CHEMICAL PRODUCTS CORPORATION

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March 14, 2005

Associate Director for Communications
Office of the Director
National Institutes of Health
Building 1, Room 344
1 Center Drive
Bethesda, Maryland 20892

Subject: **Request For Correction of NTP TR-494 Abstract**

Dear Madam or Sir;

This letter is a Request For Correction of the Abstract of NTP Techical Report 494 (TR494) available to the public on the NTP website, and possibly elsewhere. This Request for Correction is submitted by Chemical Products Corporation (CPC), a Georgia corporation located in Cartersville, Georgia, under the auspices of HHS and NIH "Guidelines for Ensuring the Quality of Information Disseminated to the Public" (Information Quality Guidelines).

The TR494 Abstract, peer reviewed on February 17, 2004, fails to transparently and objectively address the unfortunate circumstance of a non-mutagenic compound, Anthraquinone (AQ), being contaminated with strong mutagens in the test material employed for the TR494 studies.

TR494 was initially peer reviewed in May, 1999 and then withdrawn in September, 2003 in response to a Request for Correction submitted by CPC (see Attachment 1). NTP has posted a revised peer-reviewed Abstract of TR494 on NTP's web site. CPC respectfully submits that this peer-reviewed abstract presents

scientifically untenable conclusions as a result of insufficient information being provided in the draft TR494.

CPC requests that the TR494 Abstract on the NTP website be immediately withdrawn, and that the 2004 draft TR494 peer-reviewed on February 17, 2004 be withdrawn and revised to bring it into conformance with NIH's and OMB's Information Quality Guidelines.

NTP now acknowledges that 1) the test compound, Anthraquinone, CAS # 84-65-1, is not mutagenic, and 2) that the TR494 test material was contaminated with at least one mutagen; but this information is not presented transparently or objectively in the TR494 Abstract on the NTP website.

Upon the withdrawal of the 1999 TR494, Deputy Director Samuel H. Wilson stated in his September 8, 2003 letter to CPC, "The presence of this contaminant raises doubt as to the effect(s) of anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in draft TR-494"; these critical issues are not addressed in a transparent or objective manner in the 2004 TR494 or the TR494 Abstract presented on NTP's website and, thus, the conclusions presented in the TR494 Abstract are untenable.

The 2004 draft TR494 asserted that the effect of contamination of the TR494 test material with mutagens was insufficient to confound the conclusions originally presented in the 1999 TR494. NTP's assertion, accepted by NTP's Board of Scientific Counselors Technical Reports Review Subcommittee on February 17, 2004, was made in the absence of data quantifying the mutagenicity exhibited by the TR494 test material or the identity of one of the contaminants found in the test material.

Even though the compound Anthraquinone, CAS # 84-65-1, is not mutagenic in Salmonella typhimurium strains TA98 and TA100 with or without S9 activation, the test material employed in the TR494 studies is mutagenic in Salmonella typhimurium strains TA98 and TA100, both without and with S9 activation. NTP inexplicably failed to present crucial mutagenicity assay data for the TR494 test material in the 2004 draft TR494 peer reviewed on February 17, 2004. Mutagenicity assay data on an

aliquot of the TR494 test material obtained by CPC demonstrate that the TR494 test material is strongly mutagenic in Salmonella typhimurium strains TA98 and TA100 both with and without S9 metabolic activation. This data, generated by BioReliance Corporation, was furnished to NTP in August, 2000 by CPC.

The NTP Board of Scientific Counselors Technical Reports Review Subcommittee (by a vote of 7 yes, 6 no) inexplicably accepted NTP's intimations of weak mutagenicity in the TR494 test material and the conclusions presented in 2004 draft TR494, "some evidence of carcinogenic activity of anthraquinone in male rats and clear evidence of carcinogenic activity in female rats and male and female mice" even though 2004 draft TR494 did not present mutagenicity assay data for the TR494 test material and did not identify one of the contaminants in the TR494 test material. The Board only requested that the title of TR494 be changed to "Anthracene-derived Anthraquinone", and that it be made clear throughout the report that the material used in the study was anthracene-derived anthraquinone. This is a scientifically untenable resolution of interpretation of the TR494 studies being confounded by the presence of strongly mutagenic contaminants because 1) the chemical structure and CAS number presented in the TR494 Abstract is that of Anthraquinone, CAS # 84-65-1 without regard to its origin, and 2) only a small fraction of anthracene-derived anthraquinone is contaminated with mutagens as demonstrated by information in EPA's TSCA database.

The conclusions presented in the TR494 Abstract have not been supported in "an accurate, clear, complete, and unbiased manner" in the peer-reviewed 2004 draft TR494, and are thus untenable.

Deficiencies in the TR494 Abstract

The peer-reviewed TR494 Abstract now available to the public fails to meet NIH and OMB Information Quality Guidelines requirements for transparency and objectivity in at least the following 7 critical respects:

1. Although NTP now acknowledges that the compound Anthraquinone, CAS # 84-65-1 is not mutagenic, this information is not presented transparently or objectively in

the TR494 Abstract. The first two sentences in a paragraph in the TR494 Abstract state, "Anthraquinone (97% pure) was mutagenic in S. typhimurium strains TA98 and TA100, with and without rat and hamster S9 metabolic activation enzymes. A second Salmonella test with a 100% pure anthraquinone sample showed no mutagenic activity in strains TA98, TA100, or TA102, with or without rat liver S9 enzyme". This can only be described as disingenuous obfuscation; the 2004 draft TR494 stated on page 27, "9-nitroanthracene, which was present at about 0.12% in the anthraquinone sample tested by Zeiger *et al.* (1988), was positive in the *Salmonella* mutation assay over a concentration range of 10 to 1,000 µg/plate using strains TA98 and TA100, with and without 30% hamster or rat liver S9 activation enzymes (Zeiger *et al.*, 1988)". Thus, NTP is fully aware that the "Anthraquinone (97% pure)" sample found to be mutagenic was contaminated with at least one identified mutagenic impurity, yet this information is not presented in the TR494 Abstract on the NTP website.

2. Although NTP acknowledged in the 2004 draft TR494 that the TR494 test material was contaminated with a mutagen, the fact that the TR494 test material is mutagenic in Salmonella typhimurium strains TA98 and TA100 with and without S9 activation is not disclosed in the TR494 Abstract presented on the NTP Website. Mutagenicity assay data for the TR494 test material is not presented in either the 2004 draft TR494 or in the TR494 Abstract.

3. NTP has not presented mutagenicity assay data on the TR494 test material. TR494 presents mutagenicity assay data in the 2004 draft TR494 for pure anthraquinone, 1-nitroanthacene, 2-nitroanthracene, 9-nitroanthracene, 1-hydroxyanthracene, and 2-hydroxyanthracene; **but not for the TR494 test material.** TR494 asserts that the undefined degree of mutagenicity of the test material was not sufficient to confound the conclusions presented in the 1999 TR494 Abstract which are restated in the 2004 TR494 Abstract.

The aliquot of the TR494 test material submitted by CPC to BioReliance Corporation for preincubation mutagenicity assay in October 1999 was found to be mutagenic without and with S9 metabolic activation. Stronger mutagenicity was

found without S9 activation than with S9 activation (an 11-fold increase in revertants in TA98 without activation and a 6-fold increase with activation). Assuming 0.1% mutagenic contaminants (as asserted in 2004 drat TR494) in the 2500 microgram aliquot of TR494 test material that yielded 225 revertants, the contaminants in the TR494 test material yeilded **80 revertants/microgram in strain TA98 without S9 activation.** The attribution of all mutagenicity in the TR494 test material to 9-nitroanthracene, which yielded only 0.4 revertants/microgram in strain TA98 without S9 activation in the assay reported in Appendix E of 2004 draft TR494, is untenable.

4. Appendix J of the 2004 draft TR494 discloses that HPLC/UV analysis of the TR494 test material "showed a purity of 99.5% with impurities of 0.3% and 0.2%". One was identified as 9-Nitroanthracene, but NTP did not determine the identity of the second contaminant. NTP presented the hypothesis in 2004 draft TR494 that the mutagenicity found in the test material was entirely the result of 0.1% 9-Nitroanthracene (based on GC/FID analysis), and was not sufficient to confound the conclusions found in the 1999 draft TR494. We are amazed that the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee accepted this hypothesis without requiring information detailing the magnitude of mutagenicity exhibited by the contaminated test material or the identity of the second contaminant; this must surely stand as the quintessential example of inadequate peer review.

NTP was informed by CPC in late 2000 that a portion of the TR494 AQ powder aliquot furnished to CPC had been purified to remove all detectable 9-Nitroanthacene; this purified TR494 test material had nonetheless demonstrated mutagenicity in Salmonella typhimurium TA98 in a preincubation mutagenicity assay. Thus, at least one additional mutagenic impurity is present in the TR494 test material. We must reluctantly conclude that NTP did not conduct a mutagenicity assay on the TR494 test material, or identify the second contaminant found by HPLC, because NTP's overriding objective in 2004 draft TR494 was to provide support for the untenable conclusions presented in the 1999 draft TR494.

5. Even though NTP conducted further analytical characterization of the TR494 test material, and HPLC analytical data reported in Appendix J of the 2004 draft TR494 found that the TR494 test material contained 0.5% impurities rather than the 0.1% impurity level reported in the 1999 draft TR494, the TR494 test material is referred to in the TR494 Abstract only as "99.9% pure" Anthraquinone.

6. In the same paragraph that discusses "Anthraquinone (97% pure) and 100% pure Anthraquinone, the TR494 Abstract states, "Significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male and female mice exposed to anthraquinone (99.9% pure) in feed for 14 weeks." This "99.9% pure" test material is not transparently identified as the TR494 test material known by NTP to be contaminated with at least one mutagen.

7. The February 17, 2004 peer review accepted (by a vote of 7 yes, 6 no) the conclusions presented in the TR494 Abstract as written with the proviso that the TR494 Anthraquinone test material be identified as "Anthracene-derived Anthraquinone". Not only does this modification lack the scientific objectivity required by the Information Quality Guidelines (the chemical structure presented in the TR494 Abstract certainly applies to all Anthraquinone, regardless of its origin), but it does not objectively or transparently address the issue of test material contamination confounding interpretation of the NTP studies referenced in TR494.

The following information about this Request For Correction is provided in the specific format outlined in the "Responsibility of the Complainant" section of the HHS Guidelines for Ensuring the Quality of Information Disseminated to the Public.

• A detailed description of the specific material that is proposed for correction, including where the material is located, i.e., the publication title, date, and publication number, if any, or the web site and web page address (URL), or the presentation, presenter, date and mode of delivery; - The material proposed for correction is the Draft Abstract of Technical Report 494 Toxicology and Carcinogenesis Studies of Anthraquinone (CAS No. 84-65-1) in F344/N Rats and B6C3F1 Mice (Feed Studies),

NIH Publication Number 04-3953, found on the NTP web site at http://ehp.niehs.nih.gov/ntp/members/tr494full.pdf and possibly elsewhere within NTP.

• the specific reasons for believing that the information does not comply with OMB, HHS, or NIH guidelines and is in error, and supporting documentation, if any: NIH's and OMB's Information Quality Guidelines require that Abstracts of NTP Technical Reports presented to the public be factually accurate, transparent, and objective ; the TR494 Abstract presented on the NTP website is not factually accurate, transparent, or objective as described in detail above.

• Suggested recommendations for what corrective action(s) should be taken: CPC requests that the TR494 Abstract be immediately withdrawn from the NTP web site and replaced by a single sentence, slightly edited, excerpted from the September 8, 2003 letter sent by Deputy Director Samuel H. Wilson when he informed CPC that the 1999 peer-reviewed Abstract of TR-494 would be withdrawn from NTP's website, "The presence of [this contaminant] contamination raises doubt as to the effect(s) of anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in draft TR-494."

• A description of how the person requesting the correction is affected by the information error: - CPC and its subsidiary, Chemical Products Technologies, LLC, would be adversely affected by reduced sales of their Anthraquinone suspension product to the North American paper industry for use as a catalyst in the Kraft Process to obtain increased pulp yield. We believe that the North American paper industry will be reluctant to fully realize the increased pulp recovery benefits of Anthraquinone use because of uncertainty about its safety and environmental impact engendered by the deficient TR-494 Abstract presented on NTP's website.

• Complete contact information for the requester, including name, mailing address, telephone number, e-mail address, and organizational affiliation, if any, -

This letter is submitted by Jerry A. Cook, Technical Director, Chemical Products Corporation, P.O. Box 2470, Cartersville, GA 30120-1692, telephone number 770-382-2144 extension 272, email jcook@cpc-us.com.

In response to information submitted to him by CPC in August and September, 2000, Dr. Kenneth Olden initiated laboratory testing to determine the identity of previously unidentified mutagenic contaminants in the NTP Anthraquinone test material employed in the TR-494 studies. His letter dated September 26, 2000 to CPC states, "We agree that there is still considerable uncertainty about the mutagenicity of anthraquinone". Dr. Olden committed to conducting mutagenicity tests on pure Anthraquinone samples, as well as the contaminated TR494 test material, and stated that the results of this further work would be incorporated into a rewritten TR-494. This commitment by Dr. Olden has not been honored by NTP. A clear and transparent description of the results of mutagenicity testing on the contaminated Anthraquinone employed by NTP in the TR494 studies was nowhere to be found in the 2004 draft TR494. We submit that this absence of vital information makes the conclusions presented in the TR494 Abstract scientifically untenable.

OMB's Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies states, "'Objectivity' includes whether disseminated information is being presented in an accurate, clear, complete, and unbiased manner. This involves whether the information is presented within a proper context. Sometimes, in disseminating certain types of information to the public, other information must also be disseminated in order to ensure an accurate, clear, complete, and unbiased presentation. Also, the agency needs to identify the sources of the disseminated information (to the extent possible, consistent with confidentiality protections) and, in a scientific, financial, or statistical context, the supporting data and models, so that the public can assess for itself whether there may be some reason to question the objectivity of the

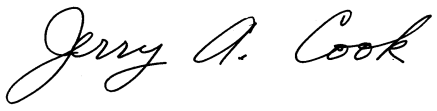
sources. Where appropriate, data should have full, accurate, transparent documentation, and error sources affecting data quality should be identified and disclosed to users.

In addition, "objectivity" involves a focus on ensuring accurate, reliable, and unbiased information. In a scientific, financial, or statistical context, the original and supporting data shall be generated, and the analytic results shall be developed, using sound statistical and research methods."

Chemical Products Corporation respectfully submits that the peer-reviewed draft Abstract of TR494 fails to meet the transparency and objectivity requirements of the HHS, NIH, and OMB Information Quality guidelines and should therefore be immediately withdrawn from NTP's website.

Please telephone me at 770-382-2144 Ext. 272 or email me at jcook@cpc-us.com if I can supply any further information or copies of any of the letters CPC has sent to NTP or received from NTP or NIH concerning NTP Technical Report 494.

Sincerely,



Jerry A. Cook
Technical Director

Attachment

Cc: Dr. John D. Graham, OIRA

Attachment 1: History relating to the 2004 TR494 Abstract

- The original draft TR494 was peer reviewed in May, 1999 and a peer-reviewed abstract was placed on the NTP website. This abstract stated that the compound Anthraquinone was mutagenic and that the TR 494 2-year studies had shown "some evidence of carcinogenic activity of anthraquinone in male rats and clear evidence of carcinogenic activity in female rats and male and female mice." The actual test material employed in these studies had not been tested for mutagenicity by NTP.

- In late 1999 and 2000, CPC determined that the compound Anthraquinone, CAS # 84-65-1, was not mutagenic in Salmonilla typhimurium strains TA98 and TA100. CPC also obtained an aliquot of the TR494 test material and submitted it to Bioreliance Laboratories for mutagenicity assay; the TR494 test material was strongly mutagenic in TA98 and TA100 both with and without S9 activation. CPC informed NTP of its determinations in letters sent in August, September, and October, 2000 (including evidence that more than one mutagen was present in the test material), but NTP took no action to remove or modify the TR494 Abstract presented on its website.

- In November, 2002 CPC submitted a Request for Correction to NIH under the auspices of the newly-enacted Information Quality Act asking that the TR494 Abstract be withdrawn from the NTP website because it failed to meet NIH and OMB Information Quality Guidelines. This Request for Correction was denied on March 19, 2003.

- On March 27, 2003 CPC submitted a Request for Reconsideration to NIH asking, once again that the TR494 Abstract be withdrawn. In September, 2003 Dr. Samuel H. Wilson responded to CPC, stating in part,
  "I have reached the following conclusions:

1. The sample of anthraquinone used in the NTP 2-year study was contaminated with 9- nitroanthracene at a level of about 0.1%.
2. The presence of this contaminant raises doubt as to the effect(s) of anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in draft TR-494. In addition, in view of imprecise statements in the text presented on the website, this abstract needs to have greater specificity than it presently has.
3. The abstract of draft TR-494 will immediately be removed from the NTP website. Further studies are underway on the metabolism of anthraquinone in rodents and on the relative mutagenic potency of this compound, its major metabolites, the contaminant 9-nitroanthracene, and two isomers of 9-nitroanthracene. Additional information from this work will eventually be incorporated into a revised abstract and technical report which will be submitted for peer review and subsequent publication."

- At the end of 2003 a "revised" draft TR494 was made available for review and public comment prior to scheduled peer review on February 18, 2003.

- CPC reviewed the "revised" draft TR494 document and determined that it did not meet NIH and OMB Information Quality Standards. CPC submitted a Request for Correction on February 5, 2003 asking that the "revised" draft TR494 be withdrawn prior to peer review and brought into compliance with Information Quality Standards prior to reissue and eventual peer review. NIH denied CPC's Request for Correction on the grounds that a document released for public comment prior to peer review, and clearly labeled as such, was not subject to the requirements of NIH's Information Quality Guidelines. NIH did not dispute CPC's assertion that the "revised" draft TR494 did not meet Information Quality Guidelines.

- CPC submitted a Request for Reconsideration in July, 2004 arguing that meaningful public comment and scientifically-sound peer review is not possible if draft NTP reports are not transparent and objective, and if vital

scientific information required to support proposed conclusions is not presented (as is the case with the 2004 draft TR494).

- On January 31, 2005 NIH denied CPC's Request for Reconsideration by affirming its opinion that Information Quality Guidelines do not apply to documents clearly labeled as being drafts made available for public review and comment prior to peer review.