hematological analyses, pathology	
Hydroquinone, in capsules	Increasing doses: 22-88 mg/kg per day (total dose of 2.38 g)	76 days	5 adult male guinea pigs (5 controls)	No toxic effects; one treated guinea pig showed "questionable depigmentation"	42
Hydroquinone	Increasing doses: 37-262 mg/kg per day (total dose of 247 mg) in drinking water	76 days	4 adult black C57 male mice (control group)	"Questionable pigmentary change" was observed; 2/4 developed alopecia on back of neck	42
Hydroquinone	Increasing doses: 37-262 mg/kg per day (total dose of 247 mg) in drinking water	76 days	4 7-week-old black C57 male mice (control group)	"General" pigmentation was observed in 2/4; all 4 developed alopecia on back of neck	42

**TABLE 4.** (Continued)

<i>Material tested</i>	<i>Dose and vehicle</i>	<i>Length of study</i>	<i>No. and species of animal</i>	<i>Results</i>	<i>Reference</i>
Hydroquinone	0.125–2.0% in diet	2 years	Groups of 12–18 rats	Similar to controls in number of live rats at experiment end	82
Hydroquinone	100 mg/kg of diet	20 weeks	Young mice	Most (number unspecified) of mice had depigmentation of fur within 4 to 20 weeks	86
Pyrocatechol	0.0625–1.0% in diet	2 years	Groups of 12–18 rats	Similar to controls in number of live rats at experiment end; at 0.25% there was “beginning” hepatic cell hyperplasia	82
Pyrocatechol	0.1–4.0 g/l in drinking water	20 weeks	Mice	No adverse effects at 0.1 g/l; at 4.0 g/l, 55% decrease in body weight; some organ weights increased but blood cell counts, bone marrow cell numbers, and spleen colony-forming units were “almost” unchanged	87

**TABLE 5.** Subchronic and Chronic Dermal Toxicity

<i>Material tested</i>	<i>No. and species of animal</i>	<i>Length of study</i>	<i>Dose of material</i>	<i>Method</i>	<i>Results</i>	<i>Reference</i>
Hydroquinone, 1–10% in creams	8 black guinea pigs	1 month	–	Applied daily 5 times a week to unepilated skin of ear and wax epilated skin of back	Nonirritant up to 3%, irritant above 5%; weak depigmentation at 1%; moderate at 3% and above	90
Hydroquinone, 2% in dimethyl phthalate	Rabbits	21 days (and 14 days further observation)	1.0–4.0 ml/kg	Applied daily by inunction; Also applied two times a week after initial UV irradiation (General Electric Uviarc portable AC ultraviolet light with an arc length of 6 inches and giving 54 W per arc inch) for 20 minutes at distance of 6 inches; over 21 days, exposure period was 10 minutes	Atypical dermatoses that were aggravated by UV appeared at first as small petechiae and observed occasionally in untreated areas; no edema; no gross local or systemic effects (separate testing without UV indicated no evidence of sensitization)	88, 89
Hydroquinone, 2% in dimethyl phthalate	3 groups of 4 rabbits	90 days	0.5–4.0 ml/kg	Applied daily by inunction	Atypical dermatoses appeared and disappeared over 90 days; initial reaction petechiae, then intense erythema; no gross local or systemic effects at 0.5 and 1.0 ml/kg; at 2.0 and 4.0 ml/kg subnormal body weight gains and lowered food intake; slight emaciation; at microscopic examination, moderate dermatitis and slight thyroid hyperplasia	88, 89
Hydroquinone, 0.5 M in dimethyl sulfoxide and 5% in hydrophilic ointment	Groups of 2–5 black guinea pigs	6 months	0.1 ml	Applied daily to 8 epilated dorsal sites and the unepilated skin of nipples	Weak to moderate depigmentation; moderately to severely irritating	91

TABLE 5. (Continued)

<i>Material tested</i>	<i>No. and species of animal</i>	<i>Length of study</i>	<i>Dose of material</i>	<i>Method</i>	<i>Results</i>	<i>Reference</i>
Creams containing 2 and 5% hydroquinone in an oil-in-water emulsion	Groups of 5 guinea pigs	3 weeks	—	Applied daily 6 days a week to epilated backs	Depigmentation visible within 8–10 days and was maximum between 14 and 20 days; no total depigmentation; inflammatory changes and thickening of the epidermis; desquamation was prominent and often seen within 1 week; 5% caused more marked depigmentation and scaling than 2%	54
5–20% hydroquinone ointment (petrolatum vehicle)	30 guinea pigs (5 controls)	50 days	—	Applied to 15–90% epilated body surface daily	Hydroquinone eliminated in the urine; body weight gains less than controls except when 15% of body surface smeared; number of lymphocytes in blood dropped, adrenals enlarged, focal fibrosis found in the myocardium of some of the guinea pigs	92
Pyrocatechol, 1–10% in creams	11 black guinea pigs	1 month	—	Applied daily 5 times a week to unepilated skin of ear and wax epilated skin of back	No depigmentation or irritation at 1%; definite weak irritant and depigmenter at 3%; definite moderate at 5%; very strong depigmenter and irritating at 7–10%	90
Pyrocatechol, 5–10% in hydrophilic ointment	Groups of 2–5 black guinea pigs	6 month	0.1 ml	Applied daily to 8 epilated dorsal sites and the unepilated skin of ears and nipples	Moderate depigmentation; moderately to severely irritating	91

A minimal to moderate skin irritation as well as a brown discoloration were observed at the application site for most animals throughout the study. Results of hematological and clinical chemistry determinations, urinalysis, and gross and microscopic examination of necropsy specimens were negative for compound effects.

### Primary Irritation

In a preliminary screening study to establish the concentration of Hydroquinone to be used in a photoallergic study, an aqueous solution of Hydroquinone was slightly irritating at 10% but not at 5.0, 1.0, or 0.5% when tested on 8 guinea pigs.<sup>(94)</sup>

A product formulation containing 2% Hydroquinone was tested on 9 rabbits using a single-insult occlusive test patch procedure. The average irritation score was 1.22 (scale 0–4).<sup>(95)</sup>

Pyrocatechol, at a dose of 0.5 g, was placed onto the intact and abraded skin of the bellies of male albino rabbits for 24 h, and the animals were observed for an additional 14 days. The PII was 5.5 (of a possible maximum of 8.0); Pyrocatechol was a primary irritant. Slight to moderate erythema and slight edema of the intact areas and necrosis of the abraded areas were observed; irritation was less at 72 h than at 24 h. After 14 days, the intact areas were free of irritation except for a slight epidermal flaking and the necrotic areas were sloughing.<sup>(7)</sup>

### Intradermal Studies

Hydroquinone, at concentrations of 0.001–0.1% in water and in a dose of 0.1 ml, was administered intracutaneously in the flank of guinea pigs. Hydroquinone was not an intracutaneous primary irritant.<sup>(96)</sup>

Groups of 8 C57 mice (black) were given 4 separate intradermal injections on their ventral surface of 50  $\mu$ g or 500  $\mu$ g Pyrocatechol in dimethylsulfoxide. Local depigmentation was observed in 5 of 8 and 7 of 8 mice, respectively.<sup>(97)</sup>

### Sensitization and Photoallergenicity

The skin sensitization potential of Hydroquinone for guinea pigs has been investigated<sup>(88,89,98)</sup> (Table 6). Hydroquinone, in dimethylphthalate at 2%, did not sensitize any of 10 guinea pigs. Hydroquinone was a "weak," "strong," and "moderate" sensitizer in a modified Draize procedure, a maximization procedure, and a single injection adjuvant test, respectively. Hydroquinone, in propylene glycol at 5%, was tested for sensitization potential using 10 guinea pigs by the Magnusson-Kligman maximization procedure. No potential for skin sensitization was found.<sup>(99)</sup> Guinea pigs sensitized to *p*-methoxyphenol also reacted to Hydroquinone.<sup>(100)</sup>

A group of 20 Hartley albino guinea pigs, equal male and female, was used to evaluate the photoallergenicity of a 10% aqueous solution of Hydroquinone. The nuchal area was clipped and gently stripped with pressure-sensitive tape, and 0.3 ml of the Hydroquinone solution was applied using a Hilltop<sup>®</sup> chamber. Induction sites were covered by occlusive patches for 2 h, uncovered, and exposed to approximately 10 J/cm<sup>2</sup> of UV (320–400 nm) light. The lumbar region was simultaneously shielded from UV exposure. Six induction treatments, on alternating days, were given. Ten guinea pigs were sham-treated as negative con-

**TABLE 6.** Sensitization

<i>Material tested</i>	<i>Concentration</i>	<i>Method</i>	<i>No. of guinea pigs</i>	<i>Results</i>	<i>Reference</i>
Hydroquinone in dimethyl phthalate	2%	Injected guinea pigs every other day or three times a week for a total of 10 injections; 2 weeks later a challenge injection; read at 24 h	10	None sensitized	88, 89
Hydroquinone	Induction injections of 2.5% intradermal challenge of 1.0%, topical challenge of 20%	Modified Draize procedure: 4 simultaneous induction injections at sites overlaying axillary and inguinal lymph nodes; 2 weeks later, intradermal and open topical challenge on opposite shaved flanks; if no sensitization, procedure repeated	10	None sensitized at first trial; 30% at second; a "weak" sensitizer	98
Hydroquinone	Intradermal inductions of 2.0%, topical induction of 10%, chamber challenge of 5%	Maximization procedure: 6 0.1 ml intradermal inductions, 2 of hydroquinone alone, 2 of hydroquinone in 50% Freund's complete adjuvant (FCA), 2 of 50% FCA alone, into the nuchal region; 7 days later a 48-h occluded patch induction over the injection sites; 2 weeks later challenge with 24-h occluded chamber on shaved flank; 3-4 challenges a week apart	10	7/10% sensitized; a "strong" sensitizer; mean patch test reaction score was 1.8 (calculated from sum of all patch test reactions positive at 4 challenges max. possible score = 3.0)	98

Hydroquinone	Intradermal induction of 2.0%, chamber challenge of 50%	Single injection adjuvant test: one intradermal induction of hydroquinone in FCA in the nuchal region; 12-14 days later 6-h occluded chamber challenge on the shaved flank; scored 18-42 h after removal; 3-4 challenges a week apart	10	4/10% sensitized; a "moderate" sensitizer; mean patch test reaction score was 1.2 (calculated from sum of all patch test reactions positive at 4 challenges max. possible score = 3.0)	98
Hydroquinone	Induction at 5% in propylene glycol, challenge at 5% in petrolatum	Magnusson-Kligman Maximization Procedure	10	None sensitized	99
Hydroquinone	Induction at 10% challenged at 2 and 0.1%	Dermal application on alternate days, 6 treatments, 9-day nontreatment period and challenge at 2 and 0.1% UVA and non-UVA exposed sites	20	20/20 sensitized at 10% and 2% challenge; 18/20 responded to 10% induction and 0.1% challenge; severity of response did not increase on separate areas exposed to UVA	94
Pyrocatechol	—	1 induction injection per week for 3 weeks into nuchal area, inguinal-axillary region, and the footpads, successively; an emulsion in FCA, total dose per guinea pig was 1 mg; 4 weeks later, topical challenge - 5 $\mu$ l of an acetone solution with a ring on animals skin, max. applied was 1 $\mu$ mol; scored at 48 h; 3 challenges 2 weeks apart	8-12	None sensitized to less than 0.2 $\mu$ mol; most animals were sensitized; sensitized animals also reacted to 3-methyl and 3-propyl catechol	101

trols. After a 9-day nontreatment period, two pairs of sites on the clipped lumbar region of each guinea pig were challenged with 0.3 ml of 2.0 and 0.1% Hydroquinone, respectively, and covered with occlusive patches for 2 h. The left side and the induction area were subsequently shielded while the right side was exposed to approximately 10 J/cm<sup>2</sup> of UV (320–400 nm) light. Sites were scored at 24 and 48 h. The 10% Hydroquinone used in the induction phase followed by the 2.0% challenge sensitized 20 of 20 guinea pigs. The severity of response when scored at 24 and 48 h after challenge was similar for both the UVA and non-UVA-treated sites. The responses at the 0.1% challenged sites indicated 18 of 20 animals were sensitized in the non-UV treated sites and 15 of 20 in the UV treated sites. The severity of response when scored at 24 and 48 h after challenge was similar for both the UVA and non-UVA-treated sites. The control groups were challenged in a similar manner. All sites were negative, except for one low-grade response in one non-UVA exposed site challenged at 2.0% Hydroquinone. Preliminary irritation screening responses of 8 guinea pigs indicated that the 10% Hydroquinone was slightly irritating at 10% but not at 5.0, 1.0 or 0.5%.<sup>(94)</sup>

The skin sensitization potential of Pyrocatechol for guinea pigs has been investigated<sup>(101)</sup> (Table 6). Guinea pigs could be sensitized to Pyrocatechol and sensitized animals also reacted to 3-methyl and 3-propyl catechol.

### Eye Irritation

A rabbit eye irritation test of an undiluted product formulation containing 2.0% Hydroquinone produced mild conjunctivitis on day 1 in 3 of 6 animals. The conjunctivitis had disappeared by the second day. A score of 1 of 110 was reported.<sup>(102)</sup>

A dose of 0.1 g of Pyrocatechol was applied to one eye of 6 male albino rabbits. The conjunctiva was moderately erythematous and edematous, moderate exudate and corneal opacity were observed, and the rabbits appeared markedly uncomfortable. At 24 h, severe conjunctivitis, swelling, and ocular discharge, iritis, and dense corneal opacities were observed. The eyes had not improved by 72 h. At day 14, the eyes had keratoconus and pannus formation. Eye irritation was numerically scored at 24, 48, and 72 h; the scores were 103, 85, and 78 (of a maximum possible of 110), respectively.<sup>(7)</sup>

### Inhalation Studies

No deaths were observed when groups of 6 female Harlan-Wistar albino rats were exposed to 1500, 2000, and 2800 mg/m<sup>3</sup> Pyrocatechol-water aerosols for 8 hours containing 13.0, 14.5, and 17.0% Pyrocatechol, respectively. Weight gains were normal over a 14-day observation period and no treatment-related lesions were seen at necropsy at the end of 14 days. At the end of the 14-day observation period, all the rats in the 2800 mg/m<sup>3</sup> group and 2 of 6 in the 2000 mg/m<sup>3</sup> group had blackened and missing toes and tail tips. At these two doses, tremors appeared within 6–7 h of exposure; these continued through the first 24 h after exposure.<sup>(7)</sup>

## Special Studies

### Animal Reproduction

Hair dyes containing 0.2% Hydroquinone (and 23 other ingredients) have been tested for teratological and reproductive effects in multigeneration studies with rats.<sup>(103,104)</sup> The procedures used in these studies have previously been described.<sup>(105,106)</sup> The results were negative.

Hydroquinone was fed to two groups of 10 female rats in their diets at concentrations of 0.003 and 0.3%. All the rats were fertile, all had litters, and none died. Gestation length, mean litter size, viability, and lactation index were similar to the untreated control group.<sup>(107)</sup>

Ten Walter Reed-Carworth Farms female rats in their first gestation were mated and then were given a total of 0.5 g of Hydroquinone (total dose) in their feed during pregnancy. Hydroquinone was not toxic for the rats. The rats were killed 22 days after mating, and the uteri were examined. One or more resorptions were observed in 100% of the Hydroquinone-treated rats and 26.8% of all implantations terminated in resorptions. This resorption rate was substantially increased over the controls; of 126 untreated pregnant rats, 40.8% had one or more resorptions and 10.6% of all implantations ended in resorptions.<sup>(108)</sup>

Hydroquinone applied topically was evaluated in a teratology study in rats. Daily dermal doses of 54, 210, or 810 mg/kg were administered to 20 animals per group from day 6–19. The positive control group received acetylsalicylic acid orally. No remarkable differences were found at necropsy between the test group and the negative controls. In the positive control group, the results were those associated with fetotoxic and teratogenic compounds.<sup>(109)</sup>

### Mutagenesis

Hydroquinone has been studied in the Ames mutagenesis assay both with and without metabolic activation; it was negative using *Salmonella typhimurium* strains TA1537, TA1538, TA98, and TA100. It was positive with one medium and negative with another for the TA1535 strain with metabolic activation<sup>(110–115)</sup> (Table 7). A concentration of 6 ppm Hydroquinone caused no increase in the frequency of penicillin- and streptomycin-resistant *S. aureus*.<sup>(116)</sup> Hydroquinone was mutagenic in the *Escherichia coli* DNA polymerase assay as it induced repairable DNA damage.<sup>(117)</sup> Hydroquinone was mutagenic in the yeast, *Saccharomyces cerevisiae*, and it caused mitotic recombination.<sup>(118)</sup>

Hydroquinone, at concentrations of 50 and 100 mM, did not induce sex-linked recessive lethal mutations in the F<sub>2</sub> and F<sub>3</sub> generations of *Drosophila melanogaster*.<sup>(112)</sup>

Hydroquinone, at a concentration of  $1.0 \times 10^{-4}$  M without metabolic activation and at a concentration of  $3 \times 10^{-5}$  M with metabolic activation, was positive in the HeLa DNA synthesis test; this assay identifies agents that cause DNA damage.<sup>(119)</sup> Hydroquinone, at concentrations of  $0.5\text{--}2.0 \times 10^{-5}$ , did not increase the frequency of Sister Chromatid Exchanges (SCE) in cultured Chinese hamster V79 cells.<sup>(120)</sup> Hydroquinone, at concentrations of  $1.6 \times 10^{-6}$  to  $2.0 \times 10^{-4}$  M, induced SCE in human lymphocytes; it also delayed cell turnover time slightly.<sup>(121)</sup> Morimoto et al.<sup>(122)</sup> reported that the induction of SCE was dependent upon the optimum activation of Hydroquinone in the test system.

**TABLE 7.** Ames Salmonella Mutagenesis Assay

Material tested	Dose and solvent	Results without metabolic activation <sup>a</sup>					Results with metabolic activation <sup>a</sup>					Comments	Reference
		TA1535	TA1537	TA1538	TA98	TA100	TA1535	TA1537	TA1538	TA98	TA100		
Hydroquinone	Up to 10 mol/plate	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	Used two minimal media; was positive on one medium for TA1535+S-9	112
Hydroquinone		...	...	...	...	(-)	...	...	...	...	(-)		110
Hydroquinone	2 μmol/plate, ethanol	(-)	(-)	...	(-)	(-)	(-)	(-)	...	(-)	(-)	Spot tests	111
Hydroquinone	0.1-1000 μg/plate	...	...	...	...	(-)	...	...	...	...	...		113
Hydroquinone	50-5000 μg/plate	...	...	...	...	...	...	...	(-)	...	...		114
Pyrocatechol	10 μg to 10 mg/plate	...	...	...	(-)	(-)	...	...	...	(-)	(-)		128
Pyrocatechol	3 μmol/plate, ethanol	(-)	(-)	...	(-)	(-)	(-)	(-)	...	(-)	(-)	Spot tests	111
Pyrocatechol	0.03-30 μmol/plate ethanol	...	...	...	(-)	...	...	...	...	(-)	...	Spot tests	111
Pyrocatechol	0.1-1000 μg/plate	...	...	...	...	(-)	...	...	...	...	...		129
Pyrocatechol	0.1-1000 μg/plate	...	...	...	...	(-)	...	...	...	...	...		113
Pyrocatechol	4 μg/plate	...	...	...	...	(+)	...	...	...	...	(+)		130
Pyrocatechol	1-10 μg/plate	...	(-)	...	(-)	(-)	...	(-)	...	(-)	(-)		115

<sup>a</sup>(-) = nonmutagenic; (±) = weakly mutagenic; ... = no data.

Oral doses of 100 mg/kg Hydroquinone to male mice did not inhibit testicular DNA synthesis.<sup>(123)</sup> Hydroquinone was negative in the mouse sperm-head abnormality test after ip administration of 0.5–2.0 mmol/kg.<sup>(120)</sup>

Hydroquinone has been described as a “mitotic poison” for the intestine, lymphoid tissue, and thymus of the mouse.<sup>(124)</sup> Abnormal metaphases were observed in intestinal cells of mice after ip or subcutaneous administration of 0.15–0.175 mg/g Hydroquinone.<sup>(125)</sup> These were seen in the bone marrow and in intestinal cells of the golden hamster after ip administration of 0.15–0.20 mg/g Hydroquinone.<sup>(126)</sup> Abnormal metaphases were also observed in vitro in chick fibroblasts ( $1 \times 10^{-7}$  M to  $1 \times 10^{-6}$  M Hydroquinone), in rat liver, bone marrow, and corneal cells after ip administration of 0.15–0.20 mg/g Hydroquinone and in rat corneal cells after instillation of one drop of a 5% Hydroquinone solution.<sup>(127)</sup>

Mice were given two ip injections of up to 110 mg/kg (1.0 mmol/kg) Hydroquinone 24 h apart. Polychromatic erythrocytes were observed in the bone marrow after two injections.<sup>(112)</sup> Hydroquinone was administered subcutaneously to mice in doses of 20–100 mg/kg daily for 6 days; the number of micronucleated cells was increased in bone marrow at all doses above 20 mg/kg Hydroquinone.<sup>(71)</sup> Hydroquinone was positive in the mouse bone marrow micronucleus test after ip administration of 0.5–2.0 mmol/kg.<sup>(120)</sup>

Pyrocatechol has been studied in the Ames mutagenesis assay both with and without metabolic activation; most researchers reported that Pyrocatechol was negative in *Salmonella* strains TA1535, TA1537, TA98, and TA100.<sup>(111,113,128,129)</sup> One researcher reported that Pyrocatechol was positive in strain TA100 with and without metabolic activation<sup>(130)</sup> (Table 7). Yoshida and Fukuhara<sup>(115)</sup> reported that Pyrocatechol was a comutagen for benzo[a]pyrene in the Ames assay, provided that a sufficient amount of the S9 mixture was used during the incubation

Pyrocatechol was negative in the *E. coli* DNA polymerase assay.<sup>(131)</sup> Pyrocatechol was mutagenic for the yeast, *S. cerevisiae*; it enhanced ultraviolet-induced mitotic crossing-over, aberrant colony formation, and mutation, and it increased ultraviolet-induced gene conversion.<sup>(132)</sup>

Pyrocatechol, at a concentration of  $2.0 \times 10^{-4}$  M, with metabolic activation was positive in the HeLa DNA synthesis test.<sup>(119)</sup> Pyrocatechol, at concentrations of  $0.5$ – $2.0 \times 10^{-5}$  M, did not increase the frequency of SCE in Chinese hamster V79 cells.<sup>(120)</sup> Increased numbers of chromatid breaks and exchanges were observed when Chinese hamster ovary cells were exposed to 0.05 mg/ml Pyrocatechol without metabolic activation; metabolic activation decreased the response.<sup>(133)</sup> Pyrocatechol, at concentrations of  $1.6 \times 10^{-6}$  to  $2.0 \times 10^{-4}$  M, induced SCE in human lymphocytes; it also delayed cell turnover time.<sup>(121)</sup> Pyrocatechol was negative in the mouse sperm-head abnormality test after ip administration of 0.5–2.0 mmol/kg.<sup>(120)</sup>

Pyrocatechol was positive in a mouse bone marrow micronucleus test after ip administration of 0.5–2.0 mmol/kg.<sup>(120)</sup> Pyrocatechol was subcutaneously administered to mice in daily doses of 5–42 mg/kg for 6 days; the frequency of micronucleated cells in the bone marrow did not change.<sup>(11)</sup>

### **Carcinogenesis, Cocarcinogenesis, and Tumor Promotion**

Hydroquinone is currently in the chronic phase of a bioassay in the NTP carcinogenesis bioassay program. It is being administered to rats and mice by gavage.<sup>(134)</sup>

Hydroquinone has been tested for carcinogenicity using a cholesterol pellet bladder implantation method. The pellet remained in the bladder for at least a year. Ten-milligram cholesterol pellets containing 20% Hydroquinone were implanted into the bladders of mice; 19 mice survived to 25 weeks, and 6 of the mice had bladder carcinomas (a tumor incidence of 32%). Cholesterol pellets alone were also implanted; 77 mice survived to 25 weeks, 4 mice had adenomas or papillomas, and 5 had carcinomas (a tumor incidence of 12%). The researchers stated that Hydroquinone was positive for carcinogenicity.<sup>(135)</sup> In respect to the carcinogenic effect in mice upon urinary implantation of cholesterol pellets containing Hydroquinone, the International Agency for Research on Cancer (IARC) stated<sup>(17)</sup>:

Experiments involving a possible action of the vehicle or a physical effect of the agent, such as in studies by subcutaneous injection or bladder implantation, are included [in this evaluation], however, the results of such tests require careful consideration, particularly if they are the only ones raising a suspicion of carcinogenicity.

The carcinogenic potential of a hair dye containing 0.2% Hydroquinone (and 23 other ingredients) has been investigated.<sup>(136)</sup> The procedures used in this testing program have been described previously.<sup>(105,106)</sup> The results were negative.

Hydroquinone has been tested for tumor-initiating activity. Twenty-four male mice of the S strain received a single application on the clipped back of 0.3 ml of a 6.7% solution of Hydroquinone (20 mg) in acetone as an initiator. Three weeks later, the promoter, croton oil, was painted on the same area of the skin in 18 weekly applications of 0.3 ml of a 0.5% solution in acetone. One week later, 22 survivors were killed and necropsied. One mouse had a tumor; no evidence of tumor-initiating activity of Hydroquinone was observed.<sup>(137)</sup>

Fifty female Swiss mice were topically treated with 150  $\mu$ g benzo(a)pyrene (BaP) as an initiator and 14 days later with 0.1 ml of a Hydroquinone solution in acetone (5 mg Hydroquinone) as a tumor promoter; the Hydroquinone solution was applied 3 times a week until the mice had been on test for 409 days. BaP and Hydroquinone were applied to the back. Fifty mice were treated with acetone alone, 100 were untreated, and two groups of mice were treated with BaP and phorbol myristate acetate (PMA) and anthralin as positive controls. Hydroquinone had no tumor-promoting activity.<sup>(138)</sup>

The cocarcinogenic potential of Hydroquinone has been investigated. Hydroquinone had a weak inhibitory action on BaP tumorigenicity. Hydroquinone, in a dose of 5 mg, was applied with and without 5  $\mu$ g BaP in a single solution in acetone three times a week for 368 days to the clipped backs of groups of 50 female Swiss mice. The experiment included mice treated with PMA and anthralin with BaP as positive controls for cocarcinogenicity and mice treated with BaP alone. The first papilloma was observed at 254 days in mice treated with Hydroquinone and BaP; 7 mice had papillomas, a total of 11 papillomas were observed, and 3 mice had squamous carcinomas. No papillomas or squamous carcinomas were seen in mice treated with Hydroquinone alone. The first papilloma was observed at 251 days in mice treated with BaP alone; 14 mice had papillomas, a total of 16 papillomas were observed, and 10 mice had squamous carcinomas. In this bioassay Hydroquinone inhibited the tumorigenicity of BaP.<sup>(138)</sup>

Hydroquinone reduced the number of melanoma transplantations. Groups of 40 BALB/c female mice were given Harding-Passey melanoma transplants. Two groups were administered 16 mg/kg and 80 mg/kg Hydroquinone in saline subcutaneously daily for 9 days starting the next day. One group was untreated and one group was treated with saline. After 140 days, all surviving mice were killed and melanomas were weighed. Only the group given 80 mg/kg Hydroquinone survived to 140 days (comparable to survival of normal female mice). The incidences of successful melanoma transplantation in the combined control groups (untreated and saline treated), the 16 mg/kg Hydroquinone group, and the 80 mg/kg Hydroquinone group were 91.7%, 55.6%, and 23.7%, respectively. However, the weights of the successfully transplanted tumors did not differ among groups.<sup>(139)</sup>

Pyrocatechol has been tested for carcinogenicity using the bladder implantation method. Ten-milligram cholesterol pellets containing 20% Pyrocatechol were implanted into the bladder of mice; 19 mice survived to 25 weeks, 1 mouse had a papilloma, and 3 developed carcinomas (a tumor incidence of 20%). Cholesterol pellets alone were also implanted; 77 mice survived to 25 weeks, 4 mice had adenomas or papillomas, and 5 had carcinomas of the bladder (a tumor incidence of 12%). Pyrocatechol was not carcinogenic in mice with bladders implanted with cholesterol pellets.<sup>(135)</sup>

A 75- $\mu$ g dose of 7,12-dimethylbenz(a)anthracene (DMBA) in acetone was topically applied to a group of 30 female Swiss mice as an initiator. After 10 days a 1% Pyrocatechol solution in acetone was topically applied as a promoter, and it was applied 5 times a week for 67 weeks. No tumor-promoting activity of Pyrocatechol was observed.<sup>(140)</sup>

Fifty female Swiss mice were treated topically with 150  $\mu$ g BaP as an initiator and 14 days later with 0.1 ml of a Pyrocatechol solution in acetone (2 mg Pyrocatechol) as a promoter; the Pyrocatechol solution was applied 3 times a week until the mice had been on test for 448 days. The initiator and the Pyrocatechol solution were applied to the back. Fifty mice were treated with acetone alone, 100 were untreated, and two groups of mice were treated with BaP and with PMA and anthralin as positive controls. Pyrocatechol was inactive as a tumor promoter.<sup>(138)</sup>

Pyrocatechol has cocarcinogenic activity. A group of 50 female Swiss mice received topical applications of 2 mg Pyrocatechol together with 5  $\mu$ g BaP in 0.1 ml acetone 3 times a week for 52 weeks. Twenty-six mice survived to 52 weeks. A group of 50 mice also received BaP alone; 42 mice survived to 52 weeks. The experiment also included a group of untreated control mice and a group of acetone treated mice. Eighty-six skin papillomas were found in 35 mice receiving Pyrocatechol and BaP; 31 mice had squamous cell carcinomas. Fourteen papillomas occurred in 13 mice receiving BaP alone; 10 squamous carcinomas of the skin were observed. No skin tumors were observed in the untreated or acetone-treated mice. Pyrocatechol was active as a cocarcinogen and enhanced the carcinogenicity of BaP.<sup>(141)</sup>

Pyrocatechol, in a dose of 2 mg, was applied with and without BaP in a single solution in acetone three times a week for 368 days to the clipped backs of groups of 50 female Swiss mice. The experiment included mice treated with PMA and anthralin with BaP as positive controls for cocarcinogenicity and mice treated with BaP alone. The first papilloma was observed at 299 days in mice

treated with Pyrocatechol and BaP; 36 mice had papillomas, a total of 90 papillomas were observed, and 31 mice had squamous carcinomas. Without BaP, 1 mouse treated with Pyrocatechol had a papilloma at 292 days and 1 mouse had a squamous carcinoma. The first papilloma was observed at 251 days in mice treated with BaP alone; 14 mice had papillomas, a total of 16 papillomas were observed, and 10 mice had squamous carcinomas. Pyrocatechol was cocarcinogenic.<sup>(138)</sup>

Mice were given Sarcoma-180 cells by subcutaneous injection. Twenty-four hours later and every other day for a total of 7 treatments, the mice were injected in the same place with 0.2 ml of a 5 mg/ml solution of Pyrocatechol. Pyrocatechol inhibited tumor growth.<sup>(142)</sup>

## CLINICAL ASSESSMENT OF SAFETY

### General Effects

A variety of tests and clinical observations have been reported. The results are given in Tables 8 and 9 and summarized in the following text.

Patch tests on the back with 1% Hydroquinone in petrolatum were conducted during 1970–1976 on 53 denture-wearing subjects with burning mouth syndrome. The reactions were scored at 48 and 72 h. Only 1 subject had a positive reaction; erythema and infiltration without papules or vesicles were observed.<sup>(146)</sup>

In Salvador, Brazil, 536 dermatological patients were patch-tested with occlusive patches containing 5% Hydroquinone in aqueous solution. The reactions were evaluated on removal of the patches at 48 h and at 96 h. Positive reactions were observed in 8.9% of the patients; Hydroquinone was a sensitizer in patients with dermatitis.<sup>(147)</sup>

Five blacks and 3 whites with freckles applied 10 and 30% Hydroquinone in petrolatum on their backs in a continuous patch test for 1 month. Two blacks developed depigmentation; more depigmentation was produced by the 30% than by the 10% Hydroquinone. Two blacks developed dermatitis, and one of them had secondary pigmentation. In the other patients, no skin changes were observed.<sup>(42)</sup>

Two and five percent Hydroquinone bleaching creams were used on the hyperpigmented skin of 56 black and white patients; the patients were also instructed to use a sunscreen. Both Hydroquinone concentrations were moderately effective in depigmenting the skin of 44 of the 56 patients. Erythema and tingling at the site of application was observed by 32% of the patients using the 5% Hydroquinone bleaching cream; 8% of those using the 2% Hydroquinone bleaching cream had mild reactions. In 1 patient, a questionable leukoderma developed; in another patient there was a possibility of sensitization.<sup>(41)</sup>

A primary skin irritation of a cosmetic formulation containing 2% Hydroquinone gave an average irritation index of 0.89 for 19 subjects (scale 0–4).<sup>(144)</sup> The same cosmetic formulation was used in a repeat insult patch test conducted on 90 subjects. The undiluted formulations (0.1 ml) was applied three times per week, occluded for 24 h after each application with Werbil patches, for 3 consecutive weeks. Following a 4-week nontreatment period, the subjects were

**TABLE 8.** Human Irritation and Sensitization

<i>Ingredient</i>	<i>Concentration (%)</i>	<i>Vehicle</i>	<i>Test method</i>	<i>Population type</i>	<i>No. of subjects</i>	<i>Results</i>	<i>Reference</i>
Hydroquinone	1, 2.5, 3.5, 5, and 7	Lotions, creams, ointments	48-h closed patch	Dark-skinned subjects	840	Classified as sensitizer and mild primary irritant; "adverse effects at 3% were negligible"	143
Hydroquinone	5, 6, and 7	Soft paraffin	Open patch, applied at 0 and 24 h	Indians (random selection)	200	6/200 mild erythema at 5.0% at 72 h	143
Hydroquinone	2	Cosmetic formulation	Primary skin irritation	Random	19	PII = 0.89 of 4.0 max	144
Hydroquinone	2	Cosmetic formulation (same as Ref 144)	Repeat insult patch test	Random	90	69/90 showed one or more mild reactions during induction phase 22/90 had a mild reaction on challenge	145

**TABLE 9.** Summary of Clinical Patient Data

<i>Ingredient</i>	<i>Concentration (%)</i>	<i>Vehicle</i>	<i>Test method</i>	<i>Population type</i>	<i>No. of subjects</i>	<i>Results</i>	<i>Reference</i>
Hydroquinone	1	Petrolatum	24-h occluded patch	Denture wearers with burning mouth syndrome	53	1/53 positive reactions erythema and infiltration without papules	146
Hydroquinone	2, 3, and 5	Ointment	Clinical treatment	White males with freckles and lentigines; normal black males	94 white 43 black	Depigmentation at all concentrations; amount increased with concentration; many patients had transient inflammatory reactions before depigmentation started 0/60 sensitized at 2% 0/39 sensitized at 3% 3/39 sensitized at 5%	55
Hydroquinone	2 and 5	Bleaching cream	Used on hyperpigmented skin with sunscreen	Black and white patients	56	44/56 depigmented at both concentrations 5/56 had erythema at 2% 18/56 had erythema at 5%	41
Hydroquinone	5	Ointment with tretinoin and dexamethasone	Clinical treatment	Selected black patients	100	Irritation first month of treatment; all subjects were depigmented; irradiated depigmented sites became hyperpigmented	56
Hydroquinone	5	Ointment with tretinoin and dexamethasone	Clinical treatment	19 patients with melasma; 25 with acne; 11 with pseudofolliculitis; 11 with senile lentigines	66 total	Positive medical benefits for all patients; no contact sensitization or photosensitization was observed	57
Hydroquinone	5	Aqueous	48-h occluded patch test	Dermatological patients	536	48/536 were positive for sensitization	147
Hydroquinone	10 and 30	Petrolatum	Continuous patch test for one month	5 black patients, 3 white	8	2/5 black patients developed depigmentation at 10%; 2/5 blacks developed irritation	42

challenged under conditions similar to the induction period. Sixty-nine of the 90 subjects had one or more mild irritant reactions during the induction patch phase, and 22 subjects reacted to challenge. Of the challenge reactions noted, none were greater than mild, and only three had the same severity of response at both the 24 and 48 h scorings (one mild, the other two barely perceptible). Within the experimental limits the investigator concluded that "this cosmetic product did not have any potential for inducing allergic sensitization."<sup>(145)</sup>

The freckles and lentiginosities of 94 white men and the normal skin of 43 black men were treated with 2, 3, and 5% Hydroquinone ointments. More depigmentation was observed with the 3 and 5% Hydroquinone ointments than with the 2% ointment. No depigmentation was observed in the darker black skins. In many patients, transient inflammatory reactions that lasted for about a week were observed before depigmentation; a few inflammatory reactions were not followed by depigmentation. No patients treated with the 2% (60 patients) or 3% (39 patients) Hydroquinone ointments became sensitized. Two of 38 patients treated with the 5% ointment became sensitized to Hydroquinone. The use of a sunscreen prevented repigmentation.<sup>(55)</sup>

Kligman and Willis<sup>(56)</sup> applied a 5% Hydroquinone cream (that also contained 0.1% tretinoin and 0.1% dexamethasone) two times a day to the skin of more than 100 black subjects. Irritation was observed only during the first month of treatment. Depigmentation occurred, and after the Hydroquinone treatment ceased, repigmentation began. Irradiated depigmented sites became hyperpigmented. Darker individuals were more susceptible to the depigmenting effect. The Hydroquinone cream was effective in treating melasma, postinflammatory hyperpigmentation, and ephelides and was not effective in treating senile lentiginosities. Two blacks with vitiligo were treated with the Hydroquinone cream. The unaffected areas were depigmented; the treatment was successful. A 10% Hydroquinone cream was more irritating and depigmentation was more rapid; a 2% cream was less irritating and less potent for depigmentation.

A 5% Hydroquinone cream (contained 0.1% tretinoin and 0.1% dexamethasone) was used to treat 19 women with melasma; 18 achieved satisfactory lightening. The cream was also used to treat postinflammatory hyperpigmentation in blacks; almost all of 25 acne patients and 8 of 11 men with pseudofolliculitis had satisfactory lightening. With the use of the Hydroquinone cream, mild to moderate irritation was observed. No contact sensitization or photosensitization was observed. A sunscreen was necessary to prevent repigmentation.<sup>(57)</sup>

In blacks, facial ochronosis and pigmented colloid milium were observed after the chronic oversaturation of the skin with creams containing Hydroquinone and exposure to sunlight.<sup>(39,58)</sup>

Open and closed patch tests with Hydroquinone were performed on dark-skinned subjects. The open patch test was conducted with 5, 6, and 7% Hydroquinone in soft paraffin on 200 Indians. The application sites were examined at 24 h, the Hydroquinone was reapplied, and the sites were examined again at 72 h. Six positive reactions (mild erythema) were observed at 72 h in patients given 5% treatment. Closed 48-h patch tests were also conducted with 1, 2.5, 3.5, 5, and 7% Hydroquinone in lotions, creams, or ointments on 840 dark-skinned subjects; positive reactions were observed, and their frequency varied with the Hydroquinone concentration and with the formulation. Hydroquinone was a

mild primary irritant and a sensitizer. The investigators stated that 3% Hydroquinone produced negligible adverse effects.<sup>(143)</sup>

Some investigators have stated that Hydroquinone caused inflammatory reactions followed by depigmentation. However, others<sup>(148)</sup> found that when an inflammatory reaction followed the use of Hydroquinone creams to lighten black skin, the invariable result was hyperpigmentation.

Occupational depigmentation from Hydroquinone followed its use by photographic developers. Other industrial exposures to Hydroquinone have not caused depigmentation.<sup>(149)</sup> In a review of Hydroquinone uses and abnormal reactions, it was stated that adverse reactions to the use of this ingredient as a bleaching cream are rare and that Hydroquinone may cross-react with resorcinol and Pyrocatechol.

Ocular lesions have been observed in workers involved in the manufacture of Hydroquinone and exposed to Hydroquinone dust and quinone vapors. Conjunctival and corneal staining and precipitation of pigment were observed, usually in persons employed for at least 5 years. In severe cases, vision was reduced. No other systemic effects were noted. Quinone may be the major factor in causing ocular injury, but the Hydroquinone dust may play a contributing role.<sup>(150,151)</sup> Corneal changes, particularly alteration of curvature, was observed long after exposure to Hydroquinone had ceased and the ocular stain and pigment had disappeared.<sup>(152)</sup> No studies document serious ocular injury caused by airborne Hydroquinone in the absence of quinone vapor.<sup>(26)</sup>

No systemic effects have been observed after occupational exposure to 2 mg Hydroquinone dust/m<sup>3</sup>.<sup>(153)</sup> The TLV for Hydroquinone is 2 mg/m<sup>3</sup> and the Short-Term Exposure Limit (STEL) is 4 mg/m<sup>3</sup>.<sup>(153,154)</sup> NIOSH<sup>(26)</sup> has recommended a 15-minute ceiling of 2 mg/m<sup>3</sup>.

Upon contact with skin, Pyrocatechol may cause an eczematous dermatitis. Absorption through the skin has resulted in symptoms resembling those induced by phenol except that convulsions were more marked.<sup>(62)</sup>

Thirteen workers were exposed to Pyrocatechol and phenol vapors for 2 years. Most of the workers complained of respiratory problems. Nine of 12 had chronic inflammation of the upper respiratory tract. No other systemic effects were observed.<sup>(74)</sup>

The TLV for Pyrocatechol set by the ACGIH<sup>(153,154)</sup> is 5 ppm.

## SUMMARY

Hydroquinone and Pyrocatechol are two benzenediol isomers, 1,4-benzenediol and 1,2-benzenediol. Both ingredients are used in cosmetics as couplers in oxidative hair dyes at concentrations of less than 1.0%. Hydroquinone, a known skin-depigmenting agent, is also used in cleansing preparations at concentrations between 1 and 5%.

Both Hydroquinone and Pyrocatechol inhibit bacterial growth. Prior to 1960 Pyrocatechol was used for its antibacterial properties in some drugs.

Both compounds are absorbed from the gastrointestinal tract, and Pyrocatechol is also readily absorbed through the skin. Small amounts of nonmetabolized

Hydroquinone are excreted in the urine of rabbits; however, most of the compound is excreted as Hydroquinone ethereal monosulfate and as the monoglucuronide. In rabbits, Pyrocatechol is excreted mainly as the ethereal sulfate and monoglucuronide. The biological half-life of Pyrocatechol in humans is between 3 and 7 h; excretion is predominantly in the urine.

The results of acute oral studies in animals indicate that Hydroquinone is practically nontoxic to moderately toxic; the data from subchronic feeding studies of Hydroquinone indicated that it was not toxic at 1%, slightly toxic at 2% and toxic at 5%. Pyrocatechol was moderately toxic in acute studies. In subchronic feeding studies, Pyrocatechol at a dietary concentration of 0.25% produced hepatic cell hyperplasia in rats, but survival was not affected at 1.0%. The results of a 20-week study in mice were a loss of weight and blood effects when 4.0 g/L Pyrocatechol was included in the drinking water. There were no adverse effects at 1.0 g/L.

No adverse local systemic effects were produced in rabbits when 2.0% Hydroquinone was applied to intact and abraded skin (3.9–9.4 ml/kg). The results of subchronic and chronic dermal studies of Hydroquinone in animals for time intervals up to 6 months indicated that the ingredient was a weak depigmenter at 1.0%. Other animals studies indicated that the time required for depigmentation was dependent upon both the concentration and the dispersion medium used. When 2.0% Hydroquinone was tested in rabbits using a single-insult patch test, a PII of 1.22 (scale 0–4) was reported. Guinea pigs were sensitized to Hydroquinone when injected at concentrations above 2.0%. The severity of the sensitivity reaction induced at 10% Hydroquinone was not increased when exposed to UVA light. The acute dermal LD<sub>50</sub> of Pyrocatechol was 0.8 g/kg. Pyrocatechol did not depigment rabbit skin at 1.0% but did at 3.0%; skin irritation was observed at 5.0%. Undiluted Pyrocatechol was a primary irritant in rabbits. Guinea pigs were sensitized when Pyrocatechol was injected at concentrations above 0.2  $\mu$ mol.

In a rabbit eye irritation test, an undiluted product formulation containing 2.0% Hydroquinone produced mild conjunctivitis in 3 of 6 animals evaluated at 24 h. The conjunctivitis had subsided on the second day. Undiluted Pyrocatechol was a severe ocular irritant in rabbits at doses of 0.1 g.

When Hydroquinone (0.003–0.3%) was included in the diet of two groups of 10 pregnant female rats, no differences were found between the test and control groups relative to gestation length, mean litter size, viability, and lactation index. In a second study 0.5 g of Hydroquinone included in the diets of a group of 10 mated female rats produced no significant difference in resorptions when compared to control groups. Hydroquinone was evaluated in a teratology study in which daily dermal exposure of pregnant rats (20 animals/group) was up to 810 mg/kg; no remarkable difference was found between the control and test groups.

The results of mutagenesis assays of Hydroquinone have varied with the assay system used. In four *S. typhimurium* strains, both with and without activation, the mutagenesis assay was negative. One strain tested was positive, with activation using one medium, but not with a second medium. Hydroquinone did not increase antibiotic resistance in *S. aureus*. Hydroquinone was mutagenic in the *E. coli* DNA polymerase and *S. cerevisiae* mitotic recombination assays. Hydro-

quinone produced positive results both with and without activation in the HeLa DNA synthesis test but was not considered mutagenic in assays using Chinese hamster cells. Hydroquinone induced SCE and delayed cell turnover time in human lymphocyte studies. Oral doses of Hydroquinone did not inhibit testicular DNA synthesis in male mice and was nonmutagenic in the mouse sperm-head abnormality test. Hydroquinone is considered a mitotic poison.

In multigeneration rat studies of topically applied hair dyes containing 0.2% Hydroquinone, no effect on reproduction was observed and embryotoxicity and teratogenesis were not produced. The F<sub>1A</sub> animals were used for carcinogenic assay of the hair dyes. The results were negative. Hydroquinone, when applied topically, was neither a tumor promoter nor a cocarcinogen in Swiss mice. Harding-Passey melanoma transplants were decreased when Hydroquinone was administered after implantation.

Like Hydroquinone, the mutagenicity of Pyrocatechol varies with the test system used. In most studies, Pyrocatechol was nonmutagenic with and without metabolic activation in the Ames assay. However, positive results have been reported for one test strain. The compound was negative in the *E. coli* DNA polymerase assay but was positive in the yeast, *S. cerevisiae*. Pyrocatechol was negative in the HeLa DNA synthesis test and with Chinese hamster V79 cells. The compound increased the numbers of chromatid breaks and exchanges in Chinese hamster ovary cells and induced SCE and delayed cell turnover time in human lymphocyte cultures. The compound given by ip injection to mice was negative in the sperm-head abnormality test but was positive in the bone marrow assay.

In three separate studies in mice, topically applied Pyrocatechol was not a tumor promoter. Topically applied Pyrocatechol was a cocarcinogen for mouse skin in two separate studies.

Hydroquinone studies in humans at doses of 500 mg and 300 mg to males and females, respectively, for 5 months produced no signs of toxicity.

Positive sensitization reactions to Hydroquinone were reported in 8.9% of 536 dermatologic patients challenged with a 5.0% solution. At higher concentrations (10 and 30%) dermatitis was produced in 2 of 5 black subjects. A cosmetic formulation containing 2% Hydroquinone produced one or more mild irritation reactions in 69 of 90 subjects in the induction phase of a sensitization test. In this latter study 22 subjects had a mild reaction when challenged by the same formulation and scored at 24 h. Only 3 of the 22 subjects had either mild or barely perceptible reactions at 48 h. The use of ointments containing 2, 3, and 5% Hydroquinone in 94 white and 43 black men with normal skin produced at least minimal depigmentation in white but not black subjects. Two of 38 patients treated with an ointment containing 5.4% Hydroquinone became sensitized. Other studies on dark-skinned subjects have confirmed these sensitization results.

Ocular lesions but no other systemic effects have been found in workers involved in the manufacture of Hydroquinone. Occupational exposure to Pyrocatechol has been associated with dermatitis and chronic inflammation of the upper respiratory tract. Recommended limits for occupational exposure of Hydroquinone and Pyrocatechol have been set at 2 and 5 ppm, respectively.

## DISCUSSION

During the review of these two ingredients, members of the Expert Panel were aware that the Food and Drug Administration had reviewed studies relating to the safety of Hydroquinone and had concluded that Hydroquinone was safe and effective as an agent to bleach or lighten the skin at concentrations between 1.5 and 2.0% in OTC drugs. The CIR Expert Panel has not evaluated the safety of Hydroquinone to lighten the skin, which is regarded by FDA as a drug use of this ingredient. Hydroquinone is a weak depigmenter at 1.0%. Following prolonged exposure it is a skin irritant and a sensitizer in some individuals. Pyrocatechol has toxicological characteristics similar to those of Hydroquinone. The available eye irritation data indicated that Hydroquinone is a mild ocular irritant at low concentrations. Pyrocatechol is extremely irritating to the eye at high concentrations. The Panel noted that the major use of these two ingredients is in rinse-off-type products for intermittent application of relatively short exposure times, at concentrations of 1.0% and less. The Panel considered these uses of Hydroquinone and Pyrocatechol to be safe, provided the formulations containing Pyrocatechol do not come into contact with the eye.

## CONCLUSION

The CIR Expert Panel concludes that Hydroquinone and Pyrocatechol are safe for cosmetic use at concentrations of 1.0% and less in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair.

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## Addendum to the Final Report on the Safety Assessment of Hydroquinone

**Summary:** An assessment of the safety of Hydroquinone was first published in 1986 (*J Am Coll Toxicol* 5:123-65). The ingredient was found to be safe for use at limited concentrations for certain formulations. This addendum reviews new data and presents a revised conclusion regarding safety. Hydroquinone is an aromatic compound used principally in hair dyes and colors, but it is also in lipsticks, skin fresheners, and other skin care preparations. Hydroquinone in an aqueous solution was shown to be absorbed through human skin at a rate of  $0.55 \pm 0.13 \mu\text{g}/\text{cm}^2/\text{h}$ . Hydroquinone is rapidly absorbed and excreted in urine in rats following oral administration. Absorption from an alcohol vehicle is greater than from an aqueous solution. Hydroquinone was found to be cytotoxic to rat hepatoma cells in culture, and nephrotoxic in male rats dosed orally by gavage. Oral administration of Hydroquinone to rats resulted in dose-dependent mortality, lethargy, tremors, and increased liver and kidney weights. Oral administration did not produce embryotoxic, fetotoxic, or teratogenic effects in rats. In rats, dermal application produced slight to severe irritation. In a guinea pig maximization test, induction with 2% Hydroquinone injected intradermally, followed by challenge with 0.5% Hydroquinone, showed extreme sensitization. In 80 patients known to be sensitive to aromatic compounds, 0.5% Hydroquinone elicited no reactions. Hydroquinone can cause depigmentation of skin. Various genotoxicity assays show that Hydroquinone can induce sister chromatid exchanges, chromosomal aberrations and loss, and increased frequency of mitotic crossovers. It also induced DNA strand breaks and inhibited DNA and RNA synthesis in rabbit bone marrow mitochondria. Forward mutation assays with or without metabolic activation were positive, but the results with the Ames test, a mouse test for somatic mutations, and other tests were negative. Hydroquinone, given to rats orally by gavage five times per week for up to 103 weeks at doses of 25 or 50 mg/kg, resulted in a significant increase of renal adenomas in males given 50 mg/kg and of mononuclear cell leukemia in females with both doses. At doses of 50 or 100 mg/kg on the same schedule, there was a significant increase in hepatocellular adenomas in both male and female mice. Other studies of Hydroquinone showed no significant difference in tumors between control and exposed groups, and marginal to no activity as a tumor promoter. It is concluded that Hydroquinone is safe at concentrations of  $\leq 1\%$  for *aqueous* cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on, nondrug cosmetic products. **Key Words:** Safety assessment—Hydroquinone.

The Cosmetic Ingredient Review (CIR) Expert Panel has previously concluded that Hydroquinone (CAS No. 123-31-9) is safe for cosmetic use at  $\leq 1\%$  in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair (Elder, 1986). The carcinogenicity data used to reach the original con-

TABLE 1. Product formulation data for Hydroquinone (FDA, 1993)<sup>a</sup>

Product category	Total no. of formulations in category	Total no. containing ingredient
Hair dyes and colors	1,112	185
Lipstick	937	2
Skin fresheners	246	1
Other skin care preparations	848	18
1993 Totals		206

<sup>a</sup> CIR requests that the cosmetic industry provide current formulation data on each product category.

clusion were primarily from a dermal study of a complete hair dye formulation that contained 0.2% Hydroquinone. In 1989, the National Toxicology Program (NTP) issued a report on a carcinogenicity study in which Hydroquinone was administered orally by gavage. This literature update includes the results of that study and other relevant data that have been published since 1983 and not cited in the previous safety evaluation.

The safety of Hydroquinone was reviewed primarily for its use in hair dyes, not for other uses, as demonstrated in the following excerpt from the Discussion of the original report:

“. . . members of the Expert Panel were aware that the Food and Drug Administration had reviewed studies relating to the safety of Hydroquinone and had concluded that Hydroquinone was safe and effective as an agent to bleach or lighten the skin at concentrations between 1.5 and 2.0% in OTC drugs. The CIR Expert Panel has not evaluated the safety of Hydroquinone to lighten the skin, which is regarded by FDA as a drug use of this ingredient.”

This report deals with the use of Hydroquinone in cosmetic leave-on preparations.

Hydroquinone is an aromatic compound (Nikitakis, 1988) that is used in hair dyes and colors, lipsticks, skin fresheners, and other skin care preparations (FDA, 1993). In 1981, it was reported to the Food and Drug Administration (FDA) that Hydroquinone was used in 170 cosmetic ingredient formulations, including 147 hair dye formulations (FDA, 1981). The number of cosmetic formulations in which Hydroquinone is used has increased since 1981; in 1993, it was reported to the FDA that Hydroquinone was used in 206 formulations, 185 of which were hair dye formulations (Table 1).

Concentration of use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). However, product formulation data submitted to the FDA in 1984 indicated that Hydroquinone was used at concentrations of  $\leq 1\%$  in hair dyes and colors requiring caution statements and at 1–5% in skin cleansing products and other skin care preparations (FDA, 1984).

## CHEMISTRY

### Analytical Methods

The purity of Hydroquinone, derivatized and underivatized samples, has been determined using gas chromatography with a flame ionization detector (GC/FID)

(Eastman Kodak Company, 1988, 1989). The structure of Hydroquinone was confirmed by gas chromatography–mass spectrometry (GC/MS), using electron impact ionization (EI).

#### Rate of Hydroquinone Disappearance During Its Use in Hair Dyes

Hydroquinone, which acts as a regulating agent allowing some control of the color-forming coupling reactions, is a “consumable” in the hair dyeing procedure, with its actual concentration decreasing as the color-forming reactions proceed (L’Oreal, 1990).

To determine how much of the Hydroquinone is consumed, three commercial hair dye formulations were used to measure the rate of disappearance of Hydroquinone during the hair dyeing procedure (L’Oreal, 1990). Two of the formulations contained 0.08% (w/w) and the third contained 0.15% (w/w) Hydroquinone. An oxidation cream containing 6% (w/w) hydrogen peroxide was used for oxidation of the mixtures.

The test article was prepared by mixing 15 g of the cream oxidant with 15 g of the hair dye. It was then uniformly applied with a paintbrush to a 30 cm long (15 g) lock of natural hair. After 5, 10, 20, and 30 min, 2.5 g samples of the hair dye mixture were collected from the hair lock and assayed in a high-performance liquid chromatography (HPLC) system. (Results of the system calibration showed that it responded linearly with respect to concentration of Hydroquinone for the range 0.8–80  $\mu\text{g/ml}$ .)

For each of the three formulations, the percentage of residual Hydroquinone was determined as a function of time. The amount of Hydroquinone that remained in the mixture decreased sharply as the oxidation reaction proceeded. Within 4 min, 50% of the Hydroquinone was already consumed. After 30 min, the residual Hydroquinone was <3% of the initial amount, regardless of which formulation was used. During the initial 20 min, the amount of Hydroquinone remaining varied for the two formulations containing 0.08%. However, the difference in the amount remaining for these two formulations decreased after 20 min.

### GENERAL BIOLOGY

#### Absorption, Distribution, Metabolism, Excretion

Hydroquinone occurs as 4-hydroxyphenyl- $\beta$ -D-glucopyranoside (arbutin) in several types of plants, including cranberries, blueberries, and pears, and it is likely that Hydroquinone is found in other sources in the diet, such as coffee and whole wheat bread (Hill et al., 1993). Consumption of a diet containing foods with a higher natural Hydroquinone concentration leads to a greater concentration of Hydroquinone in the urine.

The *in vitro* rate of percutaneous absorption of an ~5% aqueous solution of [ $^{14}\text{C}$ ]Hydroquinone was determined for humans using stratum corneum and for rats using full thickness skin (Barber et al., 1992). Five samples were used for each. The rate of absorption was  $0.522 \pm 0.13 \mu\text{g/cm}^2/\text{h}$  for human stratum corneum and  $1.09 \pm 0.65 \mu\text{g/cm}^2/\text{h}$  for rat skin. The Hydroquinone solution produced

little or no damage to the skin samples, with "damage ratios" of near 1 for both the human and rat skin. The permeability constant ( $K_p$ ) for Hydroquinone through human stratum corneum was  $9.58 \times 10^{-6}$  cm/h. Using the terminology of Marzulli et al. (1969), Hydroquinone would be classified as a "slow" permeant for human skin.

Preparations containing [ $^{14}\text{C}$ ]Hydroquinone and varying amounts of a penetration enhancer, 1-dodecylazacycloheptan-2-one (Azone), and a sunscreen, the 2-ethylhexyl ester of 4-(dimethylamino)benzoic acid (Escalol 507), were prepared in an alcoholic vehicle and used to determine the percutaneous absorption of Hydroquinone in humans (Bucks et al., 1988). Each solution contained 2% (w/w) Hydroquinone, 26.8% (w/w) water, 0.2% (w/w) ascorbic acid, and 67.5–71.0% (w/w) ethanol (95%). Solutions 1 and 3 contained 3.0% (w/w) Escalol 507 and solutions 1 and 2 contained 0.5% (w/w) Azone. (Solution 4 contained neither the sunscreen nor the enhancer.) Six male subjects were used per group. A single 100- $\mu\text{l}$  dose of each [ $^{14}\text{C}$ ]Hydroquinone solution (4.8  $\mu\text{Ci}$ ) was applied to a 16- $\text{cm}^2$  area of skin on the forehead (125  $\mu\text{g}/\text{cm}^2$ ) for 24 h. The subjects collected all their urine for the intervals 0–4, 4–8, 8–12, and 12–24 h and days 1–5 following application.

The average percutaneous absorption of Hydroquinone was estimated from the amount eliminated in the urine. Hydroquinone was readily absorbed through the skin from an alcoholic vehicle, with estimated absorption of  $35 \pm 17\%$  for solution 1,  $66 \pm 13\%$  for solution 2,  $26 \pm 14\%$  for solution 3, and  $57 \pm 11\%$  for solution 4. Peak elimination occurred within the first 12 h following application, with total elimination occurring within 5 days. Escalol 507 significantly inhibited the absorption of Hydroquinone while Azone did not significantly affect absorption.

Six fasted male beagle dogs were used to determine the dermal absorption of [ $\text{U-}^{14}\text{C}$ ]Hydroquinone (Eastman Kodak Company, 1985a). Approximately 40  $\mu\text{Ci}$ , 15 ml, of a 4.5 g/L Hydroquinone in saline solution was added to a sealed glass absorption cell that was attached to the shaved thorax of each dog. (Cotton gauze was placed inside the cell for uniform distribution of the solution.) A 55.6- $\text{cm}^2$  area of skin was exposed to Hydroquinone for 60 min and then washed and rinsed. Blood samples and urine were collected at intervals for 48 and 120 h, respectively. Radioactivity was then measured by liquid scintillation counting.

The urinary excretion of radioactivity was low, with the greatest concentrations being detected in the 24- and 48-h collections. The total amount of  $^{14}\text{C}$  excreted after 48 and 120 h was equivalent to  $\sim 149$  and 170 nmol (11.9 and 13.6  $\mu\text{g}$ ) of Hydroquinone, respectively. A measurable concentration of [ $^{14}\text{C}$ ]Hydroquinone ( $<0.025$   $\mu\text{g}/\text{ml}$ ) was not detected in the blood after 60 min dermal exposure.

Fasted male beagle dogs were used to determine the distribution of [ $\text{U-}^{14}\text{C}$ ]Hydroquinone after intravenous (i.v.) administration (Eastman Kodak Company, 1985a). Approximately 3 or 4 ml of a [ $^{14}\text{C}$ ]Hydroquinone solution was administered rapidly through an indwelling foreleg i.v. catheter at doses of 1 mg/kg (10 or 100  $\mu\text{Ci}$ ) and 10 mg/kg (30  $\mu\text{Ci}$ ) to eight and three dogs, respectively. Expired air was collected continuously for 8 h from the low-dose group. After dosing, urine samples were collected at 4, 8, and 12 h and blood samples were collected at 8 and

12 h from both groups. Urine, feces, and blood samples were collected daily for 6 or 7 days. Major organs and tissues were excised from two dogs and assayed for residual radioactivity by liquid scintillation counting.

The radioactivity in the blood comprised 7–8% of the dose after 4 h for both groups; this value decreased to ~1% by 24 h. Blood  $^{14}\text{C}$  concentrations declined slowly from 19.0 nmol Eq/ml at 5 min to 1.1 nmol Eq/ml by 24 h following the 1 mg/kg dose and from 133.2 nmol Eq/ml at 5 min to 11.4 nmol Eq/ml by 24 h following the 10 mg/kg dose. There was an apparent absorption, distribution, and elimination phase during the first 24 h. The estimated mean half-life values for the  $\alpha$  and  $\beta$  phases were 1.3 and 7.2 h, respectively, for 1 mg/kg Hydroquinone and 1.0 and 8.0 h, respectively, for 10 mg/kg Hydroquinone.

Urinary excretion was rapid initially, with the 8- and 24-h cumulative excretion totals being 20 and 26%, respectively, for the 1 mg/kg dose and 53 and 60%, respectively, for the 10 mg/kg dose. The majority of the radioactivity was eliminated within 4 h following dosing. Between days 2 and 5, only an additional 6–7% of the radioactivity was excreted at either of the doses. At a dose of 1 mg/kg Hydroquinone, 34.5% of the radioactivity was accounted for by urinary excretion after 7 days. For the 10 mg/kg dose, 65.7% of the radioactivity was accounted for by urinary excretion after 5 days.

After 48 h, 4.6 and 2.5% of the radioactivity was recovered in the feces of the 1 and 10 mg/kg dose group animals, respectively. After 4–5 days, these values were 7.5 and 6.1%, respectively. The amount of radioactivity in the expired air of animals of the 1 mg/kg dose group was below the limit of detection (<0.05% of the dose). The overall recovery of  $^{14}\text{C}$  at 4–7 days was 42.0 and 71.8% for the low- and high-dose groups, respectively.

Twenty-four hours after i.v. administration of 1 mg/kg [ $^{14}\text{C}$ ]Hydroquinone, the skin contained an equivalent of 10.4% of the dose, the liver 0.6%, and the intestine 0.5%.

Using the 48-h excretion data from both the previous dermal and i.v. absorption studies, the average percutaneous absorption rate was determined to be ~0.15 nmol/cm<sup>2</sup>/min (1.1  $\mu\text{g}/\text{cm}^2/\text{h}$ ) (Eastman Kodak Company, 1985a).

Fasted female Fischer 344 rats, five per group, were given a single oral dose of 5, 25, or 50 mg/kg [ $^{14}\text{C}$ ]Hydroquinone (10  $\mu\text{Ci}$ ) in degassed, distilled water by gavage (Eastman Kodak Company, 1985b). Six rats, two per dose group, were given vehicle only and served as controls. Urine, feces, and expired  $\text{CO}_2$  were collected for 24 h. At the end of this period, all animals were killed and various tissues were analyzed for radioactivity.

[ $^{14}\text{C}$ ]Hydroquinone was rapidly absorbed and excreted. At all concentrations,  $\geq 86.7\%$  of the dose was excreted in the urine within the first 8 h after administration and  $\geq 91.8\%$  was excreted within 24 h of administration. After 8 h, 0.3–1.9% of the dose was recovered in the feces and after 24 h, 2.3–2.9% of the dose was recovered in the feces. Approximately 0.26–0.36% of the dose was recovered as expired  $\text{CO}_2$  after 24 h.

Upon examination of selected tissues, the liver had the greatest concentration of radioactivity and contained 0.51–0.62% of the dose. The intestine and kidneys contained  $\leq 0.06\%$  of the dose, whereas other examined tissues contained  $\leq 0.02\%$

of the dose. Between 0.64 and 0.90% of the dose was recovered in the carcass. A total of 1.26–1.64% of the dose was found in the examined tissues and carcass. The radioactivity was extractable in the small intestine (20–39%), carcass (14–24%), liver (1.7–3.7%), and kidneys (0–2.4%).

After 24 h, the overall recovery was  $\geq 96.1\%$ . Elimination of Hydroquinone was not dose-related for female Fischer 344 rats. Very little of the dose was excreted as unchanged Hydroquinone. After 8 h, Hydroquinone glucuronide accounted for 43.4–49.0% of the dose in the urine, and Hydroquinone sulfate accounted for 28.8–35.9%. Only 2.1–3.0% of the dose was excreted as unchanged Hydroquinone. Other metabolites of Hydroquinone were not detected.

Lightly anesthetized fasted male Fischer 344 rats, five per group, were given a single dose of 5, 25, or 50 mg/kg [ $U-^{14}C$ ]Hydroquinone (10  $\mu$ Ci) in degassed, distilled water by intratracheal instillation (Eastman Kodak Company, 1985c). Six rats, two per dose group, were given vehicle only and served as controls. Urine, feces, and expired  $CO_2$  were collected for 48 h. At the end of this period, all animals were killed and various tissues were analyzed for radioactivity.

As with the female rats in the previous study, [ $U-^{14}C$ ]Hydroquinone was rapidly absorbed and excreted by male Fischer 344 rats. At all concentrations,  $\geq 80.74\%$  of the dose was excreted in the urine within 8 h of administration and  $\geq 91.68\%$  was excreted within 48 h of administration. In the feces, 0.56–1.5% of the dose was recovered after 8 h; this value was 1.3–2.84% after 48 h. Approximately 0.13–0.20% of the dose was recovered as expired  $CO_2$  in 48 h. A total of  $\geq 93.86\%$  of the dose was excreted in the urine, feces, and expired air after 48 h.

Upon examination of selected tissues, the liver contained 0.12–0.43% of the dose, the lungs contained  $\leq 0.13\%$ , the kidneys contained  $\leq 0.08\%$ , the intestine, brain, testes, and fat contained  $\leq 0.04\%$ , and the femur, heart, and spleen each contained  $< 0.01\%$  of the dose. Between 0.93–1.71% of the dose was recovered in the carcass, and a total of 1.33–2.46% of the dose was found in the examined tissues and carcass. Significant extractable radioactivity was found in the lungs and the carcass.

After 48 h, the overall recovery was  $\geq 96.04\%$ . Urinary conjugates were the major metabolites of Hydroquinone. Hydroquinone glucuronide accounted for 48.76–67.21% of the dose in the urine, and Hydroquinone sulfate (ethereal) accounted for 19.00–22.07%. Only 2.00–2.85% of the dose was excreted as unchanged Hydroquinone.

A two-phase study was conducted using fasted male Fischer 344 rats to determine the effect of the route of administration of 50 mg/kg [ $U-^{14}C$ ]Hydroquinone (10  $\mu$ Ci) on blood elimination kinetics (Eastman Kodak Company, 1986). Prior to Phase I, a one-piece jugular catheter was implanted in each rat using a modification of the procedure by Upton (1975). In Phase I, lightly anesthetized rats, 3–4 per group, were given a single dose of Hydroquinone by gavage, intratracheal (i.t.) administration, or femoral i.v. injection. (For the rats receiving an i.v. injection, a second catheter was implanted in the femoral vein 2 days after implantation of the first catheter.) Blood samples were collected at various times for up to 8 h after dosing. At the end of this period, the animals were killed and their lungs removed.

In Phase II, three rats per collection period were dosed with Hydroquinone by gavage. At 10 min, 20 min, 40 min, 1 h, 2 h, or 4 h after dosing, blood was obtained from anesthetized rats via cardiac puncture and the rats were then killed.

In Phase I, >80% of the dose was excreted in the urine. For all routes of administration, distribution equilibrium was attained after 1.5–2.5 h. After i.v. administration of [<sup>14</sup>C]Hydroquinone, the blood concentration time curves of total radioactivity were biphasic, with mean half-lives of 18.7 and 326 min. After administration by gavage, the average absorption rate constant was 1.3 min, the  $T_{\max}$  for radioactivity in the blood was 6.5–7.5 min, and the decline of blood radioactivity was biphasic, with average half-lives of 14.8 and 626 min. Following i.t. administration, radioactivity absorption into the blood was very rapid; the maximum concentrations of radioactivity were obtained by the first sampling period at 2 min. The blood concentration time curves for i.t. administration were triphasic, with mean half-lives of 1.1, 22.1, and 425 min.

In Phase II, the mean concentrations of <sup>14</sup>C in the whole blood, plasma, plasma ultrafiltrates, and blood cells were determined. During 0–4 h, the concentration of <sup>14</sup>C in the blood, plasma, and plasma ultrafiltrates decreased. In blood cells, the radioactivity concentration declined during the first hour, and then remained constant. At this time, ~64% of the radioactivity in the blood was associated with the cells. At 4 h, the <sup>14</sup>C concentration in the plasma was greater than twofold the amount in the plasma ultrafiltrate.

In the plasma filtrate from the animals treated with [<sup>14</sup>C]Hydroquinone, at least four electrochemically active compounds were present that were not present in the plasma ultrafiltrate from untreated animals. Free Hydroquinone represented <1% of the total radioactivity in the plasma ultrafiltrate, declined rapidly during the first hour, and remained below the limit of detection (viz., 10 ng/g plasma ultrafiltrate) through 4 h. The conjugates of Hydroquinone, predominantly Hydroquinone glucuronide, were present in the plasma ultrafiltrate 40 min after administration of [<sup>14</sup>C]Hydroquinone by gavage.

Fischer 344 rats were used to determine the metabolic fate of Hydroquinone following a single oral dose, multiple oral doses, or dermal application (Eastman Kodak Company, 1988b). Each group consisted of 16 rats, eight per sex.

In the single oral dose study, two groups of rats were dosed with either 25 or 350 mg/kg [<sup>14</sup>C]Hydroquinone (10  $\mu$ Ci) by gavage. For the multiple oral dose study, rats were dosed with 25 mg/kg nonradioactive Hydroquinone for 14 days and 25 mg/kg [<sup>14</sup>C]Hydroquinone (10  $\mu$ Ci) on day 15 by gavage. The animals were fasted 4 h after dosing. For the dermal application, annular columnators were secured to the shaved dorsal surfaces of the rats, providing an exposure area of 2 cm<sup>2</sup>. Two groups of rats were then given applications of 25 mg/kg (58–86  $\mu$ l) or 150 mg/kg (338–497  $\mu$ l) [<sup>14</sup>C]Hydroquinone (20  $\mu$ Ci); the application site was covered by an occlusive patch for 24 h and then rinsed. (Technical difficulties were encountered in containing the Hydroquinone solutions to the 2 cm<sup>2</sup> application site.)

In each group, eight rats, four per sex, were used for collection of excreta and the remaining eight were used for collection of blood samples. The studies were conducted for at least 48 h and were generally terminated when ~90% of the radioactivity was recovered in the excreta. Excreta, such as urine and feces, and

cage rinsings were collected at 8 and 24 h after dosing and then every 24 h for up to 7 days. Blood samples were obtained from the orbital sinus at intervals for up to 96 h. At study termination, the animals were killed and various tissues were analyzed for radioactivity.

Radioactivity was rapidly excreted in the urine following oral administration of [ $^{14}\text{C}$ ]Hydroquinone. There was no significant difference observed between males and females. In the single oral dose study using 25 mg/kg Hydroquinone, ~81–82% and 89% of the dose was recovered in the urine after 8 and 24 h, respectively. In the group dosed with 350 mg/kg Hydroquinone, urinary radioactivity excretion after 8 h was 54 and 45% of the dose by males and females, respectively, and 85 and 82% of the dose, respectively, after 24 h. Following repeated dosing with 25 mg/kg, males and females excreted 77 and 87% of the dose in the urine, respectively, after 8 h, and 91 and 93% of the dose, respectively, after 24 h. Approximately 1–3% of the radioactivity was recovered in the feces after 24 h. (For one low-dose female, the urine and feces were not well separated and artifactually high radioactivity values were reported in the feces.)

Due to the difficulty in containing the Hydroquinone solution to the test site during dermal administration, the amount of Hydroquinone recovered by rinsing varied greatly after 24 h, from 23.1 to 98.5% of the radioactivity, regardless of sex. After dermal application of either 25 or 150 mg/kg Hydroquinone, mean values of the recovered radioactivity ranged from 61 to 71% following rinsing at 24 h. The mean total recovery of  $^{14}\text{C}$  in the urine ranged from 7.8 to 12.8%, and was recovered primarily in the initial 48 h. The mean 7-day  $^{14}\text{C}$  recovery in the feces was 1.7–3.7% of the dose, and the mean value recovered in the chamber rinsings ranged from 3.8 to 8.9%. (Due to application difficulty, the recovered radioactivity in the urine, feces, and chamber rinsings may not have been solely from the intended application site.)

The mean amount of radioactivity recovered in the tissues and carcass was 0.51–0.96% of the dose following oral administration. The liver and the kidneys had the greatest radioactivity concentrations, with greater concentrations being recovered from females than males. After all doses, ~0.2% of the dose was recovered from the livers of female rats. For males, 0.075% of the dose was recovered from the liver after single and repeated dosing with 25 mg/kg Hydroquinone, and 0.24% was recovered from the liver following a single administration of 350 mg/kg Hydroquinone. Approximately 0.02 and 0.01% of the dose was recovered from the kidneys of female and male rats, respectively, after single and repeated doses of 25 mg/kg, whereas 0.04 and 0.03% of the dose was recovered from the kidneys of the female and male high-dose group rats, respectively. The  $^{14}\text{C}$  concentrations in the other examined tissues were not significantly different from the concentration in the carcass.

The mean radioactivity recovered from the application site ranged from 0.1 to 2.2% of the dose, and 2.6–12.9% of the dose was recovered from the carcass and other tissues. The percentage of  $^{14}\text{C}$  recovered from the application site and from other tissues was highly variable following dermal application. The total  $^{14}\text{C}$  recovered after dermal administration was 90.1–94.6% of the dose.

After oral administration of [ $^{14}\text{C}$ ]Hydroquinone, the blood radioactivity con-

centration declined biphasically and was near or below the limits of quantitation ( $\sim 0.03\%$  of the dose) 24 h after dosing. After 8 h, the mean blood concentrations ranged from 0.1 to 0.4% of the dose. After a single oral dose of 25 mg/kg Hydroquinone, peak blood concentrations were 7.62  $\mu\text{g Eq/g}$  blood for males, reached at 0.24 h, and 7.19  $\mu\text{g Eq/g}$  blood for females, reached at 0.32 h. Following a single oral dose of 350 mg/kg Hydroquinone, peak blood concentrations were 39.2  $\mu\text{g Eq/g}$  blood for males, reached at 0.56 h, and 45.8  $\mu\text{g Eq/g}$  blood for females, reached at 0.80 h. After repeated oral doses of 25 mg/kg Hydroquinone, peak blood concentrations were 6.61  $\mu\text{g Eq/g}$  blood for males, reached at 0.16 h, and 6.03  $\mu\text{g Eq/g}$  blood for females, reached at 0.24 h. The half-life of the  $\alpha$  phase ( $\alpha T_{1/2}$ ) ranged from 0.2 to 1.7 h for males and females, with the greater values being observed for the high-dose group. Estimates of the half-life of the  $\beta$  phase ( $\beta T_{1/2}$ ) varied and could not be determined accurately because of the appearance of a second peak in the blood concentration versus time curve; the range was 2.8–10.5 h.

With the exception of some female animals, blood  $^{14}\text{C}$  concentrations were below the limit of quantitation ( $\sim 0.01\%$  of the dose or 0.06  $\mu\text{g Eq Hydroquinone/g}$  blood at the low dose) following dermal application of Hydroquinone. The greatest individual values observed were 0.47  $\mu\text{g Eq Hydroquinone/g}$  blood at 0.5 h for the low-dose group and 1.13  $\mu\text{g Eq Hydroquinone/g}$  blood at 1.0 h for the high-dose group. Blood  $^{14}\text{C}$  concentrations were not detectable at later collection times.

The amount of unchanged Hydroquinone in the urine ranged from undetectable ( $\sim 0.05\%$  of the dose) to 7.12% of the dose. Hydroquinone glucuronide accounted for 45–53% of the dose whereas Hydroquinone sulfate accounted for 19–33%. A small amount of the dose was excreted in the urine as the mercapturic acid conjugate of Hydroquinone.

The systemic bioavailability of dermally relative to orally administered Hydroquinone was estimated by the ratio of the urinary excretion of radioactivity after dermal and oral administration of 25 mg/kg [ $^{14}\text{C}$ ]Hydroquinone. The percentage of dermal absorption in male rats was estimated to be 10.5% using 72-h urine  $^{14}\text{C}$  recovery data. Using cumulative 48-h  $^{14}\text{C}$  recovery data, the percentage of dermal absorption in female rats was estimated to be 11.5%.

### IMMUNOLOGICAL EFFECTS

Hydroquinone is a hepatic metabolite of benzene that is carried by the blood to the bone marrow (Thomas et al., 1989); following benzene administration, high persistent concentrations of Hydroquinone and other benzene metabolites have been found in the bone marrow (Andrews et al., 1987; Rickert et al., 1979). These metabolites may accumulate in bone marrow and adversely affect hematopoiesis (Brown et al., 1987).

Two suggested mechanisms of benzene toxicity are: (a) the direct action of benzene metabolites, including Hydroquinone, with hematopoietic cells, and (b) by the action of benzene or its metabolites on bone marrow stromal cells (Gaido and Wierda, 1987). The combined impact of benzene metabolites on specific

cellular sites may be the cause of failure of bone marrow cellular proliferation after treatment with benzene (Snyder et al., 1989).

It should also be noted that Irons et al. (1992) has stated that "the potential of Hydroquinone to alter intrinsic growth factor response and induce differentiation in a myeloid progenitor cell population may be important to the pathogenesis of acute myelogenous leukemia secondary to benzene exposure. Benzene leukemogenesis may result from the dual ability of its metabolites to promote progenitor cell differentiation and induce cytogenetic changes in replicating cells."

Additional work has shown that the metabolism by pathways leading to the Hydroquinone conjugates of benzene in the cynomolgus monkey, as in rats and mice, was saturated at relatively low doses of benzene (Sabourin et al., 1992). The profile of urinary metabolites of benzene produced by the cynomolgus monkey was qualitatively similar, but quantitatively different, from that observed using rodents. The researchers believe that, since this dose effect has been observed in three different species, it is reasonable to expect that the same enzymatic pathways will also have a limited capacity in humans. The researchers stated: "If one assumes that urinary levels of Hydroquinone conjugates [and muconic acid] parallel tissue levels of Hydroquinone [and muconaldehyde], and that Hydroquinone [and muconic acid], either alone or in combination with other phenolic metabolites, are involved in benzene toxicity, then the saturation of pathways leading to Hydroquinone [and muconic acid] at low doses must be considered in any risk assessment."

Many of the studies summarized in this section also investigated the effects of benzene, phenol (a metabolite of benzene), and other metabolites of benzene, but only the results of exposure to Hydroquinone or Hydroquinone and phenol have been included.

C57BL/6 mice, four per group, were dosed either by i.v. or intraperitoneal (i.p.) injection twice daily for 3 days with 100 mg/kg Hydroquinone (Irons et al., 1983). Hydroquinone was immunosuppressive; spleen and especially bone marrow cellularity was reduced. A suppression of lipopolysaccharide (LPS) and LPS plus dextran sulfate (DXS)-stimulated plaque-forming cells was observed.

Groups of six male B6C3F1 mice were given i.p. injections, twice daily at 6-h intervals, of Hydroquinone and Hydroquinone with phenol (Eastmond et al., 1987). Hydroquinone administration (100 mg/kg, 2 times/day) produced a transient mild suppression of bone marrow cellularity after 3 days; after 12 and 36 days of dosing, bone marrow cellularity of the test group was not significantly different from that of the controls. Coadministration of 25-75 mg/kg Hydroquinone and 75 mg/kg phenol produced a significant decrease in cellularity after 12 days of dosing; a dose-response relationship was evident.

Two groups of five male DBA/2 mice were dosed concomitantly with 50 mg/kg Hydroquinone in Dulbecco's phosphate-buffered saline (PBS) without calcium or magnesium (PBS A) (5 ml/kg) and 50 mg/kg phenol in corn oil (5 ml/kg) by i.p. injection twice daily for 2 days (Pirozzi et al., 1989). A group of five male DBA/2 mice was dosed with 2 mg/kg indomethacin, an inhibitor of the cyclooxygenase component of prostaglandin H synthase (PHS), 1 h before and 3 and 6 h after being dosed with Hydroquinone/phenol. Two groups of five mice were dosed with

4 or 8 mg/kg of meclofenamate, also an inhibitor of the cyclooxygenase component of PHS, 1 h before dosing with Hydroquinone/phenol. Two control groups were dosed with vehicle, one group was given vehicle consisting of corn oil and 4.2% ethanol in PBS A and the other vehicle consisted of corn oil, water, and PBS A. The mice were killed and their femurs removed 17 h after the last dose. Administration of Hydroquinone/phenol significantly depressed bone marrow cellularity when compared to the controls. Neither indomethacin nor meclofenamate prevented the marrow depression caused by Hydroquinone/phenol.

Groups of DBA/2 mice, six to eight mice per group, were given a single i.p. injection of 50 mg/kg Hydroquinone in PBS A and 50 mg/kg phenol in corn oil, Hydroquinone/phenol and 8 mg/kg indomethacin, or vehicle (Pirozzi et al., 1989). The indomethacin was injected 1 h before dosing with Hydroquinone/phenol. The mice were killed and their femurs removed 4–5 h after dosing. Hydroquinone/phenol significantly increased bone marrow prostaglandin E (PGE) concentration when compared with controls; indomethacin prevented this effect.

Male B6C3F1 mice were given an i.p. injection of 75 mg/kg [ $^{14}\text{C}$ ]Hydroquinone (10  $\mu\text{Ci}/25$  g body wt) and killed 4 or 18 h after dose administration, with liver, kidneys, blood, and bone marrow samples being analyzed for covalent binding of radioactivity to acid-insoluble macromolecules (Subrahmanyam et al., 1990). The binding of [ $^{14}\text{C}$ ]Hydroquinone metabolites to hepatic, bone marrow, and renal macromolecules, but not blood macromolecules, was significantly greater after 18 h compared with the values for 4 h. At 18 h, the amount of radioactivity covalently bound in the liver was 2-, 8-, and 10-fold greater than that in the kidneys, blood, and bone marrow, respectively.

In mice given two injections of 75 mg/kg [ $^{14}\text{C}$ ]Hydroquinone, 6 h apart, and then killed 18 h after the second dose, a significant difference in  $^{14}\text{C}$  associated with tissue macromolecules was not observed. This may have been due to dilution of the radioactivity.

Also in the study by Subrahmanyam et al. (1990), Hydroquinone and phenol were coadministered (doses not given). The amount of [ $^{14}\text{C}$ ]Hydroquinone oxidation products covalently bound to blood and bone marrow macromolecules was significantly increased with Hydroquinone and phenol coadministration as compared with the administration of Hydroquinone alone. No significant change was observed in the amount of oxidation products bound in the liver and kidney.

Marrow cells obtained from the femurs of male B6C3F1 mice were treated with  $10^{-7}$ – $10^{-5}$  M Hydroquinone for 1 h and then functional B cells were assayed after 0, 24, 48, or 72 h incubation (King et al., 1987). Control cultures were also grown. Bone marrow cell cultures were manipulated and unmanipulated, that is, depleted of sIgM $^{+}$  cells and not depleted of sIgM $^{+}$  cells, respectively.

In the manipulated cell cultures, decreases in the number of pre-B cells in culture were delayed for at least 48 h by Hydroquinone administration when test cultures were compared with the controls; the number of small pre-B cells was significantly increased after treatment with all doses of Hydroquinone. The number of large pre-B cells was not significantly affected by Hydroquinone.

Twenty-four hours after treatment with Hydroquinone, the number of sIgM $^{+}$

cells was significantly less than the control value. The same pattern of altered B-lymphopoiesis was also seen in unmanipulated bone marrow cell cultures.

After treatment with Hydroquinone, marrow-adherent cell clusters (>25 cells) were counted and the number of adherent cell clusters was less than the control value after 48 and 72 h. Hydroquinone caused a concentration-dependent decrease in B-lymphocyte colony formation in culture (BL-CFC) after 24 and 48 h when the unmanipulated cultures were compared with the controls; similar results were observed for B cell-depleted cultures after treatment with Hydroquinone. The authors stated that phenotypic analysis of pre-B cell maturation in liquid bone marrow cultures suggested that this may be due to an inability of pre-B cells to mature into sIgM<sup>+</sup> B cells.

After 72 h, BL-CFC frequency of Hydroquinone-treated cultures was similar to control values. LPS-stimulated B cell proliferation in unmanipulated cultures was not affected by Hydroquinone treatment. The number of adherent stromal cell colonies that develop in short-term liquid bone marrow cultures was reduced by Hydroquinone; this may have been the reason for Hydroquinone-induced inhibition of pre-B cell maturation.

The authors noted that  $10^{-7}$  M Hydroquinone, the lowest concentration tested in this study, inhibited pre-B cell maturation; theoretically, this is the same concentration of Hydroquinone attained by humans exposed to 10 ppm benzene for 8 h (the current Occupational Health and Safety Administration threshold limit value) in the work place. The results of preliminary studies suggested that inhibition of pre-B cell maturation by Hydroquinone may be due to alterations in their interactions with adherent accessory cells. In this study, Hydroquinone exposure was acute; chronic exposure, which would be closer to actual environmental exposure, might even inhibit B-cell generation further (King et al., 1987).

The effect of *in vitro* Hydroquinone pretreatment on the intrinsic colony-forming response of murine bone marrow cells from male C57BL/6 mice stimulated with recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF) in the absence of conditioned medium was examined (Irons et al., 1992). Nonadherent bone marrow cells or enriched progenitor cells were pretreated with  $10^{-8}$ – $10^{-2}$  M Hydroquinone for 30 min. Colonies of  $\geq 50$  cells were scored on days 8 and 14. Phenol or catechol may have been coadministered with Hydroquinone.

Hydroquinone pretreatment of bone marrow cells resulted in a significant increase in GM-CSF-induced granulocyte/macrophage colonies (CFU-GM) at concentrations of  $10^{-8}$ – $10^{-5}$  M, with the maximum increase observed at  $10^{-6}$  M. Concentrations of  $10^{-3}$ – $10^{-2}$  M suppressed CFU-GM. In the absence of GM-CSF, there was no colony formation with Hydroquinone pretreatment. Coadministration of phenol did not affect the extent of the Hydroquinone-induced increase in CFU-GM, but the optimal concentration-response was achieved at  $10^{-10}$  M, not  $10^{-6}$  M, Hydroquinone. Catechol coadministration prevented the increase in CFU-GM at  $10^{-10}$ – $10^{-6}$  M Hydroquinone and potentiated Hydroquinone-induced suppression by 100-fold in the absence of a demonstrable decrease in cell viability.

In the enriched progenitor cell population, bone marrow cells that were >96%

depleted of cells expressing lineage-specific markers, a twofold increase in GM-CSF-induced CFU-GM was obtained. Marginal increases in macrophage colony-forming units (CFU-M) obtained when bone marrow cells were pretreated with Hydroquinone were not seen with lineage-depleted cells. The authors stated that this suggested that "any effect of Hydroquinone on CFU-M either is mediated via a more differentiated responding cell population not present in lineage-depleted cells or requires accessory cell involvement."

Bone marrow cells from Swiss-Webster and C57B1/6J (C57) mice were incubated with 10, 20, or 40  $\mu\text{M}$  Hydroquinone in NCTC-109 to assess bone marrow toxicity by determining the effect of Hydroquinone on the development of the colony forming unit-erythroid (CFU-e) (Neun et al., 1992). Cultures were also incubated with 10  $\mu\text{M}$  Hydroquinone mixed with other benzene metabolites. Control cultures, which consisted of untreated bone marrow cells, were used for all experiments. Mean values of CFU-e colony formation were determined from six replicate cultures.

Hydroquinone produced differential toxicity to the CFU-e between the two strains of mice at 20 and 40  $\mu\text{M}$ . Hydroquinone was more toxic to Swiss-Webster CFU-e than to C57 CFU-e. Combinations of Hydroquinone and other benzene metabolites affected the CFU-e of Swiss-Webster mice more severely than the CFU-e of C57 mice.

The hematopoietic toxicity of Hydroquinone was evaluated by determining the median inhibitory concentration ( $\text{IC}_{50}$ ), the concentration for which the cell viability in treated cultures is 50% of the viability of untreated controls, for 48 h using a number of cell lines (Ruchaud et al., 1992). The  $\text{IC}_{50}$  of Hydroquinone at 48 h was 0.009 mM for the human promyelocytic leukemia NB4, 0.016 mM for the rat promyelocytic cell line IPC-81, 0.03 mM for the myelomonocytic cell line WEHI-3b  $\text{D}^+$ , and 0.014 mM for the murine factor-dependent cell line DA1.

Macrophage and bone marrow-derived fibroblastoid stromal cell line (LTF) cultures were exposed to 10  $\mu\text{M}$  [ $^{14}\text{C}$ ]Hydroquinone in serum-free medium for 24 h at 37°C to examine the role of selective bioactivation and/or deactivation on the macrophage-selective effects of Hydroquinone (Thomas et al., 1990). The amount of covalently bound, [ $^{14}\text{C}$ ]Hydroquinone-derived radioactivity for macrophage cultures was 16-fold greater than that for LTF cells. A peroxide concentration-dependent bioactivation of Hydroquinone was observed in macrophage homogenates, but not in LTF cell homogenates, when increasing concentrations of hydrogen peroxide were added. In the absence of hydrogen peroxide there was no difference in bioactivation of Hydroquinone; with the addition of 40  $\mu\text{M}$  hydrogen peroxide, maximal bioactivation in macrophage homogenates was fourfold greater than in LTF homogenates. Autoxidation resulted in binding amounts of >1 nmol [ $^{14}\text{C}$ ]Hydroquinone Eq/mg protein for both macrophage and LTF cells.

The role of peroxidase in the bioactivation of Hydroquinone was examined. A time-dependent increase was observed in the amount of covalently bound radioactivity in samples containing hydrogen peroxide and myeloperoxidase (MPO); this increase was not seen in samples without hydrogen peroxide and/or MPO. After 15 min of incubation, a mean of 97 nmol/ml (two experiments) Hydroquinone was removed and a stoichiometric amount of 1,4-benzoquinone was formed,

as opposed to >91% Hydroquinone being present after 15-min incubation in the absence of hydrogen peroxide. The authors postulated that the selectivity of Hydroquinone may "be due, in part, to the selective ability of macrophages to bioactivate Hydroquinone and/or the selective inability to detoxify 1,4-benzoquinone."

Human promyelocytic leukemia cells (HL-60) were pretreated with noncytotoxic doses of Hydroquinone, 0.1–5  $\mu$ M, for 4 h to examine the effect of Hydroquinone on the ability of HL-60 to differentiate to marrow stromal macrophage following induction by tissue plasminogen activator (TPA) (Oliveira and Kalf, 1992). A dose-dependent inhibition of differentiation of promyelocytes to marrow stromal macrophages, as measured by the appearance of adherence, nonspecific esterase activity, and the ability to phagocytize sheep erythrocytes was observed. Hydroquinone had no effect on the proliferation of HL-60 cells, and it did not prevent the inhibition of cell proliferation observed after the addition of TPA. Also, Hydroquinone prevented differentiation of HL-60 cells to monocytes/macrophages induced by  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub> ( $1,25$ -(OH)<sub>2</sub>D<sub>3</sub>), a physiologic inducer. However, Hydroquinone did not have an effect on induction of differentiation to granulocytes by either dimethyl sulfoxide (DMSO) or retinoic acid.

The inhibition of erythropoiesis by Hydroquinone was determined by measuring the incorporation of <sup>59</sup>Fe into developing erythrocytes (Bolcsak and Nerland, 1983). Male Swiss-Webster mice were subcutaneously injected with 0.5 ml of 1.3 mmol/kg Hydroquinone in corn oil 12, 24, 48, or 72 h before administration of <sup>59</sup>Fe; ten rats per time period were used. The <sup>59</sup>Fe uptake assays were performed using a method modified from Lee et al. (1974). Control rats were given pure corn oil.

Administration of Hydroquinone 48 h before <sup>59</sup>Fe administration significantly inhibited <sup>59</sup>Fe uptake; <sup>59</sup>Fe uptake was not inhibited at any other time. Nineteen rats were dosed with 1.2 mmol/kg Hydroquinone 48 h before <sup>59</sup>Fe administration; <sup>59</sup>Fe uptake was inhibited by 20%.

Twenty rats were dosed with 1.2 mmol/kg Hydroquinone 48 h before <sup>59</sup>Fe administration. This group of rats was also given i.p. injections of 400 mg/kg 3-amino-1,2,4-triazole, a benzene oxidation inhibitor, 1 h before test article administration and then every 24 h until <sup>59</sup>Fe administration. The inhibition of <sup>59</sup>Fe uptake by Hydroquinone was not affected by administration of 3-amino-1,2,4-triazole.

Hydroquinone-induced inhibition of erythropoiesis was investigated using female Swiss albino mice (Snyder et al., 1989). The mice, number per group unspecified, were given i.p. injections of 50, 75, or 100 mg/kg Hydroquinone, 25–100 mg/kg Hydroquinone and 50 mg/kg phenol, 50 mg/kg Hydroquinone and 25–100 mg/kg phenol, or 50 mg/kg Hydroquinone and 1 or 2 mg/kg muconaldehyde 64, 48, and 40 h before administration of 1  $\mu$ Ci of <sup>59</sup>Fe. Control mice were given saline. Blood samples were taken 24 h following <sup>59</sup>Fe administration. The <sup>59</sup>Fe uptake assays were performed using a method modified from Lee et al. (1974, 1981).

Intraperitoneal administration of Hydroquinone, especially 75 and 100 mg/kg, inhibited <sup>59</sup>Fe uptake; phenol potentiated this inhibition. Simultaneous adminis-

tration of 50 mg/kg Hydroquinone and 1 mg/kg muconaldehyde significantly decreased uptake of  $^{59}\text{Fe}$  (singly, these compounds at the same concentrations did not reduce  $^{59}\text{Fe}$  uptake); 50 mg/kg Hydroquinone and 2 mg/kg muconaldehyde completely inhibited  $^{59}\text{Fe}$  uptake. The authors concluded that the combined impact of benzene metabolites on various cellular sites may be the cause of failure of bone marrow cell proliferation.

A study performed by Guy et al. (1990) also involved administering Hydroquinone or Hydroquinone and phenol to female Swiss albino mice by i.p. injection 64, 48, or 40 h before i.v. injection of  $^{59}\text{Fe}$  (1  $\mu\text{Ci}/\text{mouse}$ , containing 20–40 ng Fe as Fe citrate). There were six mice per group, and 25–100 mg/kg Hydroquinone, 25–100 mg/kg Hydroquinone and 50 mg/kg phenol, or 50 mg/kg Hydroquinone and 25–100 mg/kg phenol was administered. Control animals were given equal amounts of corn oil or saline. The mice were killed 24 h after  $^{59}\text{Fe}$  administration and blood samples were taken. The  $^{59}\text{Fe}$  content was determined in a  $\gamma$  scintillation counter.

Hydroquinone, 75–100 mg/kg, decreased iron uptake. Iron uptake was significantly decreased compared with control values at all doses when Hydroquinone and phenol were coadministered, regardless of which compound was held constant and which was varied. The decreased iron uptake caused by the coadministration of Hydroquinone and phenol was greater than the decrease induced by Hydroquinone alone.

P388D1 cells were pretreated with Hydroquinone and Western blot analysis was performed (Renz and Kalf, 1990). At concentrations  $\geq 1 \mu\text{M}$  Hydroquinone for exposure periods of  $>30$  min, interleukin-1- $\alpha$  (IL-1- $\alpha$ ) was significantly reduced in cell lysates following LPS stimulation. The reduction of IL-1- $\alpha$  at 1  $\mu\text{M}$  Hydroquinone was not accompanied by a decrease in cell viability, total cellular protein concentration, DNA synthesis, or protein synthesis.

Rat splenic lymphocytes were treated with various concentrations of Hydroquinone and cultured for 3 days in the presence of a T-cell mitogen. Hydroquinone,  $10^{-6}$  M, suppressed the lymphoproliferative response (LPR) without being cytotoxic or reducing ATP concentrations within the cells; LPR enhancement was optimal at  $10^{-7}$  M Hydroquinone, with near-control LPR values being reached with  $10^{-8}$  M Hydroquinone.

## NEUROTOXICITY

A study was performed in which a functional-observational battery (FOB) was used to detect functional impairment of the nervous system by Hydroquinone (Eastman Kodak Company, 1988c). Sprague-Dawley rats, 10 males and 10 females per group were dosed orally by gavage 5 days per week for 13 weeks with 20, 64, or 200 mg/kg/day Hydroquinone in degassed, distilled water at a concentration of 5%. A group of 10 male and 10 female rats was given a volume of distilled water equal to that of the highest dose group and used as controls.

Clinical observations for each rat were made immediately after dosing; on non-dosing days, all animals were observed for mortality. Body weights were determined 3 days before study initiation and body weights and feed consumption were determined on days 0, 1, 4, and 7 and then weekly.

The FOB, which included observational procedures to detect any unusual response concerning body position, degree of activity, coordination of movement, gait, unusual or bizarre behavior, the presence of extraordinary signs, or changes in sensory function, was performed on all animals 3 days before study initiation, at 1, 6, and 24 h after administration of the initial dose. On study days 7, 14, 30, 60, and 91, the FOB was conducted before dosing. Quantitative grip strength was determined when the FOB was performed according to the procedure described by Meyer et al. (1979) and modified by Mattsson et al. (1986). Historical control data were used to demonstrate the sensitivity of the FOB.

At study termination, six males and six females from each group were perfused systematically for neuropathologic examination. The remaining rats underwent necropsy without perfusion. Brain and kidney weights were determined for all animals. Selected tissues from perfused animals were collected for neuropathology analysis.

Treatment-related mortality was not observed during the study. Urine was discolored brown throughout the study for males and females of all dose groups. All animals of the 200 mg/kg dose group and all females of the 64 mg/kg dose group had minimal to minor tremors; seven males of the 64 mg/kg dose group had tremors of minimal severity. Recovery from tremors was rapid. Tremors were observed between 30 min and 1 h after dosing for males and 4–25 min after dosing for females of the 200 mg/kg group. The incidence of tremors was significantly greater for all animals of the 200 mg/kg group during weeks 1–13 and for males of this group during week 14 as compared with controls. For the 64 mg/kg dose group, the incidence of tremors was significantly greater than control values during weeks 6, 7, and 9–13 for males and during weeks 2, 3, 5, 6, 9, and 11–13 for females.

Minimal to mild depression of general activity was statistically greater than that observed for the controls during weeks 1–13 for males and during weeks 4 and 8 for females of the 200 mg/kg dose group; minimal to mild depressed activity was observed for all males and five females. In the 64 mg/kg dose group, minimal depression of general motor activity was observed for one male during week 6, for two males during week 8, and for two females during week 4. Tremors and depressed activity were not observed for the 20 mg/kg dose group. Additional clinical signs were observed, but were not considered dose related.

The mean body weight of the males of the 200 mg/kg dose group was slightly reduced when compared with the mean body weight of controls from day 1 until study termination. This difference was statistically significant at necropsy for perfused males. Feed consumption for males of the 200 mg/kg dose group was statistically less than that of the controls on days 0–4. There were no significant differences in body weight or feed consumption for males in the 20 or 64 mg/kg dose group or any test females when compared with controls.

During FOB examinations, behavioral changes associated with Hydroquinone administration were observed primarily during the 1 and 6 h examinations. For females of the 200 mg/kg dose group, a statistically significant decrease in activity while being removed from the cage was observed at the 1-h FOB. A statistically significant decrease in home cage and locomotor activity was observed for males

of the 200 mg/kg dose group at the 6-h observation. Home cage and locomotor activity was decreased for the males of the 64 mg/kg dose group compared with controls at 6 h, but the decrease was not statistically significant.

Tremors were observed for two males of the 200 mg/kg dose group at the 1-h FOB; although this observation was not significantly different when compared with controls, it was thought to represent a real effect related to Hydroquinone. Tremors were also observed for one male of the 200 mg/kg dose group during the 30-day FOB examination. The incidence of tremors was significantly increased for females of the 200 mg/kg dose group at the 1-h FOB when compared with controls.

The mean forelimb grip strength of females of the 64 mg/kg dose group was significantly less than that of the controls before dosing; no other significant differences in grip strength were observed.

Additional random statistically significant behavioral differences, not related to dose or sex, were also observed; the differences generally did not persist with time.

The mean terminal body weight of perfused males of the 200 mg/kg dose group was significantly less than the controls. There were no significant differences observed for mean brain or kidney weights for dosed animals compared with the controls. No Hydroquinone-related signs were observed at neurologic examination and no lesions were observed at necropsy. There was no evidence that Hydroquinone resulted in lesions of the central or peripheral nervous system. The no-observed-effect concentration of Hydroquinone was 20 mg/kg; central nervous system stimulation was observed with 64 and 200 mg/kg Hydroquinone (Eastman Kodak Company, 1988c).

### NEPHROTOXICITY

Male and female Fischer-344 (F344) and Sprague-Dawley rats were dosed with Hydroquinone to determine its nephrotoxic effects (Boatman et al., 1992). Three to six rats per sex per group were dosed orally by gavage with 50–400 mg/kg Hydroquinone in degassed water. Indwelling jugular vein cannulas were placed in some rats using a procedure modified from Jongen and Norman (1987); these rats were allowed to recover for 48 h before the test. Another group of rats was pretreated with 10 mg/kg acivicin, a  $\gamma$ -glutamyltransferase ( $\gamma$ -GT) inhibitor, by i.p. injection 1 h before Hydroquinone administration. Two groups of rats were dosed with aqueous solutions of 125 or 250  $\mu$ mol/kg 2-(glutathion-*S*-yl)hydroquinone via indwelling jugular vein cannulas. Urine and blood samples were obtained at various times for up to 96 h.

Enzymuria and glucosuria were greatest for female F344 rats; however, male F344 rats had a similar pattern. After 8 h, in female F344 rats dosed with 400 mg/kg Hydroquinone, urinary alanine aminopeptidase (AAP), *N*-acetylglucosaminidase (NAG), alkaline phosphatase (ALP),  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), and creatinine values were significantly increased as compared with controls. Urinary NAG values were also significantly increased for female F344 rats given 200 mg/kg Hydroquinone. Urinary glucose and blood urea nitrogen (BUN) values were significantly increased after 24 and 48 h, respectively, for female F344 rats

dosed with 400 mg/kg. For male rats dosed with 400 mg/kg Hydroquinone, urinary AAP, NAG, and  $\gamma$ -GT values were significantly increased compared with controls after 8 h. After 24 h, serum creatinine values were significantly increased for female F344 rats and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values were significantly increased for male F344 rats dosed with 400 mg/kg Hydroquinone.

There were decreases in urinary osmolality with slight diuresis at 8 h for all dosed male and female F344 rats. The decreased osmolality was statistically significant for males and females dosed with 400 mg/kg Hydroquinone. Diuresis was significant for female F344 rats of the 200 or 400 mg/kg dose groups. At microscopic analysis of urine, a significant increase was observed in the epithelial cells (counts/hpf) for female F344 rats dosed with 200 mg/kg Hydroquinone and for male and female F344 rats dosed with 400 mg/kg Hydroquinone as compared with their predose values.

The only significant effect on urinary parameters for Sprague-Dawley rats was that of a decreased BUN value after 48 h for female rats dosed with 400 mg/kg Hydroquinone. No significant changes were observed in measured hematology values. Urinary osmolality and slight diuresis was observed after 8 h for rats of all dose groups.

For cannulated female F344 rats, significant increases were observed in urinary AAP, NAG, ALP, and  $\gamma$ -GT values after 8 h and urinary glucose values after 24 h for those rats dosed with 400 mg/kg and a significant increase was observed in urinary NAG values after 8 h for those rats dosed with 200 mg/kg Hydroquinone as compared with controls. For cannulated male F344 rats dosed with 200 mg/kg Hydroquinone, a significant increase was observed in urinary ALP values after 8 h. Also after 8 h, statistically significant diuresis was observed for female F344 rats dosed with 200 mg/kg and male Sprague-Dawley rats dosed with 400 mg/kg Hydroquinone.

Cannulated male F344 rats dosed with 250  $\mu$ mol/kg 2-(glutathion-S-yl) Hydroquinone had significant increases in urinary AAP, NAG, ALP, and  $\gamma$ -GT values after 8 h and in urinary glucose and BUN values after 24 and 48 h, respectively. These patterns of enzymuria and glucosuria were similar to those observed for male F344 rats dosed with 400 mg/kg. For rats dosed with 250  $\mu$ mol/kg 2-(glutathion-S-yl) Hydroquinone, significant diuresis was observed after 24 h.

For male F344 rats pretreated with acivicin and dosed with 400 mg/kg Hydroquinone, urinary AAP and NAG values were significantly increased after 8 h and urinary  $\gamma$ -GT and BUN values were significantly decreased after 8 and 48 h, respectively, as compared with control values. The increases in AAP and NAG values in the pretreated group were not as great as the increases observed for male F344 rats dosed with 400 mg/kg Hydroquinone without acivicin pretreatment. However, using a ranked data analysis, there was no significant difference in AAP, NAG, or ALP values among the Hydroquinone, acivicin, or Hydroquinone and acivicin groups.

At microscopic examination of the kidneys, alterations included cell regeneration, slight mineralization, slight focal necrosis, and granular casts within the proximal tubules of F344 rats, with the severity and occurrence of these lesions

corresponding to the severity of enzymuria and glucosuria. No significant microscopic observations were made for selected male Sprague-Dawley rats. No increase in the severity of hyaline droplet formation was observed in either strain of rat.

The nephrotoxic effects of Hydroquinone were also evaluated using male and female B6C3F<sub>1</sub> mice dosed with 350 mg/kg Hydroquinone in degassed water (Boatman et al., 1992). (The number of mice used was not given.) The dose of 350 mg/kg was chosen because of the high mortality at the dose of 400 mg/kg Hydroquinone. Blood samples from anesthetized mice and urine were collected at various intervals for 48 h.

Urinary creatinine values were significantly increased for male mice after 12 h and urinary BUN values were significantly increased for both male and female mice after 48 h as compared with control values. Because of large standard errors recorded for the measurement of serum values, none of the observed increases were statistically significant. No significant diuresis was observed for the mice.

Male and female F344 and male Sprague-Dawley rats were used to determine whether Hydroquinone induced renal cell proliferation and to study the relationship between the onset of nephrotoxicity and cell proliferation (English et al., 1992a). Groups of ten F344 rats, five/sex/group, were dosed orally by gavage with 2.5, 25, or 50 mg/kg Hydroquinone and a group of ten male Sprague-Dawley rats was dosed with 50 mg/kg Hydroquinone in degassed, distilled water at a constant volume of 5 ml/kg. Ten rats of both strains were used as controls and given vehicle only. All rats were dosed 5 days/week, with the F344 rats being dosed for 1, 3, or 6 weeks and the Sprague-Dawley rats being dosed for 6 weeks. Body weights were determined weekly. Urine samples were collected 8 and 24 h after the final dose. At necropsy, the kidneys and a 2 in section of duodenum were preserved.

Osmotic pumps were implanted subcutaneously into the mid-dorsal lumbar area of the rats in order to deliver bromodeoxyuridine (BrDU) continuously for 3 days before killing the animals. Immunohistochemical staining was done using a method modified from Goldsworthy et al. (1991). Sections were examined and scored without knowing the dose given to the animal.

There were no significant differences in body weight and feed consumption between treated and control animals. For male F344 rats, significant increases were observed in urinary ALP values after 1 week of dosing and in urinary AAP and  $\gamma$ -GT values after 6 weeks of dosing with 50 mg/kg Hydroquinone. These changes were considered suggestive of an alteration of the integrity of the proximal tubular membrane and, possibly, necrosis. No other significant changes in urinary parameters were observed for male F344 rats. No significant changes in urinary parameters were observed for female F344 or male Sprague-Dawley rats.

At microscopic examination, renal toxicity was detected in male F344 rats, but not in female F344 or male Sprague-Dawley rats. Tubular degeneration in male F344 rats appeared dose-related. There was no difference observed in the incidence and severity of hyaline droplet formation in the kidneys of treated and control rats.

A significant increase in cell proliferation was observed in tubule segment P2 for female F344 rats dosed for 3 weeks with 2.5 mg/kg ( $p < 0.01$ ) and 50 mg/kg

Hydroquinone ( $p < 0.01$ ;  $p < 0.001$ ); a significant increase was also observed in total cells for female rats of these groups. After 6 weeks of dosing, cell proliferation in tubule segments P1 and P2 and the total cells were significantly increased ( $p < 0.01$ ;  $p < 0.001$ ) for male F344 rats of the 50 mg/kg dose group. For male F344 rats of the 25 mg/kg dose group after 6 weeks, cell proliferation increased 18 and 34% in the P1 and P3 segments as compared with controls; these increases were not considered statistically significant ( $p > 0.01$ ). For female F344 rats after 6 weeks of dosing with 25 mg/kg Hydroquinone, a significant ( $p < 0.01$ ) increase in cell proliferation in tubule segment P1 and in total cells was observed. The authors stated that for female F344 rats, the increases in cell proliferation "were apparently not related to chemical administration, due to the absence of any dose-response relationship." There were no significant differences in cell proliferation between treated and control male Sprague-Dawley rats.

The nuclease  $P_1$ -enhanced  $^{32}\text{P}$ -postlabeling assay of Reddy et al. (1989), which allows detection at the level of one adduct in  $10^2$ - $10^{10}$  DNA nucleotides, was used to determine whether aromatic DNA adducts derived from Hydroquinone or its metabolites were formed in the kidneys of F344 rats following repeated Hydroquinone administration at doses that were tumorigenic in male rats (English et al., 1992b). Three groups, four rats/sex/group, were dosed orally by gavage with 2.5, 25, or 50 mg/kg Hydroquinone in degassed, distilled water at a constant volume of 5 ml/kg, 5 days/week for 5 weeks. A control group of eight rats, four per sex, was given vehicle only. Body weights were determined weekly. Urine was collected at 8 and 23 h after the final dose.

There were no significant differences in the body or kidney weights between treated and control rats. For male rats, significant increases were observed in urinary AAP values at 50 mg/kg, NAG values at 25 and 50 mg/kg, and creatinine values at 2.5 mg/kg Hydroquinone as compared with control values. These differences were suggestive of an alteration in the integrity of the proximal tubular membrane and possibly necrosis. No significant changes in urinary parameters were observed for female rats.

Isolation of DNA from whole kidney homogenates resulted in an alcohol-precipitated DNA pellet that appeared brownish; the brownish discoloration was considered indicative of coprecipitation of impurities. Thin-layer chromatograms of  $^{32}\text{P}$ -postlabeled preparations revealed one major spot and several minor spots that were chromatographically dissimilar from the major in vitro adducts of Hydroquinone [and *p*-benzoquinone]. A loss or substantial reduction in "adducts" following repurification of the DNA was considered suggestive of the adducts not arising from Hydroquinone administration.

DNA pellets isolated from kidney nuclei were not discolored. There were no significant differences in the chromatograms of postlabeled DNA from control rats or those of rats dosed with 50 mg/kg Hydroquinone, and no extra spots were observed in the treated chromatograms. There was no exposure-related increase in relative adduct labeling (RAL) values between treated and control samples in areas 6 and 7, where the major in vitro adducts of Hydroquinone [and *p*-benzoquinone] migrate. Also there was no increase in RAL values of other endogenous adducts. Significant reductions were observed in the RAL values of endogenous

adduct spots 2, 3, and 4 and zone 8 in males and spots 1, 4, and 6 and zone 8 in females. Because there were no significant increases in DNA adducts observed for the 50 mg/kg group, DNA adducts from the kidneys of rats of the 2.5 and 25 mg/kg groups were not assayed. The authors concluded that "these data suggest that Hydroquinone produces benign renal tumors in male F344 rats via a non-genotoxic mechanism."

### CYTOTOXICITY

The  $EC_{50}$  of Hydroquinone was reported to be 0.8 ppm, with a 95% confidence range of 0.5–1.2 ppm (Christian et al., 1976).

The cytotoxicity of Hydroquinone to cultured rat hepatoma cells, HTC line, was examined (Assaf et al., 1986). A dose-dependent response was observed, with a dose of 333  $\mu\text{g/ml}$ , 300  $\mu\text{M}$ , producing 40% cellular mortality after 24 h and 100% cellular mortality after 72 h. A dose of 66  $\mu\text{g/ml}$ , 600  $\mu\text{M}$ , resulted in 100% cellular mortality after 24 h.

The effect of Hydroquinone on human burst-forming unit-erythroid (BFU-E) colony formation by nonadherent bone marrow cells in plasma clots, measured as numbers of benzidine-positive colonies, was examined (Brown et al., 1987). After 7 days of incubation, the cultures were treated with  $5 \times 10^{-5}$  M Hydroquinone and maintained for another 7 days. BFU-E growth in the presence of  $5 \times 10^{-5}$  M Hydroquinone was 31% of the value obtained for the control cultures. The results of this study indicated cytotoxicity toward stem and stromal cells by Hydroquinone.

The effect of Hydroquinone on stromal cell function was examined by treating mouse bone marrow cells with  $10^{-7}$ – $10^{-4}$  M Hydroquinone on culture day 7 (Gaido and Wierda, 1987). After 3 days, the medium was removed and untreated bone marrow cells in agar were plated over the existing stromal cell layer. Stromal cell supported granulocyte/monocyte formation was decreased at concentrations of  $10^{-5}$  and  $10^{-4}$  M but increased at a concentration of  $10^{-7}$  M Hydroquinone. In a separate set of experiments,  $10^{-7}$ – $10^{-5}$  M Hydroquinone, with the same treatment procedure, did not significantly alter total adherent stromal cell number;  $10^{-4}$  M Hydroquinone decreased the cell number by 39%.

It was then determined whether the effect of Hydroquinone on stromal cell function was due to prostaglandin synthesis. The cultures were treated using the same procedure as above, with the exception that some cultures were treated with  $10^{-6}$  M indomethacin 1 h before Hydroquinone treatment. (This did not completely inhibit prostaglandin synthesis.)

Treatment with Hydroquinone alone produced results similar to those previously reported. Pretreatment with indomethacin significantly reduced the inhibition of stromal cell function by Hydroquinone, but stromal cell function was not enhanced by treatment with  $10^{-6}$  M indomethacin followed by  $10^{-7}$  M Hydroquinone. Radioimmunoassay (RIA) results were that treatment with increasing doses of Hydroquinone insignificantly increased prostaglandin  $E_2$  ( $\text{PGE}_2$ ) concentrations.

The  $\text{PGE}_2$  concentration necessary to significantly inhibit stromal cell-

dependent myelopoiesis was  $10^{-7}$  M; increasing doses of Hydroquinone did not increase PGE<sub>2</sub> to inhibitory concentrations. Pretreatment with indomethacin did not significantly decrease PGE<sub>2</sub> concentrations. The authors concluded that the toxicity of Hydroquinone to stromal cells may be due to further activation of Hydroquinone to other toxic moieties within the stroma.

The lymphoma-derived Raji cell line, the erythroleukemia cell line K 562, and the melanotic cell lines IRE 1 and IRE 2 were cultured in the presence of  $5 \times 10^{-5}$ – $5 \times 10^{-3}$  M Hydroquinone for 2–48 h (Passi et al., 1987; Picardo et al., 1987). Five experiments were completed in quadruplicate. The survival rates of the cultures treated with 0.1 mM Hydroquinone were  $62 \pm 7$ – $69 \pm 6\%$  after 24 h; the cell line K 562 was least affected and cell line IRE 1 was most affected. Treatment with 0.1 mM Hydroquinone for 24 h resulted in survival rates of  $20 \pm 4$ – $24 \pm 4\%$ , with cell line IRE 2 being least affected and Raji cell line being most affected. Preincubation with Hydroquinone in the medium for 24 and 48 h decreased the cell survival rate; addition of oxygen radical scavengers to the medium, singly or in combination, increased cell survival rates.

Day 5 macrophage cultures, day 3 LTF cultures, and day 14 primary fibroblast cultures were each exposed to various doses of Hydroquinone for 48 h; cell viability was then determined (Thomas et al., 1989). Macrophage viability was significantly reduced at doses of  $\geq 10^{-5}$  M Hydroquinone; some cell death was observed with  $10^{-6}$  M Hydroquinone. LTF cell viability was only affected at a dose of  $10^{-4}$  M Hydroquinone;  $10^{-6}$  M Hydroquinone did not affect innate colony-stimulating activity of primary fibroblast cell cultures.

In order to determine whether a decreased support of myelopoiesis by bone marrow cells after in vitro treatment with Hydroquinone was due to toxicity of bone marrow macrophages or fibroblastoid stromal cells, macrophages were exposed to  $10^{-8}$ – $10^{-4}$  M Hydroquinone and LTF cultures were exposed to  $10^{-7}$ – $10^{-4}$  M Hydroquinone for 48 h. LTF cells were reconstituted with treated macrophages. The reconstituted cultures were cocultured after 24 h and the number of granulocyte/macrophage colonies (G/M-CFU-C) that formed after 7 days was determined.

Hydroquinone reduced macrophage activity at a concentration of  $10^{-7}$  M; significant reductions were observed at doses of  $\geq 10^{-6}$  M. Treated LTF cells that were reconstituted with untreated macrophages and then cocultured were not significantly affected by Hydroquinone. When LTF cells were treated with  $10^{-4}$  M Hydroquinone, a decrease in constitutive production of colony-stimulating activity was observed.

Cultures of primary fibroblastoid cells isolated from fresh bone marrow adherent cells containing >95% fibroblastoid cells and macrophages were reconstituted with Hydroquinone-treated macrophages and cocultured. Reconstitution with macrophages that were treated with  $10^{-6}$  M Hydroquinone inhibited production of colony-stimulating activity and a significant decrease in the ability to support G/M-CFU-C formation was observed.

To determine the effect of Hydroquinone on macrophage interleukin-1 (IL-1) production, IL-1 activity was measured in conditioned medium that was removed from Hydroquinone-treated LPS-stimulated macrophage cultures. A dose of  $10^{-6}$

*M* Hydroquinone significantly reduced IL-1 activity. The authors concluded that Hydroquinone specifically interferes with bone marrow stromal macrophage activity, possibly due to a reduction in IL-1 activity (Thomas et al., 1989).

Hydroquinone, 1  $\mu$ M–1 mM, was added to keratinocytes in culture (Picardo et al., 1990a). Cytotoxic activity was observed at concentrations  $>10 \mu$ M, with the effect being correlated to the rate of decomposition. The presence of oxygen radical scavenger enzymes significantly reduced the toxicity. A significant release of arachidonic acid from cell membranes was observed after 1–2 h incubation with  $>100 \mu$ M Hydroquinone. Hydroquinone “may produce a toxic effect on human keratinocytes, partially due to the production of oxygen radicals and to the generation of oxidation products. These phenomena may induce the release of lipoxygenase substrates and lead to inflammatory reactions.”

Bone marrow cells from the femurs of male C57Bl/6J and DBA/2J mice were used to establish primary adherent stromal cell cultures; the cultures were treated with various concentrations of Hydroquinone for 24 h (Twerdok and Trush, 1990). Stromal cells derived from DBA/2 mice were significantly more sensitive to Hydroquinone than the cells from C57Bl/6 mice; the lethal concentration<sub>50</sub> (LC<sub>50</sub>) for DBA/2 cells was  $49.2 \pm 2 \mu$ M and for C57Bl/6 cells was  $95.3 \pm 13.5 \mu$ M. Induction of quinone reductase by *t*-butylhydroquinone before treatment with Hydroquinone protected against Hydroquinone toxicity.

## ANIMAL TOXICOLOGY

### Acute Toxicity

#### Oral

Groups of 4–20 rats, equal number of males and females per group, were dosed orally with fresh preparations of 180–2,100 mg/kg Hydroquinone or 1-week-old preparations of 420–940 mg/kg Hydroquinone as 5% aqueous solutions (Christian et al., 1976). The animals were observed for 3 weeks for adverse effects and mortality. The oral LD<sub>50</sub> was determined to be 743 and 627 mg/kg for male and female rats, respectively. All adverse effects were almost immediate (usually within 2–10 min) upon dosing. Clinical observations included hyperactivity and hypersensitivity to auditory and tactile stimuli, tremors, moderate clonic convulsions, severe tonic spasms, protuberant and dark eyes, and a discoloration of the nose, lips, and paws. Generally, mortality occurred within 2 h after dosing. After 24 h, some animals were still hypersensitive to tactile stimuli and had a transient decrease in body weight. All surviving animals were normal within 3 days after dosing. Results were similar for both fresh and aged solutions.

In a metabolic study (described earlier), oral administration of 350 mg/kg Hydroquinone by gavage resulted in reduced activity in male rats (Eastman Kodak Company, 1988b). Female rats had mild tremors with chewing and minimal reduced activity during a 15–45-min period after test article administration. At 3.5–4.0 h after dosing, the animals appeared normal. No adverse effects were observed following dosing with 25 mg/kg Hydroquinone.

The approximate lethal oral dose of Hydroquinone was 0.2 g/kg for rabbits and 0.08 g/kg for cats (Deichmann, 1983).

### Short-Term Toxicity

#### Oral

Male and female rats, six/sex/group, were dosed with 2,500–10,000 ppm Hydroquinone in drinking water for 8 weeks (Christian et al., 1976). A control group was given drinking water only. Clinical observations were made daily. Body weights and feed consumption were measured weekly; water was changed and consumption was measured three times weekly. Changes in microsomal hepatic enzymes were evaluated by measuring the length of the period of sleep induced by an i.p. injection of 125 mg/kg sodium hexobarbital; sleep time was considered the timed interval between loss and regaining of the righting reflex. Blood samples were taken during the study. At the termination of dosing, the animals underwent necropsy.

There was no mortality during the study, and no adverse clinical observations were noted. Growth was decreased for all rats of the 10,000 ppm and for female rats of the 5,000 ppm dose groups. There was no significant difference in feed consumption between treated and control rats; water consumption decreased proportionate to increased doses of Hydroquinone. There were no significant differences in hematologic parameters between treated and control animals. For male rats, the period of sleep induced by sodium hexobarbital decreased with increasing doses of Hydroquinone; this effect was not seen for female rats. Absolute liver and kidney weights of animals dosed with Hydroquinone increased with increasing concentrations of Hydroquinone, with the increased liver weights being significant for male rats of the 5,000 ppm dose group and male and female rats of the 10,000 ppm dose group. Organ-to-body weight ratios were significantly increased for the livers and kidneys of all rats of the 5,000 and 10,000 ppm dose groups and for the kidneys of female rats of the 2,500 ppm dose group. No significant microscopic observations were made.

F344/N rats and B6C3F<sub>1</sub> mice, five/sex/group, were dosed with Hydroquinone in corn oil by gavage 5 days/week; 12 doses were given over 14 days (NTP, 1989; Kari et al., 1992). The rats were dosed with 63, 125, 250, 500, or 1,000 mg/kg and the mice with 31, 63, 125, 250, or 500 mg/kg Hydroquinone. A group of rats and a group of mice were dosed with vehicle only and served as controls. All animals were observed daily and body weights were measured on days 0, 7, and 14. The animals were fasted overnight and killed for necropsy.

All male and female rats of the 1,000 mg/kg group and one male and four females of the 500 mg/kg group died on study. One male of the 1,000 mg/kg group died accidentally. Four male and five female mice of the 500 mg/kg group and two male mice of the 250 mg/kg group died on study. A few other mice died because of gavage accidents.

Tremors, which lasted  $\leq 30$  min, were observed for rats of the 500 and 1,000 mg/kg groups. For male rats of the 1,000 mg/kg group, the tremors were followed by convulsions and death. For the male and female mice of the 500 mg/kg group

and male mice of the 250 mg/kg group, tremors, followed by either recovery or convulsions and death, were observed. Tremors followed by recovery were observed for female mice of the 250 mg/kg group.

#### *Dermal*

F344/N rats and B6C3F<sub>1</sub> mice, five/sex/group, received 12 dermal applications of Hydroquinone in 95% ethanol over 14 days (NTP, 1989). The test article was applied to a clipped area of the scapular region. The rats were dosed with 240, 480, 960, 1,920, or 3,840 mg/kg and the mice with 300, 600, 1,200, 2,400, or 4,800 mg/kg Hydroquinone. The high dose was administered to rats and mice in two portions, with a 15–30-min interval for the test material to dry. A group of rats and a group of mice were dosed with vehicle only and used as controls. All animals were observed daily and weighed on days 0, 7, and 14. The animals were fasted overnight and killed.

No rats or mice died on study. There was no significant difference in weight gain between treated and control animals. In both the rat and mouse high-dose groups, crystals were observed on the skin and hair.

#### *Intraperitoneal*

Six male albino Swiss mice were given i.p. injections of 10 mg/kg Hydroquinone in saline 6 days/week for 6 weeks (Rao et al., 1988). A control group of six male mice was given i.p. injections of saline for the same time period. Following the last dose, the animals were weighed and killed, with blood samples being taken. All animals underwent necropsy and certain tissues were examined microscopically. Suspensions of bone marrow cells were made.

No dose-related deaths occurred on study. There were no significant differences in hemoglobin, total leukocytes, lymphocyte, polymorph, or lymphocyte/polymorph ratio values when compared with controls. Hydroquinone produced an insignificant depression in bone marrow cellularity. Microscopically, the livers of animals dosed with Hydroquinone had a loss of cytoplasmic detail of hepatocytes and focal fatty change. Hydroquinone did not produce a change in body weight or relative organ weight when compared with the controls. The adrenal glands of treated mice had moderate congestion at the corticomedullary junction. The study size did not warrant the drawing of conclusive results.

#### **Subchronic Toxicity**

##### *Oral*

Male and female rats, 20/sex/group, were dosed with 1,000–4,000 ppm Hydroquinone in drinking water for 15 weeks (Christian et al., 1976). A control group was given drinking water only. Clinical observations were made daily. Body weights and feed consumption were measured weekly; water was changed and consumption was measured three times weekly. Changes in microsomal hepatic enzymes were evaluated by measuring the length of the period of sleep induced by an i.p. injection of 125 mg/kg sodium hexobarbital; sleep time was considered the

timed interval between the loss and regaining of the righting reflex. Blood samples were taken during the study. At the termination of dosing, the animals underwent necropsy.

There was no mortality during the study, and no adverse clinical observations were noted. Growth was significantly decreased for male rats of the 4,000 ppm dose group. Males and females of the 1,000 ppm group generally grew faster and consumed more feed than other males and females. Water consumption decreased proportionate to increased doses of Hydroquinone. The only differences noted in hematologic parameters between treated and control animals were slightly reduced hematocrits and hemoglobin contents after 5 and 10 weeks for rats dosed with 2,000 and 4,000 ppm; these values were normal at study termination. The period of sleep induced by sodium hexobarbital was not affected by Hydroquinone administration. Absolute liver and kidney weights of animals dosed with Hydroquinone increased with increasing concentrations of Hydroquinone. Liver-to-body weight ratios were significantly increased for male and female rats of all dose groups; kidney-to-body weight ratios were significantly increased for male rats of all dose groups and for female rats of the 2,000 and 4,000 ppm dose groups. No significant microscopic observations were made.

F344/N rats and B6C3F<sub>1</sub> mice, 10/sex/group, were dosed with Hydroquinone dissolved in corn oil by gavage 5 days/week for 13 weeks (NTP, 1989; Kari et al., 1992). The doses for both rats and mice were 25, 50, 100, 200, and 400 mg/kg Hydroquinone. A group of rats and a group of mice were dosed with vehicle only and served as controls. Animals were observed twice daily and body weights and feed consumption were measured weekly. All animals were killed for necropsy at the termination of dosing. Tissues of rats and mice of the control, 200, and 400 mg/kg groups and selected tissues from rats of the 100 mg/kg group were examined microscopically.

All the rats of the high-dose group and three female rats of the 200 mg/kg group died on study, with most of the deaths occurring before week 7. In the 200 mg/kg dose group, male and female rats appeared lethargic after 10 and 11 weeks of dosing, respectively. Tremors, sometimes followed by convulsions, were observed in female rats at this dose. For the rats that died, tremors and convulsions were often observed before death, and there was a clear orange fluid or orange staining around the mouth. No clinical signs were observed in the other dose groups.

Relative liver-to-body weight ratios were significantly decreased for male rats in all dose groups and were significantly increased for female rats of the 100, 200, and 400 mg/kg groups when compared with control values.

Four male and five female rats of the high-dose group had red-to-brown perioral staining, one male and two females had reddened gastric mucosa, and one male had meningeal hemorrhage. In the 200 mg/kg group, two females had perioral staining, two males had intra-abdominal bleeding, one female had blood in the gastric contents, seven males and six females had toxic nephropathy, and four males and one female had inflammation and mild to moderate hyperplasia of the squamous portion of the stomach. Toxic nephropathy was observed in one female rat of the 100 mg/kg group. The renal lesions consisted of tubular cell degeneration

and degeneration in the renal cortex and were of moderate to marked severity in the male and of minimal to mild severity in the female rats.

Eight male and eight female mice of the high-dose group and two male mice of the 200 mg/kg group died on study; the death of one male mouse of the 200 mg/kg group mice was accidental. Lethargy was observed in all dosed males and in females of the three highest dose groups. Post-dose tremors, often followed by convulsions, were observed in males and females of the high-dose group. Post-dose tremors were also observed in males of the 200 mg/kg dose group. For male mice of all dose groups and female mice of the two highest-dose groups, relative liver-to-body weight ratios were significantly increased compared with control values. Ulceration, inflammation, or epithelial hyperplasia of the squamous portion of the stomach was observed in three male and two female mice of the high-dose group and in one female mouse of the 200 mg/kg group.

#### Dermal Irritation

In a metabolic study (described earlier), dermal application of 25 or 150 mg/kg Hydroquinone to male and female Fischer 344 rats for 24 h produced slight to severe erythema due to crystallization and wiping (Eastman Kodak Company, 1988b).

#### Sensitization

The sensitization potential of Hydroquinone was examined in both a Magnusson-Kligman guinea pig maximization test and a local lymph node assay (LLNA), which is a screening assay (Basketter and Scholes, 1992). Albino Dunkin-Hartley guinea pigs were used in the maximization test. Following a preliminary irritation test to determine test concentrations, the animals received a series of six intradermal injections of 2.0% Hydroquinone in 0.9% saline in the shoulder region to induce sensitization. After 6–8 days, a 48-h occlusive patch of 1.0% Hydroquinone in acetone–polyethylene glycol 400 was placed over the injection site. The animals were challenged on a previously untreated area of the flank using a 24-h occlusive patch of 0.5% Hydroquinone, which was the maximum nonirritating concentration. The challenge site was scored for erythema and edema 24 and 48 h after patch removal. Hydroquinone was classified as an extreme sensitizer, with 100% of the animals having a positive response after 14 and/or 48 h.

The LLNA was performed as described by Basketter et al. (1991). Male and female CBA/Ca mice, four per group, were used; single experiments were limited to one sex. The mice were dosed daily with a topical application of 25  $\mu$ l of 0.5, 1.0, and 2.5% Hydroquinone in acetone–olive oil (4:1, v/v) on the dorsal surface of each pinna for 3 consecutive days. Control mice were dosed with vehicle only. Four days after the first application, all mice were given an i.v. injection of 250  $\mu$ l phosphate-buffered saline (PBS) containing [ $^3$ H]methyl thymidine ( $^3$ HTdR; 20  $\mu$ Ci). The mice were killed 5 h after i.v. dosing, the draining auricular lymph nodes were excised and pooled for each group, and a single-cell suspension of lymph node cells (LNC) was prepared.  $^3$ HTdR incorporation was measured using  $\beta$ -scintillation counting. A chemical was considered a sensitizer if at least one

concentration resulted in at least a threefold increase in  $^3\text{HTdR}$  incorporation as compared with control values. Hydroquinone was positive for sensitization.

### Reproductive/Teratogenic Effects

The developmental toxicity potential of Hydroquinone was evaluated using pregnant Crl: COBS CD (SD)BR rats (Eastman Kodak Company, 1985*d*; Krasavage et al., 1992). Three groups of rats, 30 per group, were dosed orally by gavage with 30, 100, or 300 mg/kg Hydroquinone, 5% in distilled water, on days 6–15 of gestation. A group of 30 rats received a volume of distilled water equivalent to the largest volume of Hydroquinone solution given to a test animal on days 6–15 of gestation and served as a control group. Body weights and feed consumption were determined on days 0, 6, 9, 12, 16, and 19 of gestation. All animals were observed three times a day on dosing days or twice daily on nondosing days, respectively. On weekends during the nondosing periods, the animals were observed for morbidity and mortality. On day 20 of gestation, fasted animals were killed and examined. The liver and kidneys of each animal were weighed and examined microscopically.

No significant differences were observed in maternal mean body weight, corrected mean body weight, absolute organ weight, or feed consumption. Any statistically significant increases in relative organ weight were not considered toxicologically important. The only clinical observation for all animals dosed with Hydroquinone was brown discolored urine. No Hydroquinone-related lesions were observed during gross examination for any dose group, and no abnormalities were observed upon microscopic examination of the liver and kidneys of animals of the high-dose group. (The liver and kidneys of animals of the other dose groups were not examined microscopically.)

Combined male and female fetal mean body weight was significantly reduced for the 300 mg/kg dose group compared with controls. When measured separately, female fetal mean body weight was significantly less and male fetal mean body weight was slightly but not significantly less than control values. There was no significant difference in malformations or skeletal alterations between fetuses in the dose and control groups.

The no observable effect level (NOEL) for both maternal and developmental toxicity was 100 mg/kg Hydroquinone. The no observable adverse effect level was 300 mg/kg. Hydroquinone,  $\leq 300$  mg/kg, did not produce embryotoxic, fetotoxic, or teratogenic effects in Crl: COBS CD (SD)BR rats.

A two-generation study was conducted using Charles River CD (Sprague-Dawley)-derived rats to determine the reproductive effects of long-term oral administration of Hydroquinone (Bio/Dynamics, Inc., 1989*a*; Blacker et al., 1993). The  $F_0$  generation consisted of 30 males and 30 females per group which were dosed with 15, 50, or 150 mg/kg Hydroquinone in 5 ml/kg degassed distilled water at concentrations of 3, 10, or 30 mg/ml, respectively, by gastric intubation 7 days/week for 10 weeks before mating. A control group of 30 males and 30 females was dosed with 5 ml/kg of vehicle.

One male was housed nightly with one female from the same dose group until

evidence of mating was observed or until 7 days had elapsed without evidence of mating. Unmated females were then reassigned to sexually active males of the same dose group for another 7-day period. F<sub>0</sub> males were dosed daily during mating and the postmating period until they were killed. Mated F<sub>0</sub> females were dosed daily during mating and during the gestation and lactation periods. Unmated females were dosed during the postmating period until they were killed.

Two pups/sex/litter were randomly chosen at weaning (day 24) to be used as the F<sub>1</sub> parent generation. Excess pups were culled to 30 males and 30 females per group in a way that allowed each litter to be represented by at least one pup/sex. Rats chosen for the F<sub>1</sub> parent generation were administered the same dose as their parents starting on day 25 of age; however, the premating dose period of 7 days/week for 11 weeks did not formally begin until the last litter was weaned. The F<sub>1</sub> animals were mated and dosed using the same procedure as that used for mating F<sub>0</sub> animals. (Brother-sister mating was avoided.)

Animals were observed twice daily for mortality, toxicologic and pharmacologic effects, and parturition. All animals were examined in detail weekly for signs of local or systemic toxicity or pharmacologic effects. From week 8 until termination of study, F<sub>0</sub> and F<sub>1</sub> parents were observed daily for tremors following dosing.

Body weights of the F<sub>0</sub> and F<sub>1</sub> parents were measured at the time of assignment to the test group, the day of dose initiation, day 4 of dosing, and weekly during the premating period. Males were weighed weekly until killed. Female body weights were measured on days 0, 7, 14, and 20 of gestation and on days 0, 4, 14, and 21 of lactation. Feed consumption was measured weekly during the premating period for all F<sub>0</sub> and F<sub>1</sub> parents. For males, feed consumption was also measured weekly during the postmating period. For females, feed consumption was measured for the intervals gestation days 0-7, 7-14, and 14-20.

Litters were examined twice daily. Pups were counted, the number of pups/sex was determined, gross physical examinations were made, and body weights of live pups were measured on days 0, 4, 7, 14, 21, and 24 of lactation for the F<sub>1</sub> litters and on days 0, 4, 7, 14, and 21 of lactation for the F<sub>2</sub> litters. On day 4 of lactation, each litter was culled to eight pups, four males and four females, when possible.

Parent females were killed as a group after the weaning of the last litter. F<sub>0</sub> and F<sub>1</sub> adult males were killed 4 and 3 weeks, respectively, after mating period completion. F<sub>1</sub> pups not selected for the pool of animals from which the F<sub>1</sub> parent generation was to be chosen were killed on day 24 of lactation. F<sub>1</sub> pups chosen for the pool but not chosen for the F<sub>1</sub> parent generation were killed at weaning of the last litter. F<sub>2</sub> pups were killed on day 21 of lactation. Gross examination was performed on all animals. Various tissues from all F<sub>0</sub> and F<sub>1</sub> parent animals were preserved. The tissues of the control and 150 mg/kg groups were examined microscopically. For the 15 and 50 mg/kg dose groups, tissues with gross lesions and reproductive tissues from sexually inactive animals were examined microscopically.

There was no significant difference in mortality rates between F<sub>0</sub> and F<sub>1</sub> parents and the controls. In the 150 mg/kg group, one female of the F<sub>0</sub> generation and two females of the F<sub>2</sub> generation had tremors before death.

Mean body weight, mean body weight gains, and mean feed consumption for all dose groups of both the F<sub>0</sub> and F<sub>1</sub> generations, males and females, were comparable to control values during the premating period.

No toxicologically significant differences were observed in the weekly mean body weights or mean feed consumption for all F<sub>0</sub> and F<sub>1</sub> males during the mating and postmating periods. Maternal mean body weights and mean body weight gains were comparable between treated and control animals of the F<sub>0</sub> and F<sub>1</sub> generations during gestation and lactation and maternal mean feed consumption during gestation was also comparable for treated and control groups.

Tremors were observed in some animals after dosing. The occurrence of tremors in one F<sub>0</sub> male of the 50 mg/kg dose group and in F<sub>0</sub> and F<sub>1</sub> males and females of the 150 mg/kg group dose was considered indicative of an adverse effect.

No adverse effects related to Hydroquinone administration were observed at necropsy or during microscopic evaluation of either the F<sub>0</sub> or F<sub>1</sub> animals. The NOEL for parental effects was 15 mg/kg/day.

There were no significant differences in mating indices, pregnancy rates, or male fertility indices between the treated and control males and females. The mean gestation length for the treated F<sub>0</sub> and F<sub>1</sub> females was comparable to the controls. The number of live, dead, and total pups at birth, pup viability indices, and mean litter size at day 4 (pre-cull) and at post-cull intervals for both generations were comparable between treated and control groups. Litter survival indices were also comparable between treated and control groups of both generations.

No significant difference was observed in pup mean body weights or sex distribution between treated and control groups of either generation. There was no significant difference for either generation in the number of external or internal malformations for treated and control pups, and adverse effects of dose administration were not apparent. The NOEL for reproductive effects was 150 mg/kg/day.

A range-finding study was conducted to assess the toxicity of Hydroquinone using pregnant New Zealand White (NZW) rabbits (Bio/Dynamics, Inc., 1988; Murphy et al., 1992). Five rabbits per group were given 50, 100, 200, or 400 mg/kg Hydroquinone in 10 ml/kg distilled, degassed water at a concentration of 5, 10, 20, or 40 mg/ml, respectively, by gastric intubation on days 6–18 of gestation. Initially, a group was given 500 mg/kg Hydroquinone at a concentration of 50 mg/ml, but this group was eliminated and replaced by five rabbits receiving 300 mg/kg Hydroquinone, at 30 mg/ml, on days 6–18 of gestation following the death of the first two animals dosed with 500 mg/kg Hydroquinone. A control group of five rabbits was dosed with 10 mg/ml of vehicle on days 6–18 of gestation.

All animals were observed for pharmacologic and toxicologic effects and mortality twice daily and examined in detail on days 0, 6, 9, 12, 15, 18, 24, and 30 of gestation. Body weights were determined on days 0, 6, 12, 18, 24, and 30 and feed consumption on days 3, 6–10, 12, 15, 18, 24, and 29 of gestation. All animals were killed on day 30 of gestation.

Mortality was not observed in the control, 50, 100, or 200 mg/kg dose groups. There was 100% mortality in the 400 mg/kg dose group within 2–4 days after dose

initiation and in the 300 mg/kg dose group by week 13. As was mentioned earlier, the two animals dosed with 500 mg/kg Hydroquinone died after the first dose.

Hydroquinone administration did not have an adverse effect on pregnancy rate for the animals of the 50, 100, or 200 mg/kg dose groups. Pregnancy rates of the 300, 400, and 500 mg/kg dose groups were decreased compared with the controls. Some animals may have died before implantation occurred. The absence of implants in some females that died later in the gestation period may be suggestive of an adverse effect on the uterine implantation process or an embryo-lethal effect.

No significant effects on weight gain or mean feed consumption were observed for animals of the 50 mg/kg group, but significant effects were observed for animals of the 100, 200, and 300 mg/kg dose groups. Using corrected body weights, a mean weight loss was observed during the gestation days 6–30 interval for animals of the control, 50, 100, and 200 mg/kg groups.

Tremors were observed on day 1 for some animals dosed with 300, 400, and/or 500 mg/kg Hydroquinone. Excessive lacrimation was observed in four animals of the 300 mg/kg dose group at various times during dosing and may have been suggestive of a treatment-related effect. Dark brown urine was observed for some animals in all dose groups. Other observations were not considered dose related.

Red discoloration of the gastric mucosa and changes in the gastric contents were observed for some animals of the 300, 400, or 500 mg/kg dose groups. Yellowish/brown material stained the anogenital skin/hair of animals of these groups also. Other gross observations made for treated animals at necropsy were comparable to those made for controls.

No adverse effects, such as abortion or premature delivery, were observed for animals dosed with  $\leq 200$  mg/kg Hydroquinone. The mean number of corpora lutea and uterine implantation sites, mean preimplantation loss index, mean number of live fetuses per pregnant female, and the incidence of litters containing at least one resorption site were similar among the control, 50, 100, and 200 mg/kg groups. An increase in the mean number of resorption sites and the mean ratio of resorptions to implants for the 200 mg/kg group as compared with the controls may have been indicative of a treatment-related response.

Fetal mean weights were similar for the control, 50, and 100 mg/kg groups, but decreased for the 200 mg/kg dose group, suggestive of a treatment-related effect. No adverse effects due to Hydroquinone were observed during external evaluation of the fetuses of the 50, 100, and 200 mg/kg dose groups.

A study was conducted to evaluate the embryotoxic, fetotoxic, and/or teratogenic potential of Hydroquinone using NZW rabbits (Bio/Dynamics, Inc., 1989b; Murphy et al., 1992). Three groups of 18 rabbits were given 25, 75, or 150 mg/kg Hydroquinone in 8 ml/kg degassed, distilled water at a concentration of 3.125, 9.375, or 18.75 mg/ml Hydroquinone, respectively, by gastric intubation on days 6–18 of gestation. (These doses were selected based on the results of the range-finding study that was described previously.) Eighteen rabbits were dosed with 8 mg/ml of vehicle on days 6–18 of gestation and served as a control group.

All animals were observed for pharmacologic and toxicologic effects and mortality twice daily, and examined in detail on days 0, 6–19, 24, and 30 of gestation. Body weights were determined on days 0, 6, 9, 12, 16, 18, 24, and 30, and feed

consumption was recorded daily during gestation. All animals were killed on day 30 of gestation.

There was no mortality in the control or treated groups. There was no significant difference in body weights observed between the 25 and 75 mg/kg dose groups and the controls. For the 150 mg/kg group, the mean body weight was significantly lower than the control group mean body weight on days 16 and 18 of gestation and a mean weight loss was significant during the days 6-9 interval. The total mean weight loss over the entire dosing period was significantly greater than the total mean weight loss observed for the control group. Corrected gestation day 30 body weights were comparable among the treated and control groups.

Mean feed consumption of the 25 mg/kg dose group was comparable to control values at all times whereas it was significantly decreased for the 75 mg/kg dose group on days 11 and 12 and for the 150 mg/kg dose group on days 6-14 and 17 compared with controls.

No adverse effect of treatment was evident upon physical observation. A dark-staining urine was noted for many of the treated animals.

The pregnancy rates, premature delivery indices, mean number of corpora lutea, and mean number of uterine implantations per pregnant female were comparable between the control and treated groups. The mean preimplantation loss indices and mean number of viable fetuses were comparable between the control, low-, and high-dose groups, but the preimplantation loss index was considerably decreased and the mean number of viable fetuses was increased for the mid-dose group as compared with controls. These differences were not considered indicative of an adverse effect of dosing. The mean number of resorptions per pregnant female, mean resorption/implant ratio, and the mean number of females with resorptions among their uterine implants were comparable among all groups.

At necropsy, an accentuated lobular pattern of the liver was noted for some control and treated animals, with the incidence being greater in the low- and mid-dose groups as compared to controls. The toxicologic significance of this observation, especially without a dose relation, was equivocal on the basis of gross examination only. Other gross changes were not considered dose related. Absolute and relative kidney and liver weights were comparable among all animals.

No significant differences in fetal mean weights or sex distribution were observed between the control and treated groups.

The incidence of external, visceral, or skeletal malformations or ossification variations, both per fetus and per litter, was not significantly different between the control and treated groups. Dose-related external or visceral variations were not observed.

### MUTAGENICITY

Results of mutagenicity studies that have been published since 1983 are essentially the same as those reported in the existing safety assessment of Hydroquinone (Elder, 1986), and are summarized in Table 2.

The results of Ames tests with and without metabolic activation using *Salmo-*

TABLE 2. Hydroquinone (HQ) mutagenicity studies

Test	Organism and strain	Methods	Results and comments	Reference
Ames test	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	At least five concentrations were tested with or without S9. The experiment was run in triplicate. Positive and negative controls were used	Negative	Haworth et al., 1983
Ames test	<i>S. typhimurium</i> TA97, TA98, TA100, TA102, TA104, TA1535	HQ at concentrations of 50–1,000 and 1.25–40 µg/plate was tested with and without S9, respectively	Negative	Glatt et al., 1989
Ames test	<i>S. typhimurium</i> TA97, TA98, TA100	HQ and HQ plus nitrite at concentrations of 12.5–100 µg/plate and HQ plus chlorine at concentrations of 1–25 µg/plate were tested with and without S9. The vehicle, DMSO, was used as the negative control. The experiment was run in triplicate. The ability of HQ to produce H <sub>2</sub> O <sub>2</sub> and O <sub>2</sub> <sup>-</sup> was also examined	HQ was a direct-acting mutagen toward all three strains, with the chlorinated and nitrosated derivatives being more mutagenic than HQ alone. Mutagenic activity was greatly suppressed in the presence of S9. 100 µg/plate HQ was toxic to primarily all cultures. HQ was able to produce H <sub>2</sub> O <sub>2</sub> but not O <sub>2</sub> <sup>-</sup> . The amount of H <sub>2</sub> O <sub>2</sub> produced was decreased in the presence of catalase	Lin and Lee, 1992
<i>Salmonella</i> mutagenicity test	<i>S. typhimurium</i> TA97, TA98, TA100	Mutagenic potential was assayed at a dose range of 0–250 µg/plate with and without S9 according to the method of Maron and Ames (1983). Results were expressed as an average of two plates/dose	Negative	Sakai et al., 1985
Fluctuation test	<i>S. typhimurium</i> TA100	Mutagenic potential, with and without metabolic activation, was assayed using the method modified by Gatehouse	Positive with metabolic activation. Negative without metabolic activation	Koike et al., 1988
6-TG resistance induction	V79 Chinese hamster cells	At least five concentrations of HQ were tested; the optimal/maximal concentration was 3.5 µM	Positive	Glatt et al., 1989
Promutagenic chemical detection	<i>S. griseus</i> H69, FS2	<i>S. griseus</i> strains H69 (detects point mutations) and FS2 (detects frame shift mutations) were used to assess the activation of promutagenic chemicals by monitoring the reversion of the bacterial test strains to a kanamycin-resistant phenotype. HQ was tested at a concentration of 200 µg/ml	Negative with both strains	Buchholz et al., 1992

(continued)

TABLE 2. Continued

Test	Organism and strain	Methods	Results and comments	Reference
Sister chromatid exchange	CHO cells	Dose ranges of 50–800 and 0.5–5 µg/ml were tested with and without S9, respectively. Positive and negative controls were used	Positive	Galloway et al., 1987
Sister chromatid exchange	Chinese hamster Don cells		Positive	Shimada et al., 1988
Sister chromatid exchange	V79 Chinese hamster cells	The optimal/maximal concentration was 20 µM	Positive	Glatt et al., 1989
Sister chromatid exchange	Human lymphocytes	Lymphocytes from whole blood of 7 individuals were cultured with 40 µM HQ, 40 µM HQ, and 1–100 µM acetaldehyde, or 50 µM dimethyl maleate and 40 µM HQ	HQ alone significantly increased the number of SCEs in cultures from 3 of 7 of the individuals. A synergistic effect was observed when acetaldehyde (at concentrations which alone did not increase SCEs) was administered with HQ. Pretreatment with dimethyl maleate more than doubled the number of SCEs	Knadle, 1985
Chromosomal aberration	<i>A. nidulans</i>	A concentration range of 250–750 µg/ml was tested. Minimal and complete media were used and ≥100 colonies were tested at each concentration	Mitotic segregation was greatly increased. Haploid segregation was only slightly increased	Kappas, 1989
Chromosomal aberration	<i>A. nidulans</i>	A concentration range of 132–396 µg/ml HQ in distilled water was tested. A positive control was run. Minimal and complete media were used	HQ significantly increased whole chromosome segregants	Crebelli et al., 1991
Chromosomal aberration	<i>A. nidulans</i>	HQ, 1–3 mM, was tested without metabolic activation. The effect of 2 mM L-cysteine and 2 mM HQ was also examined	HQ produced an 8- to 10-fold increase in mitotic segregation. L-cysteine lowered the frequency of abnormal colonies to control values	Crebelli et al., 1987
Chromosomal aberration	<i>A. nidulans</i>	A concentration range of 250–750 µg/ml was tested without metabolic activation	HQ produced a 2- to 5-fold increase in the number of diploid mitotic crossover segregants. Haploid and nondisjunctive diploids were not increased	Kappas, 1990
Chromosomal aberration	CHO cells	Dose ranges of 150–600 and 5–20 µg/ml were tested with and without S9, respectively. Positive and negative controls were used	Positive with S9. Negative without S9	Galloway et al., 1987
Chromosomal aberration	Chinese hamster cell lines: immortal		<i>Don.Wg3H</i> : HQ induced metaphase, anaphase, and telophase defects and the absence of mitotic spindle	Parry et al., 1990

Chromosomal aberration	Don. Wg3H; primary LUCI <i>A. nidulans</i>	A concentration range of 132-396 µg/ml HQ in distilled water was tested. A positive control was run. Minimal and complete media were used	<i>LUCI</i> : HQ induced metaphase and anaphase defects and the absence of mitotic spindle HQ increased the frequency of mitotic crossovers	Crebelli et al., 1991
Aneuploidy induction	Mouse testicular cells	HQ in double-distilled water at concentrations of 80-120 mg/kg was given by i.p. injection and slides were prepared after 6, 14, or 22 h. From each animal, 100 metaphase II cells were examined for hypo- and hyperploidy. Negative controls were used	The frequency of hyperploid meiotic metaphase II (MMII) cells was significant at a dose of 100 mg/kg HQ after 6 h. When all dose groups were compared with the controls by the trend test, HQ caused significant induction of aneuploidy. The frequency of hypoploid MMII cells was not significantly increased. HQ induced nondisjunction in primary spermatocytes during meiotic metaphase I (MMI)	Miller and Adler, 1992
C-banding	Bone marrow cell micronuclei from male and female Swiss-albino mice	Three mice/sex were dosed with 80 mg/kg HQ by i.p. injection and then killed 24 h later. At least 10 smears were prepared per animal. C-banding was done at least 3 wks after slide preparation. DNA content and area of micronuclei were also determined	In respect to C-banding and DNA content, HQ behaved as a clastogen. However, considering the area measurements, HQ behaved as an aneuploidogen	Van Hummelen et al., 1992
Chromosomal aberration	Male mouse germ cells	Five adult male mice were dosed and one was used as a control for each group. Three experiments were performed; HQ was dissolved in sterile water and administered by a single i.p. injection at a volume of 0.1 ml/10 g body weight <i>Experiment 1</i> : analysis of chromosomal aberrations was performed in diakinesis-metaphase I cells 1, 5, 9, 11, 12, and 13 days after dosing with 80 mg/kg HQ <i>Experiment 2</i> : two groups of mice were dosed with 40 or 120 mg/kg HQ and testes were sampled 12 days later <i>Experiment 3</i> : mice were dosed with 40, 80, or 120 mg/kg HQ and differentiating spermatogonia were analyzed for chromosomal aberrations 24 h later	<i>Experiment 1</i> : Due to HQ treatment, it was not possible to analyze structural chromosomal aberrations in cells at diakinesis-metaphase I 24 h after dosing. Significant differences from controls were observed on days 9 and 13. Exchange configurations were only increased at 13 days after treatment <i>Experiment 2</i> : At 40 mg/kg, significant increases were observed in the number of chromosomal aberrations. A plateau was reached between 40 and 80 mg/kg. A significant difference in the number of aberrant cells observed after treatment with 120 mg/kg was not significantly different from controls <i>Experiment 3</i> : The mitotic index was significantly increased at 40 and 120 mg/kg, but not at 80 mg/kg, when compared with controls. At all doses, the frequency of aberrant cells, excluding gaps, was statistically greater than control values. HQ was clastogenic in male mouse germ cells	Ciranni and Adler, 1991

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
Chromosomal aberration	Mouse bone marrow cells	HQ in bidistilled water was tested at a dose range of 40–100 mg/kg. Five male and five female mice were used per i.p. dose per interval. The negative control group consisted of one male and one female per dose per interval	Chromosomal aberrations were increased in a dose-dependent manner; both chromatid breaks and exchanges were induced. HQ was clastogenic. A sex difference was not observed	Xu and Adler, 1990
Chromosome malsegregation	Male mouse bone marrow cells	Four mice per group were dosed with an injection of 40, 80, or 120 mg/kg HQ in water. Two h before dosing, mice were given 25 mg of BrdU by s.c. implantation. Bone marrow was removed 18 and 24 h after dosing	After 18 h, HQ significantly increased AGT values at 80 and 120 mg/kg and significantly induced hyperploid cells in the 2N = 41,42 class at 80 mg/kg	Pacchierotti et al., 1991
Chromosomal aberration	Mouse bone marrow cells	HQ was assayed in mouse bone marrow cell cultures	HQ caused a 5–10% increase in SCEs, a 3–5% increase in aneuploidies, and a borderline positive increase in polyploidies	Marrazzini et al., 1991
Chromosomal aberration	Bone marrow cells from male Swiss CD-1 mice	Three mice per group were dosed with 80 mg/kg HQ in bidistilled water orally and by i.p. injection after s.c. injection of 25 mg BrdU	HQ caused a weak increase in structural aberrations. The % aberrant cells was significantly increased with both routes of administration, with the greatest increase caused by i.p. injection; the most aberrant cells were observed 6 h after oral administration and 24 h after i.p. injection	Marrazzini et al., 1992
Chromosomal aberration	Human lymphocyte cultures	Two cultures per dose were exposed to 3–24 µg/ml HQ 24 h after the addition of 9 µg/ml BrdU and harvested after a total of 72 and 96 h	HQ induced polyploidy. Tetraploid cells were induced with a low frequency at both time periods in the dose range of 12–24 µg/ml without a dose–effect relationship; doses > 24 µg/ml produced toxic effects	Sbrana et al., 1992
c-Mitotic effects	Mouse bone marrow cells	HQ was dissolved in bidistilled water and tested at a dose range of 80–150 mg/kg. Five male mice were dosed by i.p. injection and five were used as negative controls	Mitotic index decreased insignificantly with increased doses. Cytotoxicity before mitosis rather than mitosis-arresting activity was observed at 150 mg/kg, but chromatid spreading and shortening was induced. At 100 and 150 mg/kg, metaphases with spread chromosomes were significantly increased. At 150 mg/kg, anaphase frequencies increased significantly and the chromosomes of the metaphase and anaphase cells were partially clumped	Miller and Adler, 1989

c-Mitotic effects	Human lymphocyte cultures	Two cultures per dose were exposed to 3–24 $\mu\text{g/ml}$ HQ 24 h after the addition of 9 $\mu\text{g/ml}$ BrdU and harvested after a total of 72 and 96 h	HQ induced c-mitoses. Partial c-mitoses were observed, with the greatest number seen at 24 $\mu\text{g/ml}$ . Shortening and spreading of chromatids were induced, but the chromosomes were often clustered. Mitotic indices were decreased (sometimes significantly) at all doses	Sbrana et al., 1992
Chromosome loss	Yeast strain D6	A dose range of 500–4,000 $\mu\text{g/ml}$ was tested without S9	HQ induced significant increases in white cycloheximidine resistant colonies, indicating chromosome loss	Parry et al., 1990
Chromosomal malsegregation	<i>Saccharomyces cerevisiae</i> D61.M	HQ was dissolved in DMSO and tested at a dose range of 0.05–3.0 mg/ml	Negative	Albertini, 1990
In vitro porcine brain tubulin assembly assay	Porcine brain tubulin	A dose range of 0.005–25 mM was tested	HQ had no effect on the steady-state level reached at the end of the assembly process, lag-phase, polymerization velocity, or end absorption. Tubulin disassembly was not affected in regard to reversibility of the assembly process. Depolymerization velocity appeared to be enhanced at high concentrations (>10 mM)	Brunner et al., 1991
DNA strand breakage	Supercoiled $\phi\text{X-174}$ DNA	HQ-induced DNA strand breakage was studied in the presence and absence of oxygen scavengers	HQ dose dependently caused single- and double-strand breakages. Doses of $10^{-3}$ – $1.1 \times 10^{-4}$ M were needed for complete loss of supercoiled DNA. Scavengers did not protect DNA from HQ-induced breakage	Lewis et al., 1988
DNA strand breakage			Positive	Shimada et al., 1988
DNA strand breakage	Male Sprague-Dawley rat liver cell nuclei	Cell nuclei were prepared from the livers of rats dosed with HQ and from control animals. Single strand breaks (SSB) were determined by the DNA unwinding technique (Ahnstrom and Erixon, 1973) as modified by Walles and Erixon (1984)	HQ caused a significant increase in SSB as compared with control values	Stenius et al., 1989
DNA strand breakage	Purified DNA	A concentration range of 10–1000 $\mu\text{M}$ was tested	HQ caused DNA strand breakage	Maeda et al., 1990
DNA strand breakage	Supercoiled Bluescript plasmid DNA	The ability of oxidation metabolites of HQ, generated by PHS, to induce SSB was examined. The DNA was incubated with PHS in buffer which contained 100 $\mu\text{M}$ HQ	PHS oxidized HQ to metabolite(s) that caused DNA SSB	Schlosser et al., 1990

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
DNA strand breakage	Human lung carcinoma cell line A 549	Cells were exposed to HQ in the presence and absence of inhibitors. The induction of DNA SSB and the formation of 8-hydroxydeoxyguanosine (8-OHdG) was determined	HQ, 800 $\mu$ M, caused a fast depletion of total thiol content in the cell cultures and the amount in cells remained low HQ, 400–800 $\mu$ M, produced DNA SSB, but the damage did not increase over time HQ, 100 $\mu$ M, did not induce DNA damage, but 100 $\mu$ M HQ plus cigarette smoke caused DNA SSB HQ, 800 $\mu$ M, did not result in significant 8-OHdG formation Catalase had little effect on the number of DNA SSB induced by HQ, but dimethylthiourea and <i>o</i> -phenanthroline reduced the number of HQ-induced SSB. An endonuclease activity inhibitor (AT) and an intracellular calcium chelator (BAPTA) also reduced the number of HQ-induced SSB	Leanderson and Tagesson, 1992
DNA strand breakage	Rat hepatocytes	HQ was tested at $\leq 0.4$ mM. Cells were also exposed to inhibitors	HQ-induced DNA SSB. HQ had a threshold value at low concentrations, after which the DNA damage increased. Exposure of cultures to 0.3 mM HQ produced twice the damage of that observed in the controls. DNA damage increased with longer exposure, with a lag phase of $\sim 20$ min. Pretreatment with a $Ca^{2+}$ inhibitor decreased the amount of DNA damaged produced by HQ. DNA damage was increased by post-treatment with a poly(ADP-ribose) polymerase inhibitor	Walles, 1992
DNA strand breakage	Mouse lymphoma cell line L5178YS (LY-S)	HQ was tested at 0.1 mM, the highest non-toxic dose. Four experiments were performed	HQ did not significantly increase the number of SSB	Pellack-Walker and Blumer, 1986
DNA sequencing technique	<sup>32</sup> P 5'-end-labelled DNA fragments from human c-Ha-ras-1 protooncogene	The DNA fragments were incubated with 5 mM HQ in 200 $\mu$ l of 20 mM Tris-HCl buffer at pH 8.0. DNA damaged was estimated by gel electrophoretic analysis	HQ caused slight DNA damage	Kawanishi et al., 1989

Foci experiment	Male Sprague-Dawley rats	Rats, some of which were pretreated with diethylnitrosamine (DEN), were dosed with 100 or 200 mg HQ	HQ significantly increased the number of foci when the rats were pretreated with DEN. No foci were observed when rats were not pretreated with DEN HQ, single dose of 200 mg/kg, induced lipid peroxidation. HQ had a dose-related effect on hepatic ODC induction. HQ rapidly depleted glutathione (GSH)	Stenius et al., 1989
DNA synthesis	Mouse lymphoma cell line L5178YS (LY-S)	A modification of the DNA synthesis inhibition assay described by Painter (1977) was performed with and without S9. A dose range of $1 \times 10^{-7}$ – $1 \times 10^{-4}$ M was tested. Several standard mutagens of [ <sup>3</sup> H]thymidine incorporation were used	HQ inhibited DNA synthesis by ~65%. The ED <sub>50</sub> was $1.0 \times 10^{-5}$ M and the highest nontoxic dose was 40 μM	Pellack-Walker et al., 1985
Nuclear DNA synthesis	Bone marrow cells	HQ, $\leq 24$ μM, was tested in cells that were in the DNA synthetic phase of the cell cycle	HQ inhibited nuclear DNA synthesis. The IC <sub>50</sub> was 11.2 μM	Lee et al., 1989
mtDNA synthesis	Rat liver mitoplasts and rabbit bone marrow mitochondria in vitro	HQ was tested at $\leq 10$ mM	$\leq 10$ mM HQ did not inhibit hepatic mtDNA synthesis, but $\leq 10$ mM HQ produced a dose-dependent inhibition of mtDNA synthesis in rabbit bone marrow mitochondria, with 10 mM causing 50% inhibition	Schwartz et al., 1986
DNA synthesis	Cell-free DNA synthetic system	The effect of $\leq 24$ μM HQ on DNA synthesis was examined	DNA synthesis was not inhibited	Lee et al., 1989
DNA synthesis	Bladder epithelial cells from male F344 rats	Five rats were fed 0.8% HQ in feed for 4 wks. Before being killed, all rats were given an i.p. injection of BrdU as a marker. Five rats were fed untreated feed and used as a control group	No increase in labeling indices of treated rats was observed	Kurata et al., 1990
mtDNA transcription	Rabbit bone marrow cell mitoplasts	HQ, $\leq 5.0 \times 10^{-5}$ M, was added to mitoplasts in vitro	HQ inhibited mtDNA transcription	Rushmore et al., 1983
RNA synthesis	Mouse spleen lymphocytes (from male Swiss mice)	HQ was tested at $\leq 2 \times 10^{-5}$ M	Dose-dependent inhibition was observed. HQ completely inhibited RNA synthesis at concentrations that had essentially no effect on lymphocyte viability	Post et al., 1985
RNA synthesis	Peritoneal macrophages (from male Swiss-Webster mice)	HQ was tested at $\leq 2 \times 10^{-5}$ M	Dose-dependent inhibition was observed. HQ had no effect on lymphocyte viability at concentrations $\leq 1 \times 10^{-4}$ M	Post et al., 1986

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
mRNA synthesis	Rabbit bone marrow nuclei	HQ, $6 \times 10^{-6} M$ , was incubated under conditions specific for RNA polymerase II. HQ was then tested at the concentration range of $10^{-7}$ – $10^{-3} M$	Positive. HQ inhibited the incorporation of [ $^3H$ ]UTP into mRNA over a 30-min period. The $IC_{50}$ for HQ varied from $4 \times 10^{-6}$ – $1 \times 10^{-5} M$	Post et al., 1984
8-OHdG formation in DNA	Calf thymus DNA	Calf thymus DNA was incubated with 50–400 $\mu M$ HQ. The effects of tyrosine and catalase in addition to HQ was also examined	HQ dose dependently caused hydrogen peroxide and 8-OHdG formation. The hydrogen peroxide and 8-OHdG formation decreased with the addition of tyrosine or catalase to the incubation mixture	Leanderson and Tagesson, 1990
DNA adduct formation	HL-60 cells	Cells were treated for 8 h with 100 $\mu M$ HQ	HQ produced 0.8–2.0 adducts/ $10^7$ nucleotides	Pongracz et al., 1990
DNA adduct formation	HL-60 cells	$^{32}P$ -postlabeling technique was used to determine DNA adduct formation after the addition of 0–500 $\mu M$ HQ to cell cultures for 0.5–24 h	A single DNA adduct was produced after 8 h of treatment with 500 $\mu M$ HQ; the relative adduct level was $7.8 \pm 0.8$ adducts per $10^7$ nucleotides. Adduct formation was linear and time dependent for 50–500 $\mu M$ HQ	Levay et al., 1991
DNA adduct formation	HL-60 cells	$^{32}P$ -postlabeling technique was used to determine DNA adduct formation after the addition of 0–2,000 $\mu M$ HQ for 1–72 h	A single DNA adduct was produced by treatment with 500 $\mu M$ HQ; the relative adduct level was $7.8 \pm 0.8$ adducts per $10^7$ nucleotides. DNA adduct formation was time- and concentration-dependent	Levay and Bodell, 1992
DNA adduct formation	HL-60 cells	$^{32}P$ -postlabeling technique was used to determine DNA adduct formation after 50 $\mu M$ HQ alone and in combination with 100 $\mu M$ catechol or 50 $\mu M$ 1,2,4-benzenetriol was added to cell cultures	DNA modification was increased 5-fold with the addition of catechol and 10-fold with the addition of 1,2,4-benzenetriol as compared with HQ alone	Levay and Bodell, 1991
DNA adduct formation	HL-60 cells	$^{32}P$ -postlabeling technique was used to determine DNA adduct formation after the addition of 50 $\mu M$ HQ in combination with 50–250 $\mu M$ catechol or 25–100 $\mu M$ 1,2,4-benzenetriol to cell cultures for 24 h	Combined treatment of HQ and 250 $\mu M$ catechol increased adduct formation 2.5-fold. Combined treatment of HQ and 50 or 100 $\mu M$ 1,2,4-benzenetriol increased adduct formation 2.2- and 2.7-fold, respectively	Levay and Bodell, 1992
DNA adduct formation	Calf thymus DNA	$^{32}P$ -postlabeling technique was used to determine DNA adduct formation after purified calf thymus DNA was reacted with 1 mg HQ and 1 mg <i>p</i> -benzoquinone overnight	Five adducts were produced; the relative adduct level was $5.5 \times 10^{-5}$	Levay et al., 1991

DNA adduct formation	Rat Zymbal glands (in vitro study); female Sprague-Dawley rats (in vivo study)	Nuclease P1-enhanced <sup>32</sup> P-postlabeling assay was used to detect DNA adducts. HQ was used in vitro. HQ/phenol were used in vivo; the bone marrow, Zymbal gland, spleen, and liver were examined for adducts	in vitro: HQ produced DNA adducts in vivo: HQ/phenol did not produce DNA adducts	Reddy et al., 1990
DNA binding	Calf thymus DNA	The binding of PHS catalyzed, arachidonate dependent, oxidation metabolite(s) of 100 $\mu$ M HQ to calf thymus DNA was examined. The results were compared with those obtained using heat-inactivated PHS as well as those in which indomethacin was added to the culture	DNA binding increased and was 65% greater than binding that occurred using heat-inactivated PHS. Indomethacin partially inhibited the binding	Schlosser et al., 1990
Forward mutation assay	L5178Y tk <sup>+/−</sup> mouse lymphoma cells	HQ in methanol or DMSO was tested at a concentration range of 0.625–10 $\mu$ g/ml with S9 (1 run) and at a concentration range of 0.625–25 $\mu$ g/ml without S9 (3 runs). Controls were used	Positive with S9 at 2.5–10 $\mu$ g/ml. Positive without S9 at 1.25–10 $\mu$ g/ml (above this dose it was toxic to cells)	McGregor et al., 1988a
Forward mutation assay	Mouse lymphoma L5178Y cells	A concentration range of 2.5–8.5 $\mu$ g/ml HQ was tested without metabolic activation	Positive	McGregor et al., 1988b
Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	A modified procedure of Schmid (1975) was used. Five male mice were dosed orally with 200 mg/kg HQ at a volume of 10 ml/kg. Controls were used	HQ significantly increased the number of MnPCEs as compared with controls	Gad-El Karim et al., 1986
Micronucleus test	Bone marrow cells from gravid Swiss CD-1 mice; fetal liver cells	Gravid mice were dosed with 40 mg/kg HQ by gastric intubation on day 13 of gestation. The mice were killed at various time intervals after dosing	A 1- to 3-fold increase in micronuclei of gravid mice was observed. A 3- to 6-fold increase in micronuclei of fetuses was observed	Ciranni et al., 1988a
Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	Mice were dosed with 80 mg/kg HQ in sterile distilled water orally or by i.p. injection. The mice were killed at various intervals after dosing	Oral administration produced a weak micronucleus increase. i.p. administration produced an evident micronucleus increase	Ciranni et al., 1988b
Micronucleus test	Mouse bone marrow cells		Positive	Shimada et al., 1988
Micronucleus test	V79 Chinese hamster cells	At least five concentrations were tested; the optimal/maximal concentration was 17.5 $\mu$ M	Positive	Glatt et al., 1989

(continued)

TABLE 2. Continued

Test	Organism and strain	Methods	Results and comments	Reference
Micronucleus test	Mouse bone marrow cells	Male and female mice were given a single i.p. dose of 30–100 mg/kg HQ or male mice were given doses of 15 and 75 mg/kg HQ daily for up to 3 days. The mice were killed at various intervals after dosing. Vehicle was bidistilled water and negative controls were used	A dose-dependent, nonlinear, response was observed after a single dose. No sex difference was observed. Additivity of the response was observed for multiple doses, with a significant effect produced after 3 days of dosing with 15 mg/kg. However, the three daily treatments were not as effective as the single total dose (45 mg/kg)	Adler and Kliesch, 1990
Micronucleus test	Peripheral human lymphocytes	The cytokinesis-blocked micronucleus assay was performed. An antikinetochores antibody was used to distinguish micronuclei containing whole chromosomes from those containing acentric fragments. HQ was tested alone, in combination with phenol, and with equimolar concentrations of catechol	Positive. Only minor increases were observed with the addition of phenol. A synergistic effect was observed using equimolar concentrations of HQ and catechol	Robertson et al., 1990
Micronucleus test	Human lymphocytes	A modified micronucleus assay was performed and an antikinetochores antibody was used to distinguish micronuclei that have a high probability of containing a whole chromosome from those containing acentric fragments. A dose range of 2.0–150 $\mu$ M HQ was used	An 11-fold increase in micronuclei was observed with 125 $\mu$ M HQ, with a clear dose response being seen from 25–125 $\mu$ M HQ. Significant increases in kinetochores-positive micronucleated cells were also observed with $\geq 75$ $\mu$ M HQ	Yager et al., 1990
Micronucleus test	Mouse bone marrow cells (male and female)	Mice were dosed with a single i.p. injection of 20–320 mg/kg HQ. The mice were killed at various intervals	Positive. No sex difference was observed. HQ was clastogenic in mouse bone marrow cells	Adler et al., 1991
Micronucleus test	Mouse bone marrow cells	HQ was assayed in mouse bone marrow cultures	HQ increased the number of MNPCEs $\geq 10$ times that of control values	Marrazzini et al., 1991
Micronucleus test	Male mouse bone marrow cells	Four mice per group were dosed with an injection of 40, 80, or 120 mg/kg HQ in water. Two h before dosing, mice were given 25 mg of BrdU by s.c. implantation. Bone marrow was removed 18 and 24 h after dosing	Positive. At 18 h, the number of MNPCEs was significantly increased by 80 mg/kg HQ. At 24 h, the number of MNPCEs was significantly increased at all three doses. The % PCEs was significantly decreased by 120 mg/kg HQ at 18 and 24 h	Pacchierotti et al., 1991

Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	Three mice per group were dosed with 80 mg/kg HQ in bidistilled water orally and by i.p. injection after s.c. injection of 25 mg BrdU	i.p. injection resulted in more micronuclei than oral administration. The greatest number of micronuclei in PCEs (10-fold the control value) was observed at 18 h and in normochromatic erythrocytes (5-fold the control value) at 42 h	Marrazzini et al., 1992
Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	Mice were dosed with a single i.p. injection of 40-80 mg/kg HQ or an HQ (40-80 mg/kg) and phenol (40-160 mg/kg) mixture	HQ alone gave weakly positive results. A significant dose-dependent increase in micronuclei in PCEs was observed. HQ and phenol coadministration resulted in a >2-fold increase in the number of micronuclei in PCEs compared with HQ alone	Barale et al., 1990
Micronucleus test	Cultured human lymphocytes	HQ in distilled water was tested at a concentration range of 1-20 µg/ml with S9 for various durations and without S9 for 2 h	Without S9, HQ caused a weak induction of micronuclei at a dose range of 5.5-6 µg/ml; 20 µg/ml was toxic. The presence of S9 did not increase the number of micronuclei	Migliore and Nieri, 1991
Micronucleus test	ENR and LEO human diploid fibroblasts	HQ in DMSO was tested at a concentration range of 0.15-8.1 µg/ml. The induction of CREST-positive and CREST-negative micronuclei and the total number of micronuclei was determined	At a dose of 8.1 µg/ml, HQ caused a significant increase in the number of CREST-negative and total micronuclei in LEO human diploid fibroblasts. There was a strong difference in the sensitivity to micronucleus induction between the two human diploid fibroblast strains	Bonatti et al., 1992
In vivo aneuploidy/micronucleus assay	Bone marrow cells from male CD-1 mice	Three mice/group were dosed with 20-80 mg/kg HQ by i.p. injection. The induction of MNPCEs and whether they were Kc <sup>+</sup> or Kc <sup>-</sup> was determined	At a dose of 40 and 80 mg/kg, HQ caused a significant increase in the number of MNPCEs. The number of Kc <sup>+</sup> MNPCEs was significant at all three doses and the number of Kc <sup>-</sup> MNPCEs was significant at doses of 20 and 40 mg/kg HQ. HQ was classified as highly aneuploidogenic	Gudi et al., 1992
Mouse spot test	Female C57BL/6JHan mice	The female mice were mated with T-stock males. Females were given an i.p. injection of 110 mg/kg HQ on day 10 of gestation. Animals were examined for spots one to two times/wk for 2-4 wks	Negative. Round gray spots, caused by lack of pigmentation at the base of the hairs, were observed on the backs of dosed females several days after treatment. These spots were not observed on the offspring. However, an increase of white midventral spots, suggesting cellular toxicity, were observed. The frequency of relevant spots was slightly, but not significantly, increased	Gocke et al., 1983

*nella typhimurium* were negative for mutagenicity by Hydroquinone (Haworth et al., 1983; Glatt et al., 1989), except for one study in which Hydroquinone was reported to be a direct-acting mutagen to the strains tested (Lin and Lee, 1992). In that study, the chlorinated or nitrosated derivatives of Hydroquinone were more mutagenic than Hydroquinone itself; the mutagenicity of Hydroquinone and its chlorinated and nitrosated derivatives was suppressed by the presence of S9. A *Salmonella* mutagenicity test was negative (Sakai et al., 1985) and a fluctuation test using *S. typhimurium* was positive with and negative without metabolic activation (Koike et al., 1988). A test for 6-thioguanine resistance was positive (Glatt et al., 1989). In an assay in which two genetically manipulated strains of *Streptomyces griseus*, one which detects point mutations and the other frame shift mutations, were used to activate promutagenic chemicals and determine the formation of mutagenic metabolites, Hydroquinone did not produce a positive response (Buchholz et al., 1992).

Hydroquinone induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells with and without metabolic activation (Galloway et al., 1987), in Chinese hamster Don cells (Shimada et al., 1988), and in Chinese hamster V79 cells (Glatt et al., 1989). Hydroquinone also increased SCEs in human lymphocyte cells, and a synergistic effect was observed when acetaldehyde was administered with Hydroquinone (Knadle, 1985). The number of SCEs induced by Hydroquinone was increased when the cell cultures were treated with dimethyl maleate before Hydroquinone administration.

The induction of chromosomal aberrations due to Hydroquinone was demonstrated in *Aspergillus nidulans* as increased mitogenic segregation (Kappas, 1989; Crebelli et al., 1991) without metabolic activation (Crebelli et al., 1987; Kappas, 1990) and in CHO cells with metabolic activation; in CHO cells without metabolic activation, increases were insignificant (Galloway et al., 1987). Hydroquinone induced mitotic division aberrations in two Chinese hamster cell lines (Parry et al., 1990) and it increased the frequency of mitotic crossovers in *A. nidulans* (Crebelli et al., 1991). Hydroquinone induced aneuploidy in male mouse germ cells (Miller and Adler, 1992) and acted as an aneuploidogen when assayed with micronuclei from mouse bone marrow cells (Van Hummelen et al., 1992). Hydroquinone was clastogenic for male mouse germ cells (Ciranni and Adler, 1991) and for mouse bone marrow cells. Chromosomal aberrations were induced in mouse bone marrow cells (Xu and Adler, 1990; Pacchierotti et al., 1991; Marrazzini et al., 1991; Marrazzini et al., 1992; Sbrana et al., 1992) and c-mitotic effects were observed in mouse bone marrow cells (Miller and Adler, 1989) and in human lymphocyte cultures (Sbrana et al., 1992). Hydroquinone induced chromosome loss in yeast strain D6 without metabolic activation (Parry et al., 1990), but did not induce chromosomal malsegregations using *Saccharomyces cerevisiae* D61.M (Albertini, 1990).

In an in vitro porcine brain tubulin assembly assay, Hydroquinone had no effect on the assembly of porcine brain tubulin (Brunner et al., 1991). High concentrations of Hydroquinone led to an enhancement of the depolymerization velocity.

Hydroquinone induced DNA strand breaks in a number of studies (Lewis et al., 1988; Shimada et al., 1988; Stenius et al., 1989; Maeda et al., 1990; Schlosser et

al., 1990; Leanderson and Tagesson, 1992; Walles, 1992); however, one study did not find Hydroquinone-induced DNA strand breakage (Pellack-Walker and Blumer, 1986). Slight DNA damage was observed using a sequencing technique (Kawanishi et al., 1989). Enzyme-altered rat liver foci were also observed (Stenius et al., 1989).

Hydroquinone inhibited DNA synthesis with and without metabolic activation (Pellack-Walker et al., 1985), nuclear DNA synthesis (Lee et al., 1989), and mitochondrial DNA (mtDNA) synthesis in rabbit bone marrow mitochondria but not rat liver mitoplasts (Schwartz et al., 1986). However, in a cell-free DNA synthetic system, Hydroquinone did not inhibit DNA synthesis (Lee et al., 1989); in another study, a significant increase in DNA synthesis in rat bladder epithelium cells was not observed (Kurata et al., 1990). (mtDNA) transcription synthesis was inhibited (Rushmore et al., 1983), as was RNA synthesis (Post et al., 1985, 1986) and mRNA synthesis under conditions specific for RNA polymerase II (Post et al., 1984).

Hydroquinone caused the dose-dependent formation of hydrogen peroxide and 8-hydroxydeoxyguanosine (8-OHdG) in calf thymus DNA (Leanderson and Tagesson, 1990).

Treatment of HL-60 cells with Hydroquinone produced DNA adducts (Pongracz et al., 1990; Levay et al., 1991; Levay and Bodell, 1992); the number of adducts formed was significantly increased with the addition of catechol or 1,2,4-benzenetriol (Levay and Bodell, 1991; Levay and Bodell, 1992). Adducts also formed by treating calf thymus DNA with a *p*-benzoquinone/Hydroquinone mixture (Levay et al., 1991). Reddy et al. (1990) found that Hydroquinone produced DNA adducts in vitro, but phenol/Hydroquinone did not produce adducts in vivo. The oxidative metabolism of Hydroquinone resulted in the formation of reactive product(s) that irreversibly bound to DNA (Schlosser et al., 1990).

Forward mutation assays with (McGregor et al., 1988a) and without (McGregor et al., 1988a, 1988b) metabolic activation were positive.

Positive micronucleus test results for Hydroquinone were obtained by Gad-El Karim et al. (1986), Ciranni et al. (1988a,b), Shimada et al. (1988), Glatt et al. (1989), Adler and Kliesch (1990), Robertson et al. (1990), Yager et al. (1990), Adler et al. (1991), Marrazzini et al. (1991), Pacchierotti et al. (1991), and Marrazzini et al. (1992). In the study by Robertson et al. (1990), coadministration of equimolar concentrations of Hydroquinone and catechol produced a significant synergistic effect on micronucleated cells as compared with Hydroquinone alone. In micronucleus tests reported by Barale et al. (1990) and Migliore and Nieri (1991), the results were weakly positive. A combined dose of Hydroquinone and phenol gave positive results. In one study, Hydroquinone caused a significant increase in CREST-negative and total micronuclei (Bonatti et al., 1992) and in another, it caused significant increases in kinetochore positive ( $Kc^+$ ), kinetochore negative ( $Kc^-$ ), and total micronucleated polychromatic erythrocytes (MNPCEs) (Gudi et al., 1992).

A mouse spot test for somatic gene mutations using Hydroquinone was negative (Gocke et al., 1983).

## CARCINOGENICITY

The carcinogenicity studies reviewed in this section are summarized in Table 3.

F344N rats, 65/sex/group, and B6C3F<sub>1</sub> mice, 64 or 65 males and 65 females/group, were dosed by gavage with Hydroquinone in deionized water 5 days/week for up to 103 weeks (NTP, 1989; Kari et al., 1992). The rats were dosed with 25 or 50 mg/kg and the mice were dosed with 50 or 100 mg/kg Hydroquinone; a group of rats and a group of mice were dosed with vehicle only and served as controls. Doses were selected based on the results of the subchronic study (NTP, 1989; Kari et al., 1992) summarized previously in this report.

All animals were observed twice daily and clinical signs were recorded monthly. Body weights were measured weekly for 13 weeks, and then monthly. Gross observations were made for all animals at necropsy. Microscopic examinations were performed on selected tissues from all rats, all mice of the control and high-dose groups, and all mice of the low-dose group that died on study. The microscopic examinations of tissues from the mice were performed according to an "inverse pyramid" design (McConnell, 1983*a,b*). After 15 months of dosing, 10 animals/group were randomly selected for interim necropsy and evaluation of clinical chemistry and hematologic parameters.

After 15 months, relative kidney weights of the male rats of the high-dose group were significantly greater than control values. A dose-related increase in the severity of nephropathy was observed in male rats. In female rats, there was a decrease in the occurrence of hyperplasia and neoplasms of the pituitary gland when compared with controls. For female rats of the high-dose group, hematocrit, hemoglobin concentration, and erythrocyte count were decreased when compared with control values. The alkaline phosphatase value was decreased in male rats of the high-dose group and increased in female rats of the low-dose group.

For male mice of the high-dose group, significant increases were observed in hematocrit, erythrocyte count, serum albumin concentration, total protein concentration, and serum alkaline phosphatase and sorbitol dehydrogenase activity after 15 months. For female mice of the high-dose group, serum albumin and total protein concentration were significantly increased and alanine aminotransferase and sorbitol dehydrogenase activity were significantly decreased when compared with the controls. The values for relative liver weight for all mice of the high-dose group, relative kidney weight for all treated female mice, and relative brain weight for female mice of the high-dose group were significantly increased compared with control values. Dose-related hepatic lesions, centrilobular fatty changes, and cytomegaly were observed in treated male mice. Hepatocellular neoplasms were observed in male and female mice; the number of neoplasms observed was not significantly greater than in the controls and therefore not attributed to the administration of Hydroquinone.

At study termination, there was no significant difference in the mortality rate between the dosed and control male rats; however, the number of moribund animals killed was greater for dosed male rats than controls after week 90. Body weights of male rats of the high-dose group were decreased after week 73 and of male rats of the low-dose group after week 89 when compared with the controls.

The relative brain, kidney, and liver weights of the male rats of the high-dose group were significantly increased when compared with control values.

The severity of spontaneous nephropathy for male rats of the high-dose group and papillary hyperplasia of the transitional epithelium overlying cysts of the renal papillae in all male rats was increased when compared with the controls. Renal tubular adenomas occurred in treated males but not in control males, and the incidence in the high-dose group was significantly different from controls. Tubular hyperplasia was observed in two male rats of the high-dose group. There were significantly fewer adenomas and adenomas and carcinomas (combined) of the anterior pituitary gland in male rats of the high-dose group than the controls; tumor incidence at this site was not considered to be dose related.

There was no difference in mortality between treated and control female rats. Body weights of all female rats remained similar throughout the study. A significant increase in the incidence of mononuclear cell leukemia was observed in treated female rats compared with the controls. Stage 3 leukemia, in which there is marked effacement of the spleen and advanced infiltration of the liver or other organs with neoplastic cells, was observed in 14 female rats of the high-dose group and eight female rats of the low-dose group as compared with five controls. c-Cell adenoma and carcinoma (combined) occurrence was significantly decreased in females of the low-dose group.

For rats, the authors concluded "there was evidence of Hydroquinone-related carcinogenicity in male F344/N rats, indicated by an increased incidence in tubular cell adenomas of the kidney and in female rats, as shown by an increase in mononuclear cell leukemia."

There was no difference in mortality between treated and control male mice. Body weights of male mice of the high-dose group were decreased after week 93 as compared with control values. The relative liver weights of treated male mice were increased compared with control values. In male mice, dose-related non-neoplastic hepatic lesions were observed. There was no significant difference in the occurrence of hepatic adenomas and carcinomas (combined) between test and control mice. Follicular cell hyperplasia of the thyroid gland was increased in treated mice.

There was no difference in mortality between treated and control female mice. Body weights of female mice of the high-dose group were decreased after week 20 compared with control values. Relative liver weights of females of the high-dose group were increased compared with controls. There was a significant increase in the number of hepatocellular adenomas in treated female mice. An increase in follicular cell hyperplasia of the thyroid gland was observed in dosed females.

For mice dosed with Hydroquinone, the authors concluded "there was no evidence in male B6C3F<sub>1</sub> mice, and there was evidence of carcinogenicity in female mice based on increases in hepatocellular neoplasms, mainly adenomas. Administration of Hydroquinone was also associated with thyroid follicular cell hyperplasia in both male and female mice and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice."

Hydroquinone was fed to F344 rats and B6C3F<sub>1</sub> mice in the diet to evaluate its carcinogenic potential (Shibata et al., 1991). Rats and mice, 30 male and 30 female

TABLE 3. *Hydroquinone (HQ) carcinogenicity studies*

Number, species and sex	Dose	Route of administration	Methods	Results and comments	Reference
F344N rats 65/sex/gp	25 or 50 mg/kg	Orally by gavage	Rats were dosed 5 days/wk for up to 103 wks. One group was dosed with vehicle (deionized water) and served as a control group	<i>Males:</i> Some evidence of carcinogenic activity of HQ as shown by marked increased incidence in renal tubular cell adenomas <i>Females:</i> Some evidence of carcinogenic activity of HQ as indicated by increased incidence of mononuclear cell leukemia	NTP, 1989
B6C3F <sub>1</sub> mice 64 or 65 males and 65 females/gp	50 or 100 mg/kg	Orally by gavage	Mice were dosed 5 days/wk for up to 103 wks. One group was dosed with vehicle (deionized water) and served as a control group	<i>Males:</i> No evidence of carcinogenic activity of HQ <i>Females:</i> Some evidence of carcinogenic activity of HQ as shown by increases in hepatocellular neoplasms, mainly adenomas	NTP, 1989
F344 rats 30/sex/gp	0.8% HQ	Orally in feed	Rats were fed treated diet for 104 wks. A control group was fed untreated feed	<i>Males:</i> A statistically significant number of renal tubular hyperplasias and microscopic adenomas developed <i>Males and females:</i> The average number of foci/cm <sup>2</sup> in analyzed liver sections was significantly decreased. There were no dose-related proliferative or neoplastic lesions nor any other microscopic lesions observed in the nonglandular or glandular stomach HQ has apparent carcinogenic potential for rodents; therefore it may play a role in human cancer development	Shibata et al., 1991
B6C3F <sub>1</sub> mice 30/sex/gp	0.8% HQ	Orally in feed	Mice were fed untreated diet for 96 wks. A control group was fed untreated feed	<i>Males:</i> The significant development of renal tubular hyperplasia and a nonstatistically significant increase in renal cell adenomas was observed. The incidence of hepatocellular adenomas, but not hepatocellular carcinomas, was increased. The occurrence of hepatic foci and the number of foci/cm <sup>2</sup> was significantly increased <i>Males and females:</i> The occurrence of squamous cell hyperplasia, but not tumor development, in the nonglandular epithelium was significantly increased HQ has apparent carcinogenic potential for rodents; therefore it may play a role in human cancer development	Shibata et al., 1991
Male Syrian golden hamsters 15/gp	0.5% HQ	Orally in feed	Hamsters were fed treated feed for 20 wks. A control group was fed untreated feed	There was no significant difference between treated and control animals in the incidence of mild or moderate hyperplasia of the nonglandular epithelium of the stomach. HQ had no structure-related activity in inducing proliferative neoplasms in the nonglandular portions of the stomach	Hirose et al., 1986
Male F344 rats 15 treated and 10 control animals	0.8% HQ	Orally in feed	The respective groups were fed treated or control diet for 51 wks	There were no significant differences observed between treated and control animals in the occurrence of lesions of the nonglandular or glandular stomach	Hirose et al., 1989

Male F344 rats 15 treated and 10 control animals	0.8% HQ	Orally in feed	The respective groups were fed treated or control diet for 49 wks	Squamous hyperplasia was increased for treated rats (26.7%) as compared with controls (0%)	Yamaguchi et al., 1989
Male F344 rats 5/gp	0.8% HQ	Orally in feed	Rats were fed treated feed for 8 wks. A control group was fed untreated feed. All animals were given an i.p. injection of BrdU 1 h before being killed	There was no significant difference between treated and control rats in cell proliferation in the nonglandular gastric epithelium or the pyloric glandular epithelium	Shibata et al., 1990
Tumor promotion					
Male F344 rats 20/gp	0.8% HQ	Orally in feed	Two groups of rats were given 150 mg/kg MNNG by stomach tube. Starting 1 wk later, the rats were fed treated or untreated feed for 51 wks	Larger neoplasms were observed in the nonglandular epithelia of HQ-treated rats compared with controls. However, there was no difference in the incidence of nonglandular or glandular stomach lesions between the groups	Hirose et al., 1989
Male F344 rats 15/gp	0.8% HQ	Orally in feed	Two groups of rats were given three i.p. injections of 25 mg/kg MNAN at wks 0, 1, and 2. Starting 1 wk later, rats were fed treated or untreated feed for 49 wks	A nonsignificant increase in the development of squamous cell carcinomas, including early in situ lesions, was observed in the esophagus of test animals compared with controls, but quantitative analysis reported that the number of squamous cell carcinomas was significantly greater in treated rats. The incidence of pulmonary alveolar cell hyperplasia was significantly decreased for treated animals. No significant difference was observed between treated and control rats in the incidence of nonglandular hyperplasia. HQ was marginally effective in enhancing esophageal carcinogenesis and demonstrated marginal activity in the promotion of upper digestive tract carcinogenesis	Yamaguchi et al., 1989
Male F344 rats 20 pretreated/gp; 10 nonpretreated	0.8% HQ	Orally in feed	Two groups were given 0.05% BBN in drinking water for 4 wks. Starting 3 days later, the rats were fed treated or untreated feed until the end of wk 36. A third, nonpretreated, group was fed treated feed (starting at the same time as the pretreated animals).	No differences in the incidence or number of PN hyperplasia, papilloma, or carcinoma were observed between the BBN-pretreated and nonpretreated test groups and the pretreated controls	Kurata et al., 1990
Male F344 rats 20 pretreated/gp; 10 nonpretreated	0.8% HQ	Orally in feed	Two groups were given 0.1% DHPN in drinking water for 2 wks. The pretreated and nonpretreated groups were then fed treated or untreated feed for 30 wks	Localized dark or whitish nodular hyperplastic and/or neoplastic lesions were observed in the lungs of pretreated test animals. No differences were observed between pretreated test and control rats in the incidences of pulmonary adenoma, carcinoma, or combined tumors, nor in the number of lesions observed in the thyroid gland, urinary bladder, or kidneys. Squamous cell carcinomas of the nonglandular stomach were observed in one pretreated test rat. Pulmonary, thyroid gland, urinary bladder, and/or kidney neoplastic lesions were not observed for nonpretreated HQ-dosed rats	Hasegawa et al., 1990

per species, were fed a diet containing 0.8% Hydroquinone for 104 and 96 weeks, respectively. The same number of male and female rats and mice was fed untreated diet and served as control groups. All animals were examined twice daily. Body weights were measured weekly throughout week 14, and then once every 4 weeks. Feed and water intake were determined over a 2-day period before each weighing. All surviving animals were fasted and killed at the termination of dosing. Necropsy was performed and the liver and kidneys of all animals were weighed.

No dose-related changes were observed for either species during the study, and there was no significant difference in survival for treated animals compared with controls. Body weight gains of dosed male and female rats and male mice were significantly reduced compared with the controls. Feed and water consumption were comparable for treated and control animals. The average amount of Hydroquinone intake, based on feed consumption, was 351 and 368 mg/kg/day for male and female rats, respectively, and 1,046 and 1,486 mg/kg/day for male and female mice, respectively.

There was a significant increase in the absolute and relative liver and kidney weights of dosed male rats and the relative kidney weights of dosed female rats as compared with controls. In mice, the relative liver and kidney weights of dosed females were significantly increased compared with the controls.

At necropsy, granular appearance or indentation of the kidneys was increased in treated male rats. Small nodular lesions were visible in the mucosa of the nonglandular stomach of many dosed male and female mice.

Chronic nephropathy was observed in treated and control male rats upon microscopic examination; however, it was more severe in the dosed males. Pelvic epithelial hyperplasia, a component of advanced chronic nephropathy, was increased in treated male rats. Slight chronic nephropathy was observed in female rats fed Hydroquinone. Statistically significant numbers of renal tubular hyperplasia, for which cystic forms were frequent and terms such as "dysplastic foci" and "dysplastic tubular epithelium" were considered synonymous, and microscopic adenomas, cystic or solid forms, developed in male rats fed Hydroquinone. The average number of foci per cm<sup>2</sup> of analyzed liver sections was significantly decreased for male and female rats fed Hydroquinone. Most of the hepatic foci were basophilic. There were no dose-associated proliferative or neoplastic lesions observed in the nonglandular or glandular stomach of rats, nor were any other dose-related microscopic lesions observed.

In male mice, microscopic lesions included significant numbers of renal tubular hyperplasia and an increase that was not statistically significant in renal cell adenomas. The characteristics of the renal lesions were similar to those observed in treated rats. Also in dosed male mice, a statistically significant increase in centrilobular hypertrophy of hepatocytes was observed. The number of hepatocellular adenomas, but not of hepatocellular carcinomas, was increased in male mice fed Hydroquinone compared with the controls. The occurrence of hepatic foci of the basophilic, eosinophilic, and clear cell types and the numbers of foci per cm<sup>2</sup> of liver were significantly increased for dosed male mice. In treated male and female mice, the occurrence of squamous cell hyperplasia, but not tumor

development, in the nonglandular gastric epithelium was significantly increased. There was no significant difference in tumor incidence for any other organ between treated and control animals.

The authors concluded the "study strongly suggested that since Hydroquinone has apparent carcinogenic potential for rodents, there is a possibility that it may play a role in human cancer development."

Fifteen male Syrian golden hamsters were given 0.5% Hydroquinone in the feed for 20 weeks to determine whether Hydroquinone would induce lesions in the squamous portion of the stomach and its effect on proliferation of the glandular stomach and urinary bladder (Hirose et al., 1986). A group of 15 hamsters was fed untreated feed and served as the control group. At the termination of dosing, the animals were killed and underwent necropsy, and nonglandular and glandular portions of the stomach and sections of the urinary bladder were removed for microscopic examination.

There was no significant difference in terminal body weights between the test and control groups. The liver weights of treated animals were greater than those of the controls. The number of animals with mild or moderate hyperplasia of the nonglandular gastric epithelium was not significantly different from the controls. No abnormal observations were made in any examined organs. Upon autoradiographic examination, the labeling indices in the nonglandular portion of the stomach, pyloric region of the glandular portion of the stomach, and the urinary bladder increased insignificantly when compared with control values. Hydroquinone had no structure-related activity in inducing proliferative neoplasms in the nonglandular portion of the stomach.

In order to examine the carcinogenic potential of Hydroquinone on the nonglandular and glandular portions of the stomach, 15 male F344 rats were fed a diet containing 0.8% Hydroquinone (Hirose et al., 1989). Ten male F344 rats ate untreated feed for 51 weeks and served as controls. Body weights and feed consumption were measured every 2-4 weeks. At study termination, all surviving animals were killed and underwent necropsy, and sections from the nonglandular and glandular stomach were removed for microscopic examination.

All rats survived until study termination. The terminal body weights were decreased and the relative kidney weights were significantly increased for the test animals compared with the controls. There was no significant difference in the incidences of gastric lesions (squamous portion), i.e., hyperplasia, papilloma, carcinoma in situ, and squamous cell carcinomas, between the test and control group. Also, there were no significant differences between the treated and control group in the incidences of glandular stomach lesions, i.e., adenomatous hyperplasia or adenocarcinoma in the fundic or pyloric regions.

Male F344 rats were used to examine the carcinogenic potential of Hydroquinone on the upper gastrointestinal tract (Yamaguchi et al., 1989). Fifteen rats were fed a diet containing 0.8% Hydroquinone for 49 weeks while 10 rats were fed untreated feed and served as controls. Body weights and feed consumption were measured every 2-4 weeks. All surviving animals were killed and underwent necropsy at study termination, and portions of the upper gastrointestinal tract were removed for microscopic examination.

All test and control rats survived until study termination. Terminal body weights were significantly decreased and relative kidney weights were significantly increased for test animals as compared with controls. Hyperplasia of the nonglandular portion of the stomach was increased for treated rats (26.7%) as compared with controls (0%).

Five male F344 rats were fed 0.8% Hydroquinone in the diet for 8 weeks to determine the carcinogenic potential on the nonglandular and glandular portions of the stomach; a control group of five rats was fed untreated feed (Shibata et al., 1990). At the end of 8 weeks, each rat was given an i.p. injection of bromodeoxyuridine and killed 1 h later. No rats died on study. No significant differences in body weight or feed consumption were found between the treated and control groups. Cell proliferation in the nonglandular gastric and the pyloric glandular epithelium were similar for the treated and control groups.

### TUMOR PROMOTION

Male F344 rats were used to examine the effects of Hydroquinone on the nonglandular and glandular portions of the stomach after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) administration (Hirose et al., 1989). Two groups of 20 rats were dosed with 150 mg/kg MNNG by stomach tube and then, starting 1 week later, were fed either 0.8% Hydroquinone in feed or untreated feed for 51 weeks. The group given untreated feed served as MNNG-pretreated controls. Body weights and feed consumption were measured every 2–4 weeks. At the termination of dosing, all surviving animals were killed and underwent necropsy; sections of the nonglandular and glandular stomach were removed for microscopic examination.

Fifteen pretreated controls and 16 pretreated test animals survived until study termination. Terminal body weights of the test animals were decreased as compared with controls. The relative liver and kidney weights of MNNG-pretreated rats fed Hydroquinone were greater than the control values. The neoplasms observed in the squamous epithelia of the nonglandular stomach of rats dosed with Hydroquinone after MNNG pretreatment were larger than those observed for pretreated control rats. However, there was no significant difference in the incidence of squamous epithelium lesions, i.e., hyperplasia, papilloma, carcinoma in situ, and squamous cell carcinomas, between the test and control group. Also, there were no significant differences between the treated and control group in the incidence of glandular stomach lesions, i.e., adenomatous hyperplasia or adenocarcinoma of the fundic or pyloric regions.

Male F344 rats, 15 per group, were given three i.p. injections of 25 mg/kg methyl-*N*-amyl nitrosamine (MNAN) at weeks 0, 1, and 2 of the study and then, starting 1 week later, were fed a diet containing 0.8% Hydroquinone or untreated feed for 49 weeks (Yamaguchi et al., 1989). Body weights and feed consumption were measured every 2–4 weeks. At the end of week 52 of the study, all surviving animals were killed and underwent necropsy; portions of the upper gastrointestinal tract were removed for microscopic examination.

Twelve pretreated test and 11 pretreated control animals survived until study

termination. Terminal body weights were significantly less and relative kidney weights were significantly greater for the test animals as compared with controls. In the esophagus, a nonsignificant increase in the development of squamous cell carcinomas, including early in situ lesions, was observed for test animals as compared with the controls; but using quantitative analysis, the number of squamous cell carcinomas was significantly greater in treated rats compared with controls. The incidence of pulmonary alveolar cell hyperplasia was significantly decreased for animals fed Hydroquinone as compared to MNAN-pretreated controls. There was no significant difference in the incidence of hyperplasia of the nonglandular mucosa in treated rats. Hydroquinone was marginally effective in enhancing esophageal carcinogenesis and demonstrated marginal activity in the promotion of upper digestive tract carcinogenesis.

Twenty male F344 rats were given 0.05% *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) in drinking water for 4 weeks and then, starting 3 days later, were fed a diet containing 0.8% Hydroquinone until the end of week 36 (Kurata et al., 1990). A control group of 20 rats was dosed with BBN but maintained on untreated feed. A third group of 10 rats was fed 0.8% Hydroquinone in the diet without BBN pretreatment. Dosing for nonpretreated rats started 4 weeks and 3 days after study initiation. After 36 weeks, all surviving animals were killed and various tissues were removed for microscopic examination.

All pretreated and nonpretreated test animals and 19 control animals survived until study termination. Terminal body weights were significantly decreased for BBN-pretreated test animals as compared with controls; no difference was observed for nonpretreated animals dosed with Hydroquinone. There were no differences observed in the incidence or number of papillary or nodular (PN) hyperplasia, papilloma, or carcinoma between the BBN-pretreated and nonpretreated test groups and the control group.

To evaluate the modifying effects on pulmonary tumorigenesis initiated by *N*-bis(2-hydroxypropyl)nitrosamine (DHPN), 20 male F344 rats were given 0.1% DHPN in drinking water for 2 weeks and then fed diet containing 0.8% Hydroquinone for 30 weeks (Hasegawa et al., 1990). A control group of 20 rats was dosed with DHPN and maintained on untreated feed. A third group of 10 rats was fed diet containing 0.8% Hydroquinone without DHPN pretreatment for 30 weeks. Body weights and feed consumption were measured periodically. After 32 weeks, all surviving animals were killed and the lungs, thoracic tissues, and various other tissues were removed for microscopic examination.

All rats survived until study termination. Terminal body weights were significantly decreased and absolute lung weights were significantly increased for DHPN-pretreated test animals as compared with nonpretreated test animals but not as compared with DHPN-pretreated control animals. Absolute liver weights for DHPN-pretreated test rats were significantly increased compared with DHPN-pretreated controls and significantly decreased compared to nonpretreated test rats. Localized dark or whitish nodular lesions, which were microscopically hyperplastic and/or neoplastic, were observed in the lungs of DHPN-pretreated test animals.

No differences were observed in the incidences of pulmonary adenoma, carci-

noma, or combined tumors between the DHPN-pretreated test and control groups, nor were there differences observed in the number of lesions in the thyroid glands, urinary bladder, or kidneys. Squamous cell carcinomas of the non-glandular stomach were observed in one DHPN-pretreated rat dosed with Hydroquinone. Pulmonary, thyroid gland, urinary bladder, and/or renal neoplastic lesions were not observed in the rats of the nonpretreated test group.

## CLINICAL ASSESSMENT OF SAFETY

### Sensitization

Eighty patients positive to at least one hapten of the *para* group of the ICDRG standard series (*p*-phenylenediamine (PPD), diaminodiphenylmethane, benzocaine, or PPD mix) were tested using the A1 test (reference and/or test description not given) with 50  $\mu$ l of a prepared solution of 0.5% Hydroquinone (Picardo et al., 1990b). No reactions to Hydroquinone were observed.

### Dermal Effects

Dermatitis has been caused by contact with Hydroquinone and from application of an "antiseptic oil" which contained a trace amount of Hydroquinone (Deichmann, 1983).

Hydroquinone has caused hypomelanosis-hyperpigmentation of the skin and depigmentation of black skin (Deichmann, 1983).

### Ocular Effects

Exposure to Hydroquinone in vapor or dust at concentrations of 10–30  $\text{mg}/\text{m}^3$  air has resulted in keratitis, discoloration of the conjunctiva, and corneal changes (Deichmann, 1983). The corneal changes, especially alteration of the curvature, remained after stain and pigment disappeared.

### Toxic Effects

Ingestion of 1 g Hydroquinone by an adult produced tinnitus, nausea, dizziness, a sense of suffocation, increased respiration rate, vomiting, pallor, muscular twitching, headache, dyspnea, cyanosis, delirium, green to brownish-green urine, and collapse (Deichmann, 1983). Ingestion of 5–12 g Hydroquinone has resulted in death, apparently due to respiratory failure and anoxia.

## EPIDEMIOLOGY

Between 35 and 45% of American women dye their hair, often at monthly intervals, over a period of years (CTFA, 1993). This estimate is drawn from market research data on hair dye product use, generally from females aged 15 to 60 years.

A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber, or between cancer and personal use of hair dyes. The World Health Organization's International Agency for Re-

search on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6–13, 1992, in Lyon, France (IARC, 1993).

The charge to the IARC Working Group was to ascertain whether all appropriate data had been collected and were being reviewed; to evaluate the results of the epidemiologic and experimental studies and prepare accurate summaries of the data; and to make an overall evaluation of the carcinogenicity of exposure to humans.

The IARC Working Group conclusions were: "There is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence: "Occupation as a hairdresser or barber entails exposures that are *probably carcinogenic (Group 2A)*." And: "There is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic." Hence: "Personal use of hair colourants *cannot be evaluated as to its carcinogenicity (Group 3)*." (IARC, 1993).

### SUMMARY

This Addendum to the Final Report on Hydroquinone was prepared in response to the release of an NTP (1989) report of an oral carcinogenicity study. In the original CIR report, it was concluded that Hydroquinone was safe for cosmetic use at  $\leq 1\%$  in formulations designed for discontinuous, brief use followed by rinsing from skin and hair. This conclusion applied primarily to the use of Hydroquinone in hair dye formulations. The use of Hydroquinone to lighten the skin was not addressed because such use is regarded by FDA as a drug use.

In 1993, Hydroquinone was reported to be used in 206 formulations, 185 hair dyes, two lipsticks, one skin freshener, and 18 other skin care preparations.

Hydroquinone in an alcoholic vehicle was absorbed through the skin of the forehead of male subjects; absorption of Hydroquinone from a solution that also contained Escalol 507 (a sunscreen) and Azone (a penetration enhancer) was  $35 \pm 17\%$ , from a solution containing Azone was  $66 \pm 13\%$ , from a solution containing Escalol 507 was  $26 \pm 14\%$ , and from a solution containing only Hydroquinone was  $57 \pm 11\%$ . The average percutaneous absorption rate of Hydroquinone using 48-h excretion data from dermal and i.v. absorption studies using dogs was estimated to be  $\sim 0.15 \text{ nmol/cm}^2/\text{min}$  ( $1.1 \text{ } \mu\text{g/cm}^2/\text{h}$ ). Hydroquinone was rapidly absorbed and excreted by male and female Fischer rats following oral administration; overall recovery was  $\geq 96\%$  from females after 24 h and from males after 48 h. In a study using urinary excretion data, dermal absorption was estimated to be 10.5% for male rats using 72-h data and 11.5% for female rats using cumulative 48-h data.

Hydroquinone was found to have some immunologic effects; it especially had effects on bone marrow. In an FOB, Hydroquinone was not found to cause central or peripheral nervous system lesions. Hydroquinone was nephrotoxic in male F344 rats. Hydroquinone also showed cytotoxic properties.

According to the terminology of Hodge and Sterner (1949), Hydroquinone is slightly toxic, with an oral  $\text{LD}_{50}$  of 743 and 627 mg/kg for male and female rats, respectively.

Administration of Hydroquinone to rats in drinking water (2,500–10,000 ppm) for 8 weeks resulted in significant increases in liver and kidney weights. Hydroquinone administered orally to rats (63–1,000 mg/kg) and mice (31–500 mg/kg) for 14 days resulted in tremors and deaths in the high-dose groups. Dermal administration to rats (240–3,840 mg/kg) and mice (300–4,800 mg/kg) for 14 days caused neither death nor any significant adverse effects. For mice given i.p. injections of 10 mg/kg Hydroquinone for 6 weeks, it was concluded that Hydroquinone may cause hematologic injury.

Rats given 1,000–4,000 ppm Hydroquinone in drinking water for 15 weeks had significantly increased liver and kidney weights. Oral administration of 25–400 mg/kg Hydroquinone to rats and mice for 13 weeks resulted in mortality in the high-dose groups for both rats and mice. Other adverse signs, such as lethargy, tremors, and changes in relative liver to body weight ratios, were observed.

Dermal application of 25 or 150 mg/kg Hydroquinone to rats produced slight to severe erythema.

In a Magnusson–Kligman guinea pig maximization test, Hydroquinone was classified as an extreme sensitizer. Hydroquinone was positive for sensitization in an LLNA.

Oral administration of Hydroquinone did not produce embryotoxic, fetotoxic, or teratogenic effects in rats, nor did it produce significant adverse reproductive effects in a two-generation study. Using rabbits, various teratogenic/reproductive treatment-related effects were observed at doses of 200–500 mg/kg. All dams dosed with 300–500 mg/kg Hydroquinone died. Some maternal toxicity was observed at a number of dose concentrations.

Hydroquinone induced SCEs, chromosomal aberrations, and mitotic division aberrations increased the frequency of mitotic crossovers, caused c-mitotic effects, and induced chromosome loss. It was clastogenic for male mouse germ cells and for mouse bone marrow cells. Hydroquinone induced DNA strand breaks and inhibited DNA, nuclear DNA, and mtDNA synthesis in rabbit bone marrow mitochondria. It also inhibited mtDNA transcription synthesis and RNA synthesis. Hydroquinone caused the formation of hydrogen peroxide and 8-OHdG in calf thymus DNA and produced DNA adducts in HL-60 and other cells. Forward mutation assays with and without metabolic activation were positive, as were numerous micronucleus assays. Results of the Ames test and a mouse spot test for somatic gene mutations were negative.

In an NTP study, Hydroquinone was given to rats orally by gavage five times per week for up to 103 weeks at doses of 25 or 50 mg/kg. The higher dose induced a significant incidence of renal adenomas in males and both doses caused a significant increase in the incidence of mononuclear cell leukemia in females. Mice were dosed with 50 or 100 mg/kg Hydroquinone following the same schedule as that used for the rats. The incidence of hepatocellular adenoma was significantly increased in female mice.

NTP concluded that Hydroquinone produced “some evidence of carcinogenic activity” for male and female F344/N rats and female B6C3F<sub>1</sub> mice but “no evidence of carcinogenic activity” for male B6C3F<sub>1</sub> mice in an oral carcinogenicity study.

Shibata et al. (1991) conducted a study in which rats and mice were fed diet containing 0.8% Hydroquinone for 104 and 96 weeks, respectively, and concluded that "the study strongly suggested that since Hydroquinone has apparent carcinogenic potential for rodents, there is a possibility that it may play a role in human cancer development." Hydroquinone did not induce a significant number of neoplasms in either the glandular or nonglandular stomach of hamsters fed 0.5% Hydroquinone in the diet for 20 weeks or rats fed 0.8% Hydroquinone in the diet for 51, 49, or 8 weeks.

When Hydroquinone was fed to rats after pretreatment with MNAN, Hydroquinone was marginally effective in enhancing esophageal carcinogenesis and had marginal activity in the promotion of upper digestive tract carcinogenesis. Other studies did not prove Hydroquinone to be a tumor promoter.

No reaction to Hydroquinone was observed when patients positive to at least one hapten of the *para* group of the ICDRG standard series were tested using the A1 test. Hydroquinone contact has caused dermatitis and Hydroquinone exposure can result in ocular effects. Hydroquinone has caused hypomelanosis-hyperpigmentation of the skin and depigmentation of black skin. Ingestion of 1 g Hydroquinone by humans can produce severe toxicity; ingestion of 5–10 g can be fatal.

## DISCUSSION

The CIR Expert Panel previously determined that Hydroquinone was safe for cosmetic use at  $\leq 1\%$  in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. The conclusion was reached primarily for Hydroquinone's use in hair dyes because at the time the conclusion was made, the only confirmed use of Hydroquinone in cosmetic products was in hair dye formulations. The use of Hydroquinone in skin preparations as a skin lightener was regarded as a drug use.

Following the November 30–December 1, 1992 Expert Panel meeting, at which the safety of use of Hydroquinone was discussed, FDA advised CIR that in 1993, Hydroquinone has been reported to be used in lipstick formulations, skin lotions, and other nonrinse-off cosmetic products that may not necessarily be classified as drugs. Therefore, in accordance with the CIR Procedures, the conclusion on the safety of the use of Hydroquinone in cosmetic products must address both types of products, i.e., rinse-off and leave-on products.

Since the release of the initial final report in 1986, additional data have become available. A 1989 NTP oral carcinogenicity study using rats and mice indicated that Hydroquinone showed some evidence of carcinogenicity in laboratory animals. Other data indicate that Hydroquinone may have immunotoxic effects, especially on bone marrow. In addition, new data indicate that, although Hydroquinone has been shown to be a "slow" permeant through human skin using an aqueous vehicle, it is readily absorbed through the skin from an alcoholic vehicle.

Currently, some marketed hair dye formulations that use Hydroquinone also contain one or more alcohols. However, the Expert Panel noted that data supplied by industry indicate that during the hair dyeing procedure, Hydroquinone is a

“consumable.” This means that the actual concentration of Hydroquinone decreases sharply as the color-forming reaction proceeds. Therefore, the amount of Hydroquinone that may be absorbed during the hair dyeing process is limited both by the decreasing concentration of available Hydroquinone and by the length of time the hair dye is applied before being rinsed off.

However, the actual exposure to and absorption of Hydroquinone as it is used in hair dyes would not be applicable to leave-on cosmetics. For leave-on products, absorption could occur over a period of time, even with products that do not contain alcohol. Also, in the case of lipstick formulations, Hydroquinone would be ingested and absorbed more readily.

Therefore, the Expert Panel determined that the CIR Expert Panel’s conclusion of safety for the use of Hydroquinone in cosmetic products should distinguish between the two different types of exposure. The Expert Panel confirmed its original conclusion of safety for rinse-off preparations, but added the qualification indicating that Hydroquinone was safe for use in *aqueous* rinse-off products at  $\leq 1.0\%$ . The Expert Panel noted that the regulated drug use of Hydroquinone was, at least in part, for a cosmetic purpose, i.e., to eliminate blemishes of the skin, and/or for other medical conditions, such as uneven skin pigmentation. The Expert Panel therefore decided to clarify its earlier conclusion of 1986 in which all then-reported leave-on cosmetic uses of Hydroquinone were assumed to be misclassified and considered drugs, by now stating that Hydroquinone should not be used in any leave-on type of nondrug cosmetic product. Manufacturers of leave-on drug/cosmetic products that contain Hydroquinone should seek guidance from the governmental agencies that regulate such products.

### CONCLUSION

The CIR Expert Panel concludes that Hydroquinone is safe at concentrations of 1.0% and less for *aqueous* cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on nondrug cosmetic products.

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# Final Amended Safety Assessment of Hydroquinone as Used in Cosmetics

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## Abstract

Hydroquinone is an aromatic compound that functions in cosmetics as an antioxidant, fragrance, reducing agent, or polymerization inhibitor. Hydroquinone is also used as a skin bleaching agent. Safety and toxicity information indicate that hydroquinone is dermally absorbed in humans from both aqueous and alcoholic formulations and is excreted mainly as the glucuronide or sulfate conjugates. Hydroquinone is associated with altered immune function in vitro and in vivo in animals and an increased incidence of renal tubule cell tumors and leukemia in F344 rats, but the relevance to humans is uncertain. Quantitatively, however, the use of hydroquinone in cosmetics is unlikely to result in renal neoplasia through this mode of action. Thus, hydroquinone is safe at concentrations of  $\leq 1\%$  in hair dyes and is safe for use in nail adhesives. Hydroquinone should not be used in other leave-on cosmetics.

## Keywords

cosmetics, hydroquinone, safety

## Introduction

The Cosmetic Ingredient Review (CIR) Expert Panel first assessed the safety of hydroquinone (HQ) as a cosmetic ingredient in 1986<sup>1</sup> and concluded that HQ was found to be safe for use at limited concentrations for certain formulations (primarily hair dyes). A subsequent review was conducted following the completion of the National Toxicology Program (NTP) report on HQ. That amended safety assessment,<sup>2</sup> of HQ dealt with the use of HQ in cosmetic leave-on preparations and was published in 1994 with the conclusion from the CIR Expert Panel that HQ "... is safe at concentrations of  $\leq 1\%$  for aqueous cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on, non-drug cosmetic products."<sup>2</sup> This amended safety assessment will address the current uses of HQ in cosmetic products and assess information on the safety of HQ in cosmetic products that have become available since the last (1994) published report.

## Chemistry

### Definition and Structure

Hydroquinone (CAS No 123-31-9) is the aromatic compound that can function as an antioxidant, a fragrance ingredient, a

hair colorant, a reducing agent, and a skin bleaching agent.<sup>3</sup> The structure of HQ is presented in Figure 1. Technical names for this ingredient are presented in Table 1.<sup>3</sup>

### Physical and Chemical Properties

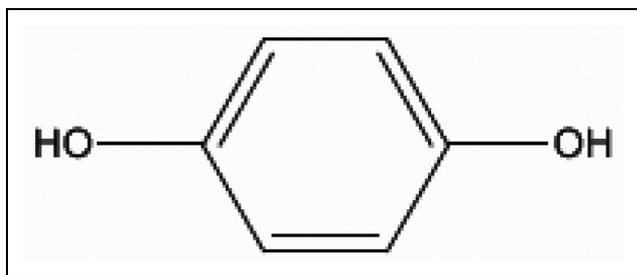
In its pure form, HQ is a colorless crystalline solid. Commercial preparations of HQ are usually white to off-white crystalline materials. The physical and chemical properties of HQ are presented in Table 2.<sup>4</sup>

### Manufacture and Production

There are 5 grades of HQ: photographic, technical, US pharmacopeia (USP), inhibitor, and polyester. Photographic and technical grades are produced in the highest volume, most commonly through either hydroperoxidation of *p*-diisopropylbenzene, hydroxylation of phenol, or oxidation of aniline.<sup>4</sup>

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**Figure 1.** The structure of hydroquinone.

**Table 1.** Technical Names for Hydroquinone<sup>3</sup>

Ingredient Name	Other Technical Names
Hydroquinone	1,4-Benzenediol 1,4-Dihydroxybenzene p-Dihydroxybenzene 4-Hydroxyphenol p-Hydroxyphenol

**Table 2.** Physical and Chemical Properties of Hydroquinone<sup>4</sup>

Property	Value
CAS No	123-31-9
Molecular weight	110.11
Color, form	Colorless, crystals
Melting point (°C)	172
Boiling point (°C)	285-287 @760 mm Hg
Vapor pressure @ 25 °C, Pa	$2.34 \times 10^{-3}$
LogP <sub>ow</sub>	0.50-0.61
Solubility, g per 100 g solvent (30°C)	
Solvent	Value
Ethanol	46.4
Acetone	28.4
Water	8.3

### Impurities

Resorcinol (1,3-benzenediol) and catechol can be present in HQ preparations depending on the method of manufacture.<sup>4</sup> In addition, impurities that impart a yellow color to the crystals are often present in technical-grade HQ but can be removed through specialized sublimation processes to produce more refined grades of HQ.

### Analytical Methods

Hydroquinone can be analyzed by a number of techniques, including spectroscopic, chromatographic, titrimetric, and electrochemical methods.<sup>4</sup>

### Reactions

In solution, HQ is rapidly converted to *p*-benzoquinone via an oxidation reaction upon exposure to air. The rate of this

oxidation is increased in alkaline solution.<sup>4</sup> Hydroquinone, which acts as a regulating agent allowing some control of the color-forming coupling reactions, is a “consumable” in the hair dyeing procedure, with its actual concentration decreasing as the color-forming reaction proceeds.<sup>5</sup>

## Use

### Cosmetic

Hydroquinone is an aromatic compound that is used in cosmetic formulations as an antioxidant, fragrance ingredient, hair colorant, and a reducing agent. Hydroquinone is present in the liquid component of artificial nail systems where it functions to inhibit the polymerization of the methacrylate monomers.<sup>6</sup> According to information supplied to the US Food and Drug Administration (FDA) as part of the Voluntary Cosmetic Registration Program (VCRP), the use of HQ has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009 (Table 3).<sup>7,8</sup> A survey of current use concentrations conducted by the Personal Care Products Council reported a use of 0.5% for HQ in other nail care products.<sup>9</sup>

Hydroquinone is added to the methacrylate monomers at a concentration of 0.02% or less than 200 ppm to inhibit their polymerization during transport and storage. In a study to determine the concentration of HQ that remains following the polymerization process, methacrylate polymer was analyzed using the titration method with UV detection (limit of detection [LOD] = 10 ppm) and the concentration of HQ in the finished product was below the LOD.<sup>11</sup>

In 2008, the European Union (EU) banned the use of HQ in hair dyes due to a lack of safety information. They have stated that there is no evidence that HQ as found in hair dye products is safe for human health.<sup>12</sup> Hydroquinone is approved for use in the EU in artificial nail systems, for professional use only, up to a maximum concentration of 0.02% after mixing.<sup>13</sup>

### Noncosmetic

**Medical.** Hydroquinone is used in topical formulations as a skin bleaching and depigmenting agent and is used in the treatment of melasma (chloasma), freckles, senile lentiginos, and postinflammatory hyperpigmentation. This use of HQ is considered a drug use that falls under the purview of the FDA. Hydroquinone is available via a prescription and over-the-counter (OTC) products that range in concentration from 0.4% to 5% HQ.<sup>14</sup> These OTC products are used to lighten the skin and are applied all over the face and body with varying use patterns among consumers. It is recommended that exposure to sunlight be avoided when using these products.

The FDA has issued a Federal Register notice on their intent to designate OTC skin bleaching products as no longer generally recognized as safe and effective (GRASE). Any products currently on the market would be considered new drugs that require an approved new drug application (NDA) for continued marketing.<sup>14</sup>

**Table 3.** Historical and Current Cosmetic Product Uses and Concentrations for Hydroquinone<sup>7-10</sup>

Product Category (FDA 2008)	1993 Uses (FDA 1993)	2007 Uses (FDA 2007)	2009 Uses (FDA 2009)	2009 Concentrations (%; Council 2009)
Hair coloring products				
Dyes and colors	185 (1112)	139 (2481)	13 (2481)	–
Makeup				
Lipstick	2 (937)	1 (1912)	–	–
Nail care products				
Other <sup>a</sup>	–	– (124)	– (124)	0.5
Skin care products				
Cleansing creams, lotions, liquids, and pads	–	1 (1368)	1 (1368)	–
Face and neck creams, lotions, etc	–	– (1195)	2 (1195)	–
Moisturizers	–	1 (2039)	2 (2039)	–
Fresheners	1 (246)	– (285)	2 (285)	–
Other	18 (848)	9 (1244)	12 (1244)	–
Total uses/ranges for hydroquinone	206	151	32	0.5

Abbreviations: FDA, US Food and Drug Administration.

<sup>a</sup> Nail adhesive.

The EU banned the use of HQ in OTC skin lightening products in 2001.<sup>15,16</sup>

**Industrial.** Hydroquinone is used in the development of black and white film, as an inhibitor of polymerization, as a stabilizer in paints, varnishes, motor fuels and oils, and as an antioxidant for fats and oils.<sup>17</sup>

## General Biology

### Inhibition of Tyrosinase

The ability of HQ to decrease melanogenesis when applied topically has made this a useful agent for decreasing skin pigmentation. Hydroquinone has been shown to be a weak substrate for the enzyme tyrosinase in kinetic studies but is able to compete with the endogenous substrate, tyrosine in vivo and inhibit the conversion of tyrosine to dopa and dopa to dopaquinone, thus decreasing melanin formation.<sup>18,19</sup>

### Absorption, Distribution, Metabolism, and Excretion

Hydroquinone is rapidly absorbed and excreted in urine in rats following oral administration.<sup>2</sup> Absorption from an alcohol vehicle is greater than from an aqueous solution. Hydroquinone in an aqueous solution was absorbed through human skin at a rate of  $0.55 \pm 0.13 \mu\text{g}/\text{cm}^2$  per h

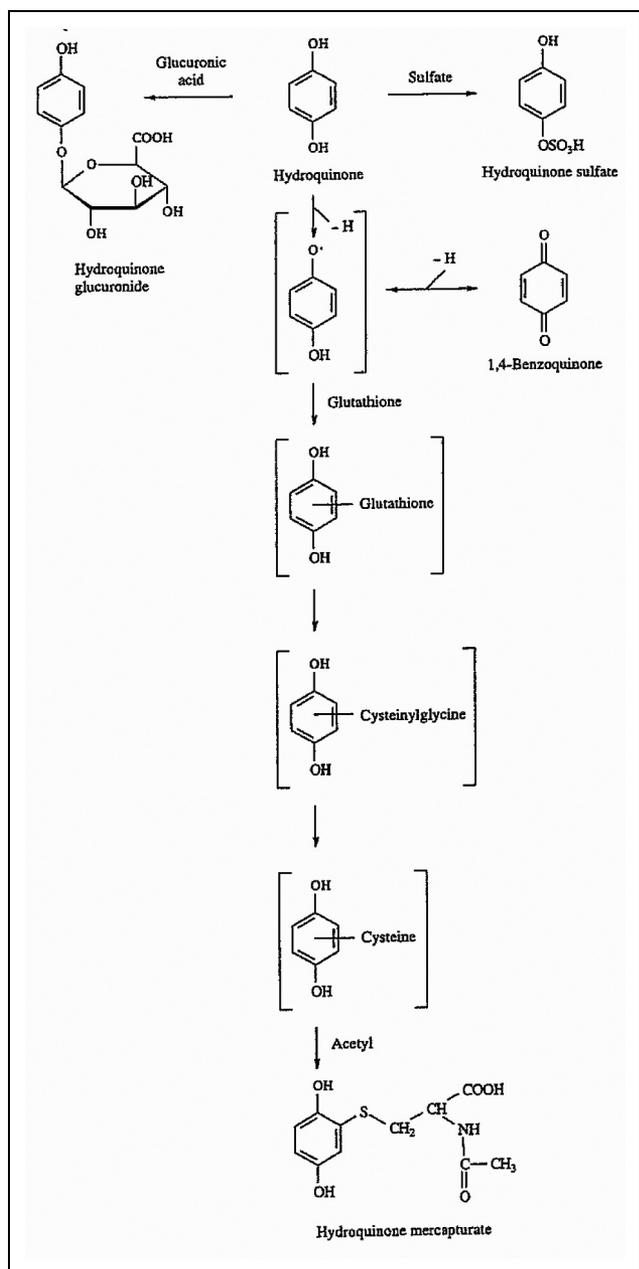
Proposed pathways for the metabolism of HQ in rats and humans are presented in Figure 2. The principal products observed from the metabolic process are the sulfate and glucuronide conjugates. Oxidation to 1,4-benzoquinone results in a reactive metabolite that forms mono- or polyglutathione conjugates.<sup>17</sup> The glutathione conjugates are believed to be the causal agents in rodent nephrotoxicity and renal carcinogenesis.

*In vitro.* Human skin (500  $\mu\text{m}$  thick) from 6 donors was used to examine the permeation of a 2% [<sup>14</sup>C]hydroquinone cream

alone and following pretreatment with 2% sodium azide.<sup>20</sup> No difference was observed in the permeation of HQ between sodium azide treated (42.7%) and untreated (43.3%) skin. A lag time of 8 hours was noted and believed to be attributed to the time it takes for the HQ cream to penetrate through the skin and into the receiving fluid. Using this model system, 100% of the dose was accounted for.

Metabolism was also examined in this system, with no significant difference in HQ recovery with ( $28.1\% \pm 18.4\%$ ) and without ( $28.5\% \pm 12.9\%$ ) sodium azide treatment. The amount of benzoquinone recovered was significantly decreased, however, from  $10.8\% \pm 5.7\%$  without sodium azide pretreatment to  $4.1\% \pm 2.0\%$  with sodium azide pretreatment.<sup>20</sup>

The metabolic rate constants for the conversion of HQ to the monogluthathione conjugate (HQ-SG) and subsequently to the mercapturic acid (HQ-Cys) in hepatocyte cultures isolated from F344 rats and humans were measured.<sup>21</sup> The substrate was added to the culture system and the disappearance and subsequent appearance of downstream metabolites were followed using sequential sampling (from 5 to 75 minutes) and high-performance liquid chromatography (HPLC) analysis. In this model system, glucuronidation was the initial favored step in both rat and human hepatocytes, with human hepatocytes having a higher  $V_{\text{max}}$  and intrinsic clearance ( $V_{\text{max}}/K_m$ ) than rat hepatocytes (15.2 vs 3.85 nmol/min per  $10^6$  cells, respectively). The human hepatocytes also demonstrated a higher capacity for metabolism of the HQ-SG to HQ-Cys than the rat hepatocytes. Acetylation was favored over deacetylation in both species. Overall, the authors concluded that the capacity for the metabolism of HQ and HQ-SG is greater in humans than in rats, suggesting a greater capacity for the detoxification of the glutathione conjugates. These metabolic constants were incorporated into an existing physiologically based pharmacokinetic (PBPK) model, which subsequently predicted that the body burden of these metabolites would be much higher in rats than in humans.<sup>21</sup>



**Figure 2.** Proposed metabolism of hydroquinone.<sup>17</sup>

*In vivo.* Male F344 rats (number not specified) were given either a single dose of 1.8 mmol/kg [<sup>14</sup>C]HQ in corn oil or 14 daily doses of HQ (1.8 mmol/kg in corn oil) followed by a single dose of 1.8 mmol/kg [<sup>14</sup>C]HQ on day 15 by gavage.<sup>22</sup> Urine was collected for 72 hours and analyzed for total radioactivity. Following a single gavage dose, 21% of the dose was recovered in the 0- to 5-hour urine sample with an additional 35% excreted in the next 19 hours. The major metabolites identified, via HPLC-UVEC, in the 0- to 24-hour urine samples were HQ glucuronide (21%), HQ sulfate (15%), and HQ mercapturate (13%). Following subchronic administration, 46% of the radioactivity was recovered in the 0- to 5-hour urine sample and 31% excreted in the next 19 hours. Subchronic

**Table 4.** Concentrations of Arbutin in Foods Used in the Human Diet Experiments<sup>24</sup>

Food Product	Arbutin Concentration (μg/g)
Tea	0.14 ± 0.02
Coffee	0.31 ± 0.04
Pear (Bosc)	3.84 ± 0.74
Pear (d'Anjou)	15.09 ± 11.69
Wheat cereal	1.04 ± 0.09
Wheat germ	10.65 ± 3.61
Whole wheat bread	2.04 ± 0.35

treatment changed the metabolic profile, with an increase in the amount of radioactivity excreted as the glucuronide conjugate (2 fold) and the glutathione conjugate (1.4 fold), while the percentage of the dose that was excreted as the sulfate remained the same. The authors state that these findings lend support to their hypothesis that subchronic administration of HQ increases the rate and extent of HQ metabolism to nephrotoxic glutathione conjugates.<sup>23</sup>

Hydroquinone occurs as the glucose conjugate, 4-hydroxyphenyl-β-D-glucopyranoside (arbutin), in the leaves of several plants, including cranberries, blueberries, and some varieties of pear. Arbutin is easily hydrolyzed to D-glucose and HQ in hot, dilute acid.<sup>4</sup> To determine the contribution of the diet to total HQ levels in the body, Deisinger et al (1996) measured the concentration of arbutin and HQ in foods and in human plasma and urine samples, respectively, using gas chromatography with an electron capture detector (GC-ECD).<sup>24</sup> They found certain foods to be high in arbutin as shown in Table 4. The researchers measured the concentration of HQ in 2 groups of human volunteers (2/gender per group) who consumed a high- or low-HQ breakfast. Blood samples were collected at 30, 50, and 120 minutes after completion of the meal and urine was collected every 2 hours for 7 to 8 hours after the morning meal. A meal low in HQ was provided for lunch for both the high- and low-HQ breakfast groups. Hydroquinone and phenol concentrations were determined in acid hydrolyzed samples and HQ concentrations were statistically significantly elevated at the 1- and 2-hour time points (exact values not given) in blood plasma, as compared to background concentration. Mean plasma total phenol concentrations did not change with time. A similar pattern was observed for HQ in the urine samples. The concentration of HQ was statistically significantly increased at 2 to 3 hours after the high-HQ meal and decreased slowly after 3 hours. Urinary total phenol excretion rates averaged ~800 μg/h and showed high interparticipant variability but little mean variability over time. Selected, unhydrolyzed blood and urine samples were analyzed and very little free HQ or phenol was found in these samples. It was determined from this that HQ absorbed from these exposures is rapidly conjugated and is not detectable as free HQ or phenol. Plasma and urine levels of HQ and phenol in the low-HQ breakfast group showed a decline (not statistically significant) in their levels over time.

The *in vivo* bioavailability of HQ was examined in 3 different experiments using a total of 14 healthy males (age

**Table 5.** Radioactivity Recovery Following Topical Application of 25  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]Hydroquinone to Human Forehead Skin for 24 Hours<sup>20</sup>

Time (h)	Percentage Administered Dose <sup>a</sup>	
	Urine	Skin Surface Wash
24	37.0 $\pm$ 9.8	5.2 $\pm$ 3.2 <sup>b</sup>
48	7.1 $\pm$ 2.1	
72	0.9 $\pm$ 0.6	
96	0.4 $\pm$ 0.2	
Total	45.3 $\pm$ 11.2	5.2 $\pm$ 3.2

<sup>a</sup> Values are mean  $\pm$  SD (n = 6).<sup>b</sup> Dosed skin site washed with soap and water after 24-hour dosing period.**Table 6.** Radioactivity Recovered Via Tape Stripping, Following Topical Application of 1  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]Hydroquinone to Human Forearm Skin<sup>20</sup>

Time (h)	Percentage Administered Dose <sup>a</sup>	
	Skin Wash Recovery	Skin Tape Strips
0	82.3 $\pm$ 8.1	1.2 $\pm$ 0.4
1	67.5 $\pm$ 25.3	5.4 $\pm$ 2.5
3	54.8 $\pm$ 18.0	8.6 $\pm$ 4.5
6	53.6 $\pm$ 17.3	15.8 $\pm$ 4.2
24	15.0 $\pm$ 4.5	6.6 $\pm$ 2.1

<sup>a</sup> Values are mean  $\pm$  SD (n = 4).

18-80) following dermal application without occlusion, as the product is normally applied.<sup>20</sup> In group A (n = 6), 25  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]hydroquinone cream (containing 2.5 mg HQ) was applied to a 25  $\text{cm}^2$  area of skin on the forehead. The next day, the area was washed by cotton balls with a 50% soap solution and rinsed with deionized water. The cotton balls were collected and analyzed for radioactivity. Four-day urine samples were collected by the volunteers and submitted to the researchers for analysis. Table 5 provides the results of this study. A total of 45.3%  $\pm$  11.2% of the dose was recovered in the urine. 5.2%  $\pm$  3.2% was recovered from the cotton ball wash. Thin layer chromatography was used to identify the metabolites in urine and the majority of the recovered dose was excreted as the glucuronide conjugate with lesser amounts of the sulfated conjugate and the parent compound identified.

In group B (n = 4), 5 skin sites (1  $\text{cm}^2$  each) on the ventral forearm were treated with 1  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]hydroquinone cream (containing 0.1 mg HQ). The treated surfaces were washed 5 times and tape stripped 10 times with cellophane tape at time intervals of 0, 1, 3, 6, and 24 hours. The tape strips were analyzed for radioactivity. Table 6 provides the results of this study. The researchers concluded that while HQ can be removed from the skin through washing early after exposure, absorption over time will decrease the amount that can be removed.

In group C (n = 4), 25  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]hydroquinone cream (containing 2.5 mg HQ) was applied to a 25  $\text{cm}^2$  area of skin on the left forearm. Catheters were placed in the vein draining the

treated area on the left forearm, the ipsilateral site, and in the same area on the right forearm, the contralateral site. Blood samples (10 mL) were taken from both sites at 0 hour (prior to dosing) and at 0.5, 1, 4, and 8 hours after dosing. The treated skin site was washed by cotton balls with a 50% soap solution and rinsed with deionized water. The cotton balls were not analyzed for radioactivity in this substudy. Urine samples were collected at 24, 48, 72, and 96 hours after dosing, by the volunteers and submitted to the researchers for analysis. The researchers found that initially, the concentration on the ipsilateral side was higher than on the contralateral side; but by 4 hours, the concentration was the same on both sides. The peak plasma concentration of 0.04  $\mu\text{g-Eq/mL}$  occurred at the 4-hour time point. The researchers calculated that 8.0%  $\pm$  4.1% of the dose was excreted in the urine for the 8-hour dosing period and that 45.3% of the dose was absorbed. The researchers assumed 100% excretion in making these determinations.

## Immunological Effects

Hydroquinone inhibited IL-2-dependent T cell proliferation in primary human T lymphoblasts (HTLs) in vitro.<sup>25</sup> Exposure of HTLs to 50  $\mu\text{mol/L}$  HQ decreased interleukin 2 (IL-2)-dependent proliferation by >90%. Cell viability was not affected by this concentration. Hydroquinone reversibly inhibited DNA synthesis in these cells, but this effect could be reversed up to 6 hours after treatment by removing HQ. No effect on glutathione levels was observed with up to 24 hours of treatment. Hydroquinone did not block binding of 125I-IL-2 to the cells but interfered with the IL-2-dependent progression of the HTLs through S phase of the cell cycle.

Macrophage-mediated immune responses were also inhibited by HQ treatment.<sup>26</sup> Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) production was inhibited by a 6-hour treatment with 100  $\mu\text{mol/L}$  HQ in mouse RAW264.7 cells. Hydroquinone treatment decreased lipopolysaccharide (LPS)-mediated nitric oxide (NO) production, but HQ alone up to 100  $\mu\text{mol/L}$  had no effect on NO levels. Human U937 cells (a macrophage cell line) and platelets showed a decrease in cell adhesion molecules in response to HQ treatment. The authors believe these effects occur through inhibition of the PI3K/Akt signaling pathway because inhibition of this pathway produced similar effects to those seen with HQ and a synergistic effect was observed with HQ and inhibitors of this pathway.

## Animal Toxicology

Oral administration of HQ to rats resulted in dose-dependent mortality, lethargy, tremors, and increased liver and kidney weights.<sup>2</sup> Hydroquinone was found to be cytotoxic to rat hepatoma cells in culture and nephrotoxic in male rats dosed orally by gavage.

## Acute Toxicity

*Oral.* Hydroquinone was found to induce indicators of nephrotoxicity in male and female F344 rats but not in male

**Table 7.** Functional Observational Battery (FOB) of Sprague-Dawley Rats Treated With 0, 20, 64, or 200 mg/kg per d Hydroquinone 5 d/week for 13 weeks: Statistically Significant Differences<sup>28</sup>

Observation	Change	N	Dose Group	FOB Observation Period
Differences attributed to hydroquinone exposure				
Home-cage activity	Decreased	6/10	200 mg/kg males	6 h
Behavior while removing from cage	Decreased	10/10	200 mg/kg females	1 h
Tremors	Increased	10/10	200 mg/kg females	1 h
Locomotor activity	Decreased	9/10	200 mg/kg males	6 h
Urine stains	Urine discolored papers	10/10	200 mg/kg males	1 and 6 h, days 1, 30, and 60
	under home cages brown	10/10	64 mg/kg males	Days 1, 30, and 60
	when left to stand overnight	10/10	20 mg/kg males	Days 1 and 60
		10/10	20, 64, and 200 mg/kg females	Days 30 and 60
Differences not attributed to hydroquinone exposure				
Urination	Increased	–	200 mg/kg males	1 h
			20 mg/kg males	1 h
Defecation	Increased	–	20 mg/kg males	1 h
	Decreased		200 mg/kg males	1 h
Spontaneous vocalizations	Increased	–	200 mg/kg females	Day 91
Approach response	Increased	–	64 mg/kg females	Day 30
Auditory orientation	Increased	–	200 mg/kg males	Day 60
	Decreased		20 and 200 mg/kg females	Day 91
			64 mg/kg females	Days 30 and 60
Olfactory orientation	Increased	–	64 mg/kg males	Day 7
Visual orientation	Increased	–	64 mg/kg males	Days 30, 60, and 91
Pinna touch response	Decreased	–	200 mg/kg males	Day 14
Tail pinch response	Increased	–	200 mg/kg	Day 30
Grip strength quantitative	Decreased	–	64 mg/kg females	Pre-exposure

or female Sprague-Dawley (SD) rats or B6C3F1 mice.<sup>27</sup> Rats (4-6/group) were gavage dosed with 0, 200, or 400 mg/kg HQ in water. Mice (number not specified) were gavage dosed with 0 or 350 mg/kg HQ in water. Urine and blood samples were collected from all animals. For rats, samples were collected at 0 (prior to dosing), 8, and 24 hours, at 24-hour intervals up to 96 hours after dosing. For mice, urine was collected 16 hours prior to dosing and at 12, 24, and 48 hours after dosing. The samples were examined for markers of kidney toxicity. Female F344 rats were the most sensitive to the indicators examined with statistically significant increases in the urinary activities of the enzymes, alanine aminopeptidase (ALP), alkaline phosphatase (AAP),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT), and *N*-acetyl glucosaminidase (NAG) at the 400 mg/kg dose level 8 hours after dosing. Creatinine (8 hours) and glucose (24 hours) were also statistically significantly increased at the 400 mg/kg dose. Blood urea nitrogen (BUN) was statistically significantly increased at the 400 mg/kg dose at the 48-hour time point. While many of these same parameters were increased in the 200 mg/kg dose group, only the increase in NAG was statistically significant at this dose level.

Male F344 rats had a similar pattern with statistically significant differences at the 400 mg/kg dose, but the change was of a smaller magnitude than that observed with the female F344 rats. At the 8-hour time point, AAP, NAG, and  $\gamma$ -GT activities were statistically significantly increased. Urinary creatinine and BUN were not changed by HQ treatment in

male F344 rats. No significant differences were observed at the 200 mg/kg dose level.

Blood urea nitrogen was decreased in female SD rats at the 400 mg/kg dose level. No other changes were observed in the other parameters in male or female SD rats.

Blood urea nitrogen was statistically significantly increased in male and female B6C3F1 mice at the only dose tested (350 mg/kg). The urinary enzyme activities were also increased, but the differences were not statistically significant.

Urinary osmolality was decreased in all groups of rats dosed at the 400 mg/kg level, with statistically significant changes observed in the male and female F344 rats and the female SD rats. Statistically significant changes in diuresis were observed only in female F344 rats at both dose levels. No statistically significant changes in red or white blood cell counts in the urine were observed in any of the treated animals.

Histopathologically, F344 rats of both genders exhibited changes such as cell regeneration, slight mineralization, slight focal necrosis, and the presence of granular casts in the kidneys. The authors described any histopathological changes observed in male SD rats as minimal at either dose level. The kidneys from female SD rats and from mice were not examined.

A range-finding study was conducted in 7 female SD rats (age 10-13 weeks, 1 animal/group) using a single gavage dose of 500, 400, 375, 350, 300, 200, or 100 mg HQ/kg body weight (bw).<sup>28</sup> Table 7 provides the results of this study. Mortality occurred in the 500 and 400 mg/kg bw dose groups. Based on these results, an acute oral study was designed using 7 male

SD rats (age 9 weeks) and 5 female SD rats (age 14-15 weeks). The animals received a single gavage dose at concentrations of 3.75%, 3.45%, 3.15%, and 2.85% in degassed water, which correspond to dose groups of 375, 345, 315, and 285 mg/kg, respectively. Animals were observed 3 times on the day of dosing and once per day on days 1 to 14. Body weight was measured on days 0 (prior to dosing), 7, and 14.

Mortality occurred within 1 hour of dosing in both male (1 of 5) and female (3 of 5) rats at 375 mg/kg and in female rats at 345 (1 of 5) and 285 (1 of 5) mg/kg. The researchers observed mild-to-moderate tremors in all animals after dosing and noted minor convulsions in 1 of 5 males and 3 of 5 females at 375 mg/kg, 1 of 5 males and 1 of 5 females at 345 mg/kg and for 2 of 5 females at 285 mg/kg. With the exception of the male at 345 mg/kg, the animals that convulsed died prior to the 1-hour observation. No signs of tremors or convulsion were observed at the 4-hour observation. Brown discolored urine stains were present in the cages of all surviving animals between day 0 and day 2. From day 3 to the end of the study, all surviving animals appeared to be clinically normal. At necropsy, the researchers noted minor-to-moderate thymus hemorrhage for some of the animals that died on the day of dosing (details not provided) and minor hydronephrosis of the right kidney for a single 345 mg/kg male rat that survived to the end of the study. The hydronephrosis was not considered treatment related, however, as the animal was not in the highest dose group.

**Dermal.** New Zealand White SPF rabbits (5/gender) were shaved to expose the dorsal skin and a 2000 mg/kg bw limit dose of HQ was applied to the skin, covered and wrapped to secure the treatment in place for 24 hours.<sup>28</sup> The residual HQ was removed with running water after 24 hours. Animals were observed once daily from days 1 to 14 and body weights were measured on days 0 (prior to treatment) 7, and 14.

The researchers observed brown discolored urine stains in the cages of all animals on day 2. There were no adverse dermatological effects at the site of application, no adverse neurobehavioral effects, no changes in weight gain, and no mortalities during the study. No treatment-related findings were observed at necropsy. The dermal LD<sub>50</sub> was reported as >2000 mg/kg.

### Subchronic Toxicity

**Dermal.** Dermal exposure to a HQ cream did not produce renal toxicity in a subchronic study.<sup>29</sup> F344 rats (20/gender/group) were shaved and treated dermally with 0%, 2%, 3.5%, and 5% HQ in an oil-in-water emulsion cream (1.6 mL/kg bw on a 4 × 6 cm area on the back, with occlusion) 5 days/week for 13 weeks. Body weights and feed and water consumption were monitored and the animals were observed for clinical signs of toxicity and dermal irritation. Urine was collected from all animals to look for markers of cell damage, including NAG,  $\gamma$ -GT, ALP activities, and creatinine concentrations. The authors implanted 5 animals/gender/group with osmotic pumps loaded with 20 mg/mL BrdU (delivery rate 10  $\mu$ L/h).

Animals were then treated dermally with HQ cream as described above and killed 3 days later during the 3rd, 6th, and 13th weeks of treatment to look at renal cell proliferation. No clinical signs of toxicity were observed in any of the treated animals. Erythema was observed in male and female treated animals with increasing concentrations producing increased severity. This finding abated when exposure stopped. In addition, brown discoloration of the skin and scaly skin were observed with higher incidences occurring in the higher treatment groups although no clear dose-related trend was evident. A minimal to minor epidermal hyperplasia was observed at the site of application. No evidence of exogenous ochronosis was observed in this study.

No significant differences in absolute or relative organ weights were observed. Serum levels of protein and alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities were statistically significantly increased as compared to controls in the 5% HQ-treated male rats, but the authors did not feel this represented an adverse effect of HQ treatment because the magnitude seen was not clinically relevant. At 13 weeks, there were no differences in the urine parameters examined in male rats and only the osmolality value was statistically significantly decreased at the highest concentration tested. No histopathological changes and no changes in cell proliferation were observed in males after 13 weeks of treatment. In females, the BrdU labeling index in the 3.5% and 5% treated groups was slightly increased for cells in the outer/inner stripe of the distal tubules. No histopathological changes were observed.

### Dermal Irritation and Sensitization

In rats, dermal application produced slight-to-severe irritation.<sup>30</sup> In a guinea pig maximization test, induction with 2% HQ injected intradermally, followed by challenge with 0.5% HQ, showed extreme sensitization. Hydroquinone was classified as an extreme sensitizer in a guinea pig maximization test. Briefly, albino Dunkin-Hartley guinea pigs were given a series of 6 intradermal injections of 2.0% HQ in 0.9% saline in the shoulder region to induce sensitization. After 6 to 8 days, a 48-hour occlusive patch of 1.0% HQ in an acetone-polyethylene glycol 400 vehicle was placed over the injection site. The animals were challenged on a previously untreated area of the flank using a 24-hour occlusive patch of 0.5% HQ, which was the maximum nonirritating concentration. After 14 and/or 48 hours, 100% of the animals had a positive response.

### Reproductive/Developmental Toxicity

The literature on Reproductive and Developmental Toxicity of HQ was reviewed in a previous safety assessment and was summarized as follows:

Oral administration of Hydroquinone did not produce embryotoxic, fetotoxic, or teratogenic effects in rats, nor did it produce significant adverse reproductive effects in a

two-generation study. Using rabbits, various teratogenic/reproductive treatment-related effects were observed at doses of 200-500 mg/kg. All dams dosed with 300-500 mg/kg Hydroquinone died. Some maternal toxicity was observed at a number of dose concentrations.<sup>2</sup>

Growth retardation of offspring was reported at concentrations that also caused maternal toxicity.<sup>1</sup> The overall literature in this area has not shown reproductive or developmental effects even following high-dose HQ exposures.<sup>23</sup>

## Genotoxicity

Hydroquinone has been found to be mostly negative for mutagenicity in bacterial assays but positive in many mammalian cell assays *in vitro* and *in vivo* including micronuclei formation, sister chromatid exchange (SCE), and chromosomal aberrations.<sup>2</sup>

### *In Vitro*

Blood samples were collected from healthy male volunteers (aged 20-40 years) and used to produce whole blood cultures and isolated lymphocytes for a series of experiments examining the genotoxicity of HQ.<sup>31</sup> Cultures were treated with HQ (0.5-50 µg/mL) for 90 minutes at 37°C. Methylmethanesulfonate (MMS) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were used as positive controls. Samples were assayed for DNA damage using the Comet assay. Hydroquinone treatment resulted in dose-dependent increases in tail moment values in isolated leukocytes but had no effect on whole blood samples. Methylmethanesulfonate produced an increase in DNA damage in both types of samples, while H<sub>2</sub>O<sub>2</sub> produced damage in only the isolated leukocytes. To investigate whether peroxides may play a role in HQ-induced DNA damage, isolated lymphocytes were treated with HQ in the presence or absence of catalase (250 U/mL). The effects of HQ were almost completely suppressed in the presence of catalase, suggesting that the damage could be due in part to an HQ-generated peroxide or other catalase substrate. Cellular metabolism was not required to generate the causative agent, as treatment of lysed cells also produced the previously observed DNA damage. Finally, the isolated lymphocytes were treated with HQ at 5, 10, and 50 µg/mL and the sample split into 2. One half of the sample was immediately tested in the Comet assay, while the other half was cultured for 66 hours and stimulated by phytohemagglutinin (PHA) to look for micronuclei. The DNA damage previously observed was detected in the freshly analyzed sample, but no significant increase in micronuclei was observed in the cultured cells. These experiments suggest that there are proteins and/or enzymes in whole blood samples that can block the actions of HQ, and it is likely that a self-generated peroxide product is the DNA damaging agent in this system.

The ability of HQ to induce chromosomal aberrations in V79 cells was examined in the presence and absence of S9 mix and antioxidant enzymes.<sup>32</sup> Hydroquinone was found to

generate hydroxyl radicals in a time- and pH-dependent manner in the presence of Fe<sup>3+</sup>/EDTA with more alkaline pH producing higher levels of thiobarbituric acid (TBA) reactive products. The addition of catalase to this system decreased the concentration of TBA-reactive products, suggesting that H<sub>2</sub>O<sub>2</sub> is the specific agent generated. The presence of chromosomal aberrations was increased in the presence of 80 µmol/L HQ at pH 7.4 and 8.0, but the increase was not significant as compared to each other, despite the earlier finding that more hydroxyl radicals are produced at pH 8.0. This is believed to be due to a concomitant increase in cytotoxicity as measured by the mitotic index. The addition of S9 mix, superoxide dismutase (SOD), and SOD + catalase all significantly decreased the percentage of cells with chromosomal damage, but catalase alone did not have an effect. The authors suggest that while H<sub>2</sub>O<sub>2</sub> generation appears to play a role in the clastogenic activity of HQ, the addition of S9, SOD, or SOD + catalase did not completely abolish the chromosomal aberrations; therefore, other reactive metabolites, such as the semi-quinone radical or the quinone, may play a role.

A total of 27 nonsmoking healthy young caucasian volunteers were enrolled in a study to determine the effect of polymorphisms for the glutathione *S*-transferases (GSTs) on HQ-induced genotoxicity to lymphocytes.<sup>33</sup> Blood samples were collected and genotyped and lymphocytes isolated from the samples were tested for micronuclei induction and SCE in the presence of HQ. For the micronuclei induction experiments, lymphocytes were PHA stimulated and treated with HQ (0, 40, and 80 µmol/L) for 3 hours. Mitomycin C ([MMC] 1.5 µmol/L) was used as a positive control and performed as expected. The number of micronucleated cells was significantly increased at 40 and 80 µmol/L, which were also significantly different from each other. A strong correlation was also observed between the GST phenotype and the frequency of HQ-induced micronuclei, with GSTM1 null lymphocytes exhibiting a significantly higher level with both 40 and 80 µmol/L HQ.

Lymphocyte cultures from these same participants were also PHA stimulated and treated with 0 or 80 µmol/L HQ for 3 hours to test for SCE. The 80 µmol/L HQ treatment induced a significant increase in SCE as compared to controls. No relationship was observed between HQ-induced SCE and the GST polymorphisms examined.

Hydroquinone was tested for induction of DNA damage in HepG2 cells and found to be positive.<sup>34</sup> Cells were exposed to 0, 6.25, 12.5, 25, and 50 µmol/L HQ for 1 hour at 37°C. DNA strand breaks were significantly increased in cells treated with 6.25 up to 25 µmol/L HQ. Cells treated with 50 µmol/L HQ showed a significant increase in DNA strand breaks as compared to controls but were not increased above the 25 µmol/L HQ-treated group. Cell viability was not affected by HQ treatment and no apoptosis was observed (data not shown). The presence of DNA protein cross-links was investigated using a proteinase K posttreatment. Posttreatment with proteinase K did not affect DNA migration in cells exposed to 6.25 to 25 µmol/L HQ; however, cells exposed to 50 µmol/L HQ did

show an increase in DNA migration, suggesting that DNA protein cross-links were present in these cells.

The induction of micronuclei was also examined in HepG2 cells.<sup>34</sup> Cells were treated with 0, 6.25, 12.5, 25, and 50  $\mu\text{mol/L}$  HQ for 24 hours and evaluated for the frequency of micronuclei. Cyclophosphamide (800  $\mu\text{mol/L}$ ) was used as a positive control and performed as expected. A dose-dependent increase in micronuclei formation was observed in cells treated with 12.5 to 50  $\mu\text{mol/L}$  HQ. The researchers did further work to look at indicators of oxidative stress. They found that 8-hydroxydeoxyguanosine (8-OHdG) was dose dependently increased in cells treated with 12.5 to 50  $\mu\text{mol/L}$  HQ, reactive oxygen species (ROS) were statistically significantly increased in the 2 highest concentrations, and all treated cells showed statistically significant decreases in glutathione (GSH) content.

### *In Vivo*

Hydroquinone increased the incidence of micronuclei in the bone marrow cells of male Swiss albino mice.<sup>35</sup> Animals were given 1 intraperitoneal (ip) injection of HQ and killed 12, 24, and 36 hours after treatment. The mice (5/dose per time period) were dosed at 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 75, and 100 mg/kg body weight. The frequency of micronucleated polychromatic erythrocytes (MPCE) was highest at 24 hours after treatment and was statistically significant as compared to controls at 6.25 mg/kg body weight up to 100 mg/kg body weight for all 3 time points. The frequency of micronucleated normochromatic erythrocytes (MNCE) followed a similar pattern and was highest at 24 hours after treatment and was statistically significantly increased at 6.25 mg/kg body weight up to 100 mg/kg body weight for all 3 time points. The frequency of MNCE reached a plateau at the 75 mg/kg dose. The PCE/NCE ratio decreased in a dose-dependent manner and suggests that HQ treatment resulted in a dose-dependent inhibition of erythropoiesis.

Male Swiss albino mice (4/dose) were given a single dose of HQ (0, 0.5, 1, 2, 4, and 8 mg/kg body weight; ip) per day for 6 days and killed 24 hours after the last treatment.<sup>36</sup> Splenocytes were isolated from these animals, cultured for 72 hours and examined for micronuclei formation. No signs of HQ-induced toxicity were observed over the treatment period. A statistically significant, dose-dependent increase in the number of micronucleated binucleate splenocytes (MNBNS) was observed. The 3 highest doses of HQ also produced increases in the number of BNS.

### **Carcinogenicity**

Hydroquinone administered to rats orally by gavage 5 times/week for up to 103 weeks at doses of 25 or 50 mg/kg resulted in a significant increase of renal adenomas in males at the 50 mg/kg dose and of mononuclear cell leukemia in females with both doses.<sup>37</sup> At doses of 50 or 100 mg/kg on the same schedule, there was a significant increase in hepatocellular adenomas

in both male and female mice.<sup>37</sup> Other previously reviewed studies of HQ showed no significant difference in tumors between control and exposed groups, and marginal to no activity as a tumor promoter.<sup>1</sup>

### **Chronic**

Hydroquinone is metabolized to several glutathione conjugates including 2,3,5-tris(glutathione-S-yl)HQ (TGHQ), and this compound was tested for its ability to induce renal cancer in Eker rats.<sup>38</sup> Eker rats are susceptible to renal tumor development because they carry a germline mutation in the tuberous sclerosis 2 (*Tsc-2*) tumor suppressor gene. Eker rats have 1 mutated copy and 1 wild-type copy and loss of function of the wild-type copy is the rate limiting step for the development of preneoplastic and ultimately neoplastic lesions.

Two groups of male Eker rats (40/group) were given either 0 or 2.5  $\mu\text{mol/kg}$  TGHQ 5 d/week ip in 0.5 mL of saline for 4 months and then 0 or 3.5  $\mu\text{mol/kg}$  TGHQ for an additional 6 months. (These doses represent  $\sim$ 600-800 nmol/rat and are 0.6%-0.8% of the dose of HQ used in the NTP (1989) study.<sup>37</sup> After 4 months, 10 animals/group were killed and examined for cell proliferation in the kidneys.

2,3,5-tris(glutathione-S-yl)HQ treatment for 4 months significantly increased the labeling index of cells in the kidney, as measured by BrdU incorporation, compared with saline controls ( $133.4 \pm 30.9$  vs  $9.0 \pm 1.9$  cells/250  $\mu\text{m}^2$ ,  $P = .016$ ). Preneoplastic lesions were also observed at this time point, characterized by a peritubular fibrosis surrounding a dilated tubule. Kidney tumors were not observed at this time point.

Following 10 months of treatment, statistically significant increases in basophilic dysplasias, adenomas, and renal cell carcinomas were observed. Most of the renal cell tumors were in the region of TGHQ-induced acute renal injury and using HPLC-based DNA fragment analysis and laser capture microdissection, the researchers found that there was a loss of the wild type *Tsc-2* gene within the preneoplastic lesions.

### **Renal Tubule Cell Tumor Mode of Action**

Hydroquinone treatment has been associated with an increased incidence of renal tubule cell tumors in male F344 rats but not in female F344 rats or B6C3F1 mice. A mode of action (MOA) has been proposed for the HQ-induced renal tubule cell tumors in male F344 rats. This MOA involves HQ or a metabolite interacting with the rat kidney to exacerbate chronic progressive nephropathy (CPN). The combination of CPN, along with HQ-induced cell proliferation in the kidney, promotes neoplasm formation.<sup>39</sup>

Hydroquinone is metabolized in rats to the glucuronide, sulfate, and glutathione conjugates, with increasing levels of the glucuronide and the mercapturic acid measured in the urine following either increasing treatment time or dose.<sup>22,40</sup> Intravenous injection of the glutathione metabolite 2,3,5-(triGSyl)HQ shows that the kidney is a target of this compound, and it produces an increase in cytotoxicity and oxidative DNA damage

as measured by markers of kidney damage in the blood and 8-OHdG in the kidney, respectively.<sup>22</sup> Subchronic treatment (6 weeks) of male F344 rats with 25 and 50 mg/kg HQ (via gavage) produced increased cell proliferation in the kidneys, and dose-dependent and statistically significant increases in the incidence of degenerative and regenerative foci in the tubules.<sup>41</sup> This same treatment regimen using 2.5 mg/kg HQ did not produce these effects in male F344 rats and none of the tested doses (2.5, 25 or 50 mg/kg) produced these effects in female F344 rats or in male SD rats. Glutathione metabolites can be reabsorbed in the proximal tubules of the kidney and metabolized by  $\gamma$ -glutamyl transferase (also  $\gamma$ -GT) where the released compound could generate ROS or covalently bond to cellular targets (ie, proteins or DNA). DNA adducts were not observed in F344 rat kidney following HQ treatment using <sup>32</sup>P-postlabeling, suggesting that this is not the target macromolecule.<sup>42</sup> Protein adducts have been identified following gavage and ip treatment with HQ.<sup>43,44</sup>

The localization of a reactive molecule to the kidney along with increased cell proliferation in the kidney seems to also coincide with the occurrence of CPN in these animals.<sup>39,45</sup> Chronic progressive nephropathy is a spontaneous lesion of the kidneys that occurs with age in certain strains of rats, including F344 and SD rats. Male rats are usually more affected than females and the incidence and severity of CPN has been shown to be affected by castration, hormonal status, and caloric intake, with protein overnutrition thought to be a causal factor.<sup>46,47</sup> These lesions are characterized by an increase in basophilic tubules with a thickened basement membrane in the cortex that begin as small discrete areas that expand as the disease progresses. These foci gradually merge into areas of tubule alteration and eventually become areas of frank glomerular pathology with infiltration of inflammatory cells.<sup>48</sup> The impact of CPN on HQ-induced carcinogenicity is unclear, but there does appear to be an association between the severity of CPN and the increased tumor response.<sup>47</sup> The increased proliferation associated with nephrotoxic glutathione conjugates of HQ could leave cells more susceptible to oxidative DNA damage, with subsequent initiation and promotion of neoplasm formation.

The relevance of this process to humans has been questioned on the basis of a proposed lack of a correlative process in humans to the CPN observed in rats. While there does not appear to be a specific human disease that shares all of the features of rodent CPN, humans do experience a glomerular sclerosis of 10% to 30% of nephrons as they age.<sup>46,49,50</sup> In addition, the authors of the NTP analysis cautioned that the presence and severity of CPN in rats did not fully explain the renal tubule cell tumors observed even though a positive correlation between CPN and RTCNs was observed. Their analysis also found that there were many animals with severe CPN that did not develop renal tubule cell neoplasms.<sup>47</sup> Thus, qualitatively, the MOA proposed in animals may have some relevance to humans. Quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

## Role of HQ in Benzene Toxicity

Because HQ is a metabolite of benzene, its possible synergistic effect in benzene toxicity has been studied. Possible mechanisms of benzene toxicity have included the consideration of the role of benzene's metabolites in the resulting myelotoxicity. Benzene is a known human carcinogen, inducing leukemia and aplastic anemia. Human bone marrow is a known target, and numerous studies have been conducted to try to determine the mechanism for this effect and the responsible agents. In addition to HQ, benzene metabolites catechol and phenol have also been studied for their effects, both alone and in combination. In the models examined, a synergistic effect does appear to occur depending on the end point under study.<sup>51-55</sup> In examining HQ alone however, there does not appear to be a great deal of similarity in the toxicities observed with the individual compounds.<sup>17,56</sup> Whereas benzene produces leukemia and aplastic anemia in humans, no carcinogenic response has been identified in HQ exposed occupational populations.<sup>17,23</sup> The cancer profiles of the 2 compounds are also different in animals. In NTP studies conducted by the same route of exposure and in the same species of animals, benzene produced neoplasms at multiple sites in all 4 gender-species studies, while HQ induced neoplasia in 1 site in each with the exception of male mice; and all were at different sites.<sup>56</sup> Therefore, it is not clear what role, if any, HQ plays in benzene carcinogenesis. The experimental data suggest that HQ produced at the site of action and in combination with other benzene metabolites probably contributes to the overall toxic profile of benzene, but there are definite differences between species in sensitivity and in the capacity for activating and detoxifying processes that should be considered.

## Clinical Assessment of Safety

### Exogenous Ochronosis

Use of creams containing at least 1% to 2% HQ has been associated with exogenous ochronosis in people of Asian, Latin American, and African descent.<sup>37,57-59</sup> Ochronosis is the bluish black discoloration of tissues and has been observed in people exposed to several substances in addition to HQ including phenol, trinitrophenol, resorcinol, mercury, picric acid, benzene, and antimalarials. This form of ochronosis has been named exogenous because it does not share any of the systemic complications or the urinary abnormalities observed with the autosomal recessive metabolic disorder alkaptonuric ochronosis even though histologically, the hyperpigmentation in the skin is the same. This condition is associated with prolonged use (>6 months) of HQ containing skin-lightening products and while the mechanism is not understood may involve HQ's effects on tyrosinase, or on homogentistic acid oxidase and resulting deposition of pigment.

### Dermal Sensitization

In 80 patients known to be sensitive to aromatic compounds, 0.5% HQ elicited no reactions.<sup>1</sup> Hydroquinone (1%) did not

produce a positive reaction in a cross-reactivity study.<sup>60</sup> Twenty-two volunteers who were classified as sensitized to p-phenylenediamine ([PPD] 9 male; 13 female, 19-72 years old) were patch tested to a serial dilution of benzoquinone (0.1%, 0.2%, 0.5%, and 1.0%) and with other compounds that could be metabolized to benzoquinone including HQ.<sup>30</sup> The control population (n = 8 male and 12 female, 18-77 years old) was tested with the same compounds and concentrations and were selected for no known previous allergy to PPD or other para-benzene derivatives. Among the PPD-sensitized participants, only 1 had reactions to all of the benzoquinone dilutions, while 4 reacted to either 0.2% or 1.0% benzoquinone. Of these participants, 3 presented with erythema, swelling, and papules. In the fourth participant, vesicles were also present

## Hair Dye Epidemiology

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. Hydroquinone functions as a coupler in the hair dye reaction and is a "consumable" in the hair dyeing procedure. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information and have been considered by the CIR Expert Panel.

In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that "personal use of hair colorants cannot be evaluated as to its carcinogenicity" and that "occupation as a hairdresser or barber entails exposures that are probably carcinogenic."<sup>61</sup> The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

Rollison et al (2006) reviewed the available epidemiology literature published from 1992 through February 2005, which includes over 80 citations on personal hair dye use published since the IARC review.<sup>62</sup> The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration, and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin lymphoma. These findings, however, were not consistently observed across studies.

Several studies addressing the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other end points published since the above review also have been considered.

In February 2008, an IARC Working Group re-evaluated the epidemiology literature including studies considered in the 1993 evaluation as well as all studies subsequently published. For personal use of hair colorants, the Working Group considered the epidemiological evidence inadequate and concluded that personal use of hair colorants is "not classifiable as to its carcinogenicity to humans." The Working Group considered the studies of occupational exposures in hairdressers and barbers as providing limited evidence of carcinogenicity and reaffirmed the previous conclusion made in 1993 regarding occupation as a hairdresser or barber.<sup>63</sup>

A summary of the available hair dye epidemiology data is available at <http://www.cir-safety.org/findings.shtml>.

## Summary

Hydroquinone is reportedly used in hair dye preparations, skin care products, nail products, and as recently as 2007 in lipstick. Information provided to the FDA through the VCRP indicates that the use of HQ has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009. Hydroquinone is a component of artificial nail products because it is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon polymerization of the acrylic monomers, HQ is oxidized and is no longer detectable in the final polymer using analytical techniques for identifying trace amounts in a solid matrix. Any residual HQ is trapped in the polymer and is therefore unavailable and not likely to be absorbed.

While an earlier *in vitro* study suggested that HQ would be considered a "slow permeant," a more recent *in vivo* study demonstrated that HQ is in fact rapidly absorbed through the skin from an aqueous preparation. Hydroquinone is metabolized to the sulfate and glucuronide conjugates, with oxidation to 1,4-benzoquinone, resulting in a reactive metabolite that forms mono- or polyglutathione conjugates. The glutathione conjugates are believed to be responsible for the nephrotoxicity observed in rats. In addition to nephrotoxicity, HQ has some immunotoxic effects and has been positive in many mammalian cell assays *in vitro* and *in vivo* including micronuclei formation, SCE, and chromosomal aberrations despite being mostly negative in *in vitro* bacterial mutagenicity assays. The induction of renal cell tubule tumors in male F344 rats has raised concern regarding the nephrocarcinogenicity of HQ and has led to several mechanistic studies which suggest that the male F344 rat is more susceptible to the glutathione conjugates of HQ due to the spontaneous occurrence of CPN which nearly all rats develop as they age. There is no human disease that shares all of the features of rodent CPN, however, there are histopathological similarities between human chronic renal disease and CPN that do not allow the proposed MOA to be ruled out entirely on a qualitative basis. Quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

Hydroquinone has been reported to cause exogenous ochronosis in several ethnic populations following prolonged use (>6 months) of at least a 1% to 2% cream. These effects along

with the NTP cancer study findings have led the FDA to reconsider the GRASE label for HQ in leave-on drug products.

The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the available hair dye epidemiology data is available at <http://www.cir-safety.org/findings.shtml>.

## Discussion

The 1994 conclusion of the CIR Expert Panel included the term "aqueous" in discussing the safety of HQ as a cosmetic ingredient. This term was added following discussions of the absorption of HQ from what was termed an alcoholic vehicle (ie, ~70% ethanol), which resulted in estimated HQ absorption of up to 66%. The only other data at the time was an in vitro study that used the stratum corneum of human abdominal skin to examine HQ absorption. The preparation used in that study was described as a 5% aqueous solution of HQ, and the authors calculated a permeability constant of  $9.3 \times 10^{-6}$  which suggested that HQ was a slow permeant with respect to human skin. Since that study, commercial preparations of HQ (commercial cream containing 2.5 mg of HQ) have been shown to be readily absorbed when applied to forehead skin; that is, assuming 100% excretion, 45% of the dose was absorbed. The in vivo studies taken together demonstrate that absorption of HQ will occur if the skin is exposed, and that this absorption occurs from both aqueous and alcohol-based products.

Concerning the use of HQ in hair dye formulations, the question remains regarding the percentage of alcohol in these products. Information provided to the CIR Expert Panel in 1993 suggests that hair dye formulations at the time did contain alcohols but the percentage in the formulation was not provided. Hydroquinone is considered a consumable in the hair dye reaction process and its concentration decreases considerably over time. Therefore, the amount of HQ that may be absorbed during the hair dyeing process is limited by both the decreasing concentration available and by the length of time the hair dye is applied before being rinsed off.

The use of HQ in artificial nail products is considered a safe use because HQ is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon their use, the HQ is oxidized and is no longer present in the preparation and minimal dermal exposure and absorption is expected to occur from this application. Absorption of HQ from other leave-on cosmetic products could be appreciable, and the CIR Expert Panel reiterates that HQ should not be used in these leave-on cosmetics.

The CIR Expert Panel examined the association between oral HQ treatment and the development of renal tubule cell tumors in rats and determined that, while the qualitative relevance of the MOA in humans could not be ruled out, quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points, based on lack of strength of the associations and inconsistency of findings.

## Amended Conclusion

The CIR Expert Panel concluded that HQ is safe at concentrations of  $\leq 1\%$  for cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone is safe for use in nail adhesives in the practices of use and concentration described in this safety assessment. Hydroquinone should not be used in other leave-on cosmetic products.

## Authors' Note

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