UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 16, 2011

MEMORANDUM

SUBJECT: Ethics Review of Intentional Exposure Human Toxicity Study

TO: Jennifer McClain
Antimicrobials Division
Office of Pesticide Programs

FROM: Laura Parsons
Human Research Ethics Reviewer
Office of Pesticide Programs

REF: Gulson, B., M. McCall, M. Korsch, L. Gomez, P. Casey, Y. Oytam, A. Taylor, M. McCulloch, J. Trotter, L. Kinsley, and G. Greenoak. (2010). Small Amounts of Zinc from Zinc Oxide Particles in Sunscreens Applied Outdoors Are Absorbed through Human Skin. Toxicological Sciences. 118(1). 140-149. 10 p. (MRID 48387301)

I have reviewed the referenced document and have determined that all applicable requirements of EPA's Rule for the Protection of Human Subjects of Research (40 CFR Part 26) have been satisfied. If the study is determined to be scientifically valid and relevant, I find no regulatory barrier to EPA's relying on this research in its actions taken under FIFRA or §408 of FFDCA.

Summary Characteristics of the Research

The summary description of this research was obtained from the published literature and through email correspondence with a primary author and the Macquarie University Ethics Secretariat, the group which provided ethical oversight for this research. This research was conducted with a personal care product (sunscreen) and did not include intentional exposure to a pesticide. EPA is interested in this study because it concerns dermal absorption of nanoparticles

and therefore, if valid, this research could be used to support dermal exposure assessments for pesticide formulations which include nanoparticle technology.

In this research, 20 human subjects were exposed to sunscreens containing stable isotopes of zinc oxides for the purpose of measuring the extent of dermal absorption of different sizes of zinc particles. Metal oxide nanoparticles are commonly used in sunscreens because they reflect and absorb ultraviolet (UV) light resulting in transparent rather than opaque coatings such as those found in other metal oxide sunscreens. This research was conducted in Australia in March 2009.

There were 10 male and 10 female subjects ranging in age from 19 to 66 years of age. There were several family connections among the subjects, with three pairs of siblings and one father and son pair. Subjects wore UV protective upper body clothing with a patch removed to expose some skin on the back. Sunscreen containing stable isotope ^{68}Zn oxide either formulated with "bulk" particles (110 $\pm$ 46 nanometers in size) or with nanoparticles (19 $\pm$ 8 nanometers in size) was applied twice daily for a period of 5 days to the exposed skin on the subjects' backs. Thirty minutes after each of the two daily applications, the subjects were exposed to direct sunlight for at least 30 minutes. Participants were encouraged to wear other (nonzinc) types of sunscreen in the areas not covered by the UV-resistant clothing.

Urine and blood samples were taken before first use of the sunscreen as a control for each individual. Urine and blood samples were taken 8 days before sunscreen application, immediately before the first application, at the end of each of the 5 days of application and 6 days after the last application. Urine and blood samples were analyzed for ^{68}Zn oxide concentrations. The test material was removed from each subject's back at the end of each day with an alcohol-lanolin wipe.

The protocol was developed from a pilot study which used three subjects. While no ethics information was available regarding the pilot study, the pilot study was assumed to be conducted under the same ethics oversight as the main study, which is discussed below.

1. **Value of the Research to Society: "**This paper describes the first application of stable isotopes in nanotechnology specifically for tracing absorption or penetration of Zn from ZnO nanoparticles in sunscreen applied to healthy human skin under conditions of normal use." (p. 141). The study was conducted by researchers at Macquarie University in Sydney, Australia. The research was funded by the Commonwealth Scientific and Industrial Research Organization (CSIRO) and Macquarie University. This research addresses the question of whether nanoparticles absorb across intact skin more than larger particles since increased dermal absorption may have implications for many uses of nanotechnology including use in personal care products as well as in pesticidal uses.

2. **Subject Selection**: Twenty-two adult males and females were recruited through "personal contacts" of the researchers. (see email correspondence as Attachment 1). Two subjects withdrew before the monitoring began, leaving 20 subjects for the study. Seventeen subjects completed all the monitoring while three subjects completed part, but

not all, of the study. The ages ranged from 19 to 66 years of age. The researcher reported that none of the female subjects were pregnant. (see email correspondence as Attachment 1). There is nothing to suggest that the subject selection was in any way coercive or inequitable.

2. **Risks and Benefits**: The main risks were in blood sampling and any adverse reaction to the sunscreen formulations. The sunscreen formulations contained an enriched level of a stable isotope of zinc which does not pose a higher risk than naturally occurring zinc and "many" commercially available sunscreens contain "nanoparticulate TiO_2 and/or ZnO". (p 140). Risks were minimized by using a trained phlebotomist to collect blood samples, and questioning potential subjects regarding "adverse reactions to cosmetics". Rules for managing risks were not specifically discussed in the study, but they are discussed in the *National Statement on Ethical Conduct in Human Research* (Supplement 1, p 17) and the exposure was suspended early for one female subject who had an adverse reaction to the sunscreen.

   There were no benefits to subjects. The risk-benefit balance was not discussed in the study report. But given that the risks were small, the potential benefits to society outweigh the risks.

3. **Independent Ethics Review**: This research was reviewed and approved by the Macquarie University Human Research Ethics Committee which is an Australian National Health and Medical Research Council (NH&MRC) registered ethics committee. This research was reviewed under the *National Statement on Ethical Conduct in Human Research.*

4. **Informed Consent**: The subjects attended a meeting at the researcher's house one week before the study during which the study procedures and risks were described. The researchers stressed the need for their commitment for the five days of exposure and the several days of follow up. Brian Gulson, Ph.D., the principal author of this publication, confirmed that each of the subjects provided written consent (see email correspondence as Attachment 1); however, no further information on the consent process was available.

6. **Respect for Potential and Enrolled Subjects**: No mention was made of whether or not subjects were free to withdraw without penalty, but two subjects did withdraw early in the study. The subjects were provided with a unique identifier and only the researchers directly involved had access to these and the data. The publication did not include any information about compensation for subjects.

Applicable Standards

This research was conducted in Australia in March 2009, after EPA's amended Rule for the Protection of Human Subjects of Research became effective on April 7, 2006.

Standards Applicable to the Conduct of the Research

FIFRA §12(a)(2)(P) does not apply to this research because it did not involve the use of a pesticide. The portions of EPA's regulations regarding the conduct of research with human subjects, 40 CFR part 26 subpart A - L, do not apply since the research neither was conducted or supported by EPA nor was it conducted by a person with the intention to submit the results to EPA.

The international standards for this type of research are provided in the 2004 Helsinki Declaration.

The Australian Government Standards Governing Research with Human Subjects, which were applicable when this research was initiated, are provided in the *National Statement on Ethical Conduct in Human Research*. An excerpt of this statement is provided as Supplement 1. The full text of the statement can be found at:
http://www.nhmrc.gov.au/publications/synopses/e72syn.htm

Standards Applicable to the Documentation of the Research

EPA identified this study through a review of the public literature. No person has independently submitted the published article or any results of this research to EPA. Consequently, the requirements for the submission of information concerning the ethical conduct of completed human research contained in EPA regulations at 40 CFR part 26, subpart M do not apply.

Standards Applicable to EPA's Reliance on the Research

The Agency's rule (40 CFR part 26 subpart Q) defines standards for EPA to apply in deciding whether to rely on research—like this study—involving intentional exposure of human subjects. The applicable acceptance standards from 40 CFR part 26 subpart Q are these:

> **§26.1703. Prohibition of reliance on research involving intentional exposure of human subjects who are pregnant women (and therefore their fetuses), nursing women, or children.** Except as provided in §26.1706, in actions within the scope of §26.1701 EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.
>
> **§26.1705 Prohibition of reliance on unethical human research with non-pregnant, non-nursing adults conducted after April 7, 2006.** Except as provided in **§**26.1706, in actions within the scope of **§**26.1701, EPA shall not rely on data from any research initiated after April 7, 2006, unless EPA has adequate information to determine that the research was conducted in substantial compliance with subparts A through L of this part, or if conducted in a foreign country, under procedures at least as protective as those in

subparts A through L of this part. This prohibition is in addition to the prohibition in §26.1703.

Compliance with Applicable Standards

This research did not involve intentional exposure of any pregnant or nursing female subjects or any children. Reliance on the research is therefore not prohibited by 40 CFR §26.1703.

EPA is forbidden by 40 CFR §26.1705 to rely on data from research involving intentional exposure—such as this study— "unless EPA has adequate information to determine that the research was conducted in substantial compliance with subparts A through L of this part, or if conducted in a foreign country, under procedures at least as protective as those in subparts A through L of this part." This research was approved by the Macquarie University Human Research Ethics Committee, formerly the Macquarie University Ethics Review Committee (Human Research), which is a National Health and Medical Research Council (NH&MRC) registered ethics committee. Any research that is reviewed and approved by this committee must meet the requirements of the *National Statement on Ethical Conduct in Human Research* (Supplement 1). These requirements are similar to the requirements of 40 CFR §26 subparts A through L in terms of risk minimization, informed consent, respect for subjects and the requirements of independent ethics review. Further, the authors of the publication certified that "all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration." (p 140). Copies of the approval letters, email correspondence, and statements in the publication provide enough information to conclude that the research substantially complied with requirements of a foreign procedures that were as protective as those in 40 CFR §26 subparts A through L. Therefore, reliance on this research is not prohibited by 40 CFR §26.1705.

Conclusion

I find no barrier in law or regulation to reliance on MRID 48387301 in EPA actions taken under FIFRA or §408 of FFDCA. I defer to others for a full review of the scientific validity of this study. If it were determined not to have scientific validity, it would also not be ethically acceptable.

Attachment 1: Email Correspondence

Date (2011)	Email From	Email To	Contents	Page
Jan 3	Laura Parsons	Dr. Gulson	Request for information	7 of 16
Jan 3	Dr. Gulson	Laura Parsons	Response to request	9 of 16
Jan 12	Dr. Gulson	Laura Parsons	Links to National Ethics Statement and Answers to EPA Questions	10 of 16
Jan 31	Laura Parsons	Gulson and Thorp	Request for statement from Ethics Secretariat	12 of 16
Jan 31	Fran Thorp	Laura Parsons	Information from Macquarie University Ethics Officer	13 of 16
Feb 17	Laura Parsons	Gulson and Thorp	Request for approval letters	14 of 16
Feb 27	Dr Gulson	Laura Parsons	Copies of approval letters from May and December 2006.	16 of 16

From: Laura Parsons/DC/USEPA/US
To: bgulson@gse.mq.edu.au
Cc: Kelly Sherman/DC/USEPA/US@EPA, William Jordan/DC/USEPA/US@EPA
Date: 01/03/2011 04:58 PM
Subject: Request for further information on conduct of study "Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.

Dear Dr. Gulson,

I am a US Environmental Protection Agency staff member in the Office of Pesticide Programs working on ethics issues for pesticide research with human subjects. The Pesticide Office has a specific regulation (40 CFR part 26) governing our use of research with human subjects which went into effect in April 2006.

One of our risk assessors has reviewed your recent paper in Toxicological Sciences and believes that your research would be instructive in EPA's assessment of other uses of nano-technology. She has asked my group to review your paper for compliance with EPA's ethics regulations since our regulation requires EPA and our advisory board to review the ethical conduct of this study if we decide to use it to inform our assessments. We do not mean to imply that we believe that your research was in any way unethical.

I note that your publication indicates that the research was conducted in compliance with all government policies and the Helsinki Declaration and was approved by human ethics committees at Macquarie University and Commonwealth Scientific and Industrial Research Organization (CSIRO). I have skimmed Australia's National Statement on Ethical Conduct in Human Research (2007), and while I am not completely clear what guidance covers your particular research method or field, it is clear that our government guidelines are similar.

I am writing to you to request the records of the approvals of your research by Macquarie and CSIRO so that we can conduct our own ethics review as required by our regulation. In addition we are interested in other records or information you could provide regarding:

- How were your subjects recruited and selected?
- Were any of the female subjects pregnant or nursing?
- What risks to subjects were identified and how they were minimized?
- How were risks to subjects weighed against the benefits for the subjects and society?
- Informed consent. What were your subjects told about the research and did they give written consent? Can we see a copy of the consent form?
- How was the privacy of individuals protected?

It is our plan to review your research for scientific and ethical conduct and to present those reviews to our Human Studies Review Board (HSRB) as required for our use of your research in our pesticide risk assessments. I would be glad to answer any questions that you have about this process. Below I have copied the pertinent section governing EPA's use of human research from our Code of Federal Regulation Part 26. Also, here is a link to the HSRB website: http://www.epa.gov/osa/hsrb/

I look forward to working with you.

Thank you,

Laura Parsons
Office of Pesticide Programs

§ 26.1602 EPA review of completed human research.

(a) When considering data under FIFRA or FFDCA from research involving intentional exposure of humans, EPA shall review the material submitted under § 26.1303 and other available, relevant information and document its conclusions regarding the scientific and ethical conduct of the research.

(b) EPA shall submit its review of data from human research covered by subpart Q, together with the available supporting materials, to the Human Studies Review Board if EPA decides to rely on the data and:

(1) The data are derived from research initiated after April 7, 2006, or

(2) The data are derived from research initiated before April 7, 2006, and the research was conducted for the purpose of identifying or measuring a toxic effect.

(c) In its discretion, EPA may submit data from research not covered by paragraph (b) of this section to the Human Studies Review Board for their review.

(d) EPA shall notify the submitter of the research of the results of the EPA and Human Studies Review Board reviews.

§ 26.1603 Operation of the Human Studies Review Board.

EPA shall establish and operate a Human Studies Review Board as follows:

(a) *Membership*. The Human Studies Review Board shall consist of members who are not employed by EPA, who meet the ethics and other requirements for special government employees, and who have expertise in fields appropriate for the scientific and ethical review of human research, including research ethics, biostatistics, and human toxicology.

(b) *Responsibilities*. The Human Studies Review Board shall comment on the scientific and ethical aspects of research proposals and reports of completed research with human subjects submitted by EPA for its review and, on request, advise EPA on ways to strengthen its programs for protection of human subjects of research.

From: Brian Gulson <brian.gulson@mq.edu.au>

To: Laura Parsons/DC/USEPA/US@EPA

Cc: Maxine.Mccall@csiro.au, Ethics Secretariat <ethics.secretariat@mq.edu.au>

Date: 01/03/2011 06:06 PM

Subject: Re: Request for further information on conduct of study "Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.

Dear Laura,

Thank you for your interest in our research. I am retired (supposedly) and don't often go into the University anymore. I wll forward your request to our ethics people and look at your additional questions.

Kind regards
Brian Gulson

From: Brian Gulson <brian.gulson@mq.edu.au>
To: Laura Parsons/DC/USEPA/US@EPA
Cc: Ethics Secretariat <ethics.secretariat@mq.edu.au>
Date: 01/12/2011 07:02 PM
Subject: Re: Request for further information on conduct of study
"Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.

Dear Laura

I spoke with the ethics office at the University yesterday and they provided me with the links that cover ethics in the Uni as listed below.
A request to see the records of approval is highly unusual and as it is confidential do not provide such information to outside parties.
I cannot speak for CSIRO and have not heard from them.

"Hi Brian

thank you for your call earlier. Here are the links to the National Statement on Ethical Conduct in Human Research (2007) and the Australian Code for the Responsible Conduct of Research (2007). These are the national documents informing ethical review in Australia.

http://www.nhmrc.gov.au/publications/synopses/e72syn.htm

http://www.nhmrc.gov.au/publications/synopses/r39syn.htm

Cheers
Nicola"

With respect to the other questions:

How were your subjects recruited and selected?
#As in any human study (or that I have run), recruitment is a major issue. In this case we advertised by email within the University and CSIRO, and gave a presentation at the Surf Life Saving Club but received no response. We then used personal contacts to obtain the required numbers.

Were any of the female subjects pregnant or nursing?
#No

What risks to subjects were identified and how they were minimized?
#The main risks were in blood sampling and any adverse reaction to the sunscreen formulations. The blood samples were taken by a trained phlebotomist. Re sunscreen: the volunteers were asked in the questionaire and personally if they if they suffered adverse reactions to cosmetics. None replied in the positive at that time.

How were risks to subjects weighed against the benefits for the subjects and society?
As the risks were considered to be minimal, the potential benefits to society far outweighed the risks.

Informed consent. What were your subjects told about the research and did they give written consent?
#The subjects attended a meeting at my house 1 week before the trial started and I described the trial and any risks. I also stressed the need for their committtment for the 5 days and follow up, and for minimal contamination of samples during collection (this applied especially to urine samples as we did not accompany the subjects into the toilets).
One subject withdrew on the evening as they did not wish to provide blood samples for the duration and one had study committments and withdrew on the first day of the trial.
Each morning at the beach I reiterated the messages.

Can we see a copy of the consent form?
##This is up to the University ethics secretariat.

How was the privacy of individuals protected?
#The subjects were provided with a unique identifier and only the researchers directly involved had access to these and the data.

I hope this is sufficient and please contact me if you require further assistance.

Kind regards
Brian

From: Laura Parsons/DC/USEPA/US
To: Brian Gulson <brian.gulson@mq.edu.au>, Ethics Secretariat <ethics.secretariat@mq.edu.au>
Cc: Kelly Sherman/DC/USEPA/US@EPA, William Jordan/DC/USEPA/US@EPA
Date: 01/31/2011 03:18 PM
Subject: Re: Request for further information on conduct of study "Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.

Brian and Nicola:

Thank you for this information and the links. I had skimmed the information contained in the links as I was looking at the summary of your research before contacting you. We certainly do not intend to request any information that would be considered confidential.

Would the Ethics Secretariat provide a statement that, in their view, your research complied with the applicable provisions of the Australian ethics rules? In that case, my presentation to our Human Studies Review Board would be a cross-walk between your (Australian) ethics rules and our regulation.

I very much appreciate your time and effort on this. Please let me know if a statement from the Secretariat is possible and if I need to pose this request to someone other than Nicola.

Thank you,
Laura
.
Laura Parsons
Office of Pesticide Programs
703-305-5776

From: Ethics Secretariat <ethics.secretariat@mq.edu.au>
To: Laura Parsons/DC/USEPA/US@EPA
Cc: Brian Gulson <brian.gulson@mq.edu.au>, Kelly Sherman/DC/USEPA/US@EPA, William Jordan/DC/USEPA/US@EPA, Kandy White <karolyn.white@mq.edu.au>
Date: 01/31/2011 08:42 PM
Subject: Re: Request for further information on conduct of study "Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.
Sent by: fran.thorp@mq.edu.au

Dear Laura

Thank you for your email.

I have copied this email to Dr Karolyn White, the Director of Research Ethics at Macquarie University who may wish to add to the information I have provided below.

The Macquarie University Human Research Ethics Committee, formerly the Macquarie University Ethics Review Committee (Human Research), is a National Health and Medical Research Council (NH&MRC) registered ethics committee and any research that is reviewed and approved by this committee must meet the requirements of the National Statement on Ethical Conduct in Human Research (NHMRC 2007). In the year Brian's research was initially considered the relevant document was the National Statement on Ethical Conduct in Research in Research Involving Humans (NH&MRC 1999) which was replaced by the National Statement on Ethical Conduct in Human Research in 2007.

Please see the NH&MRC weblink link to information about the Human research Ethics Committees in Australia.

http://www.nhmrc.gov.au/health_ethics/hrecs/overview.htm

The letter of Final Approval that Brian was given for the research to proceed is the usual document researchers use to show that the research has been through the appropriate ethics review process in Australia and that the research meets the requirements of the National Statement. Any amendments made to the project since the final approval was issued were reviewed and approved under the same framework, i.e. they met the

requirements of the National Statement.

Brian will be able to supply you with a copy of the final approval letter and relevant amendment approval correspondence if this would be helpful. If you feel an additional document or statement that the research complied with Australian ethics standards is required, please contact Dr White who will be able to assist you.

Please do not hesitate to contact the Ethics Secretariat if we can be of further assistance.

Regards
Fran

Ms Fran Thorp
Human Research Ethics Officer

From: Laura Parsons/DC/USEPA/US
To: Brian Gulson <brian.gulson@mq.edu.au>
Cc: fran.thorp@mq.edu.au, Kandy White <karolyn.white@mq.edu.au>, Kelly Sherman/DC/USEPA/US@EPA, William Jordan/DC/USEPA/US@EPA, Ethics Secretariat <ethics.secretariat@mq.edu.au>
Date: 02/17/2011 10:22 AM
Subject: Re: Request for further information on conduct of study "Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.

Hi Brian,

This note below from Ms. Thorp was very helpful. Would it be possible for you to send the copy of the Letter of Final Approval that she mentions to show that your research went through the ethics process in Australia?

I can probably use our email exchanges if necessary, but a copy of your approval letter would be a neater package.

Thank you,

Laura

Laura Parsons
Office of Pesticide Programs
703-305-5776

From: Brian Gulson <brian.gulson@mq.edu.au>
To: Laura Parsons/DC/USEPA/US@EPA
Date: 02/17/2011 08:28 PM
Subject: Re: Request for further information on conduct of study "Small Amounts of Zn from ZnO Particles...." published in Toxicological Sciences.

Hi Laura

I am out of town until the end of next week but will try and track this down (came as hardcopy) when I return and email a copy to you.

Kind regards
Brian

From: Brian Gulson <brian.gulson@mq.edu.au>
To: Laura Parsons/DC/USEPA/US@EPA
Date: 02/27/2011 06:55 PM
Subject: Re: thank you

Hi Laura
Attached are some notes from the ethics committee.
The original application was in my PhD students name (Herbert Wong) who later withdrew from his candidature because of medical reasons.

Hope this is useful.
Brian