CHEMICAL PRODUCTS CORPORATION

February 6, 2004

Associate Director for Communications
Office of the Director
National Institutes of Health
Building 1, Room 344
9000 Rockville Pike
Bethesda, MD 20892

Subject: Submission of Information Quality Request For Correction of NTP Draft TR-494 NIH Publication Number 04-3953 prior to extended public comment period and peer review

Dear Madam or Sir;

This letter is a Request For Correction submitted by Chemical Products Corporation (CPC), a Georgia corporation located in Cartersville, Georgia under the auspices of NIH's Information Quality Guidelines.

NTP has posted a revised draft of Technical Report 494 (TR494), now NIH Publication Number 04-3953; this is purported to be a revision and correction of TR494, NIH Publication Number 99-3953. Draft TR494 has been made available on NTP's web site for public comment before scheduled peer review on February 18, 2004. This document lacks transparency and objectivity; these deficiencies, along with the complete absence of some of the information essential to the evaluation of the stated conclusions, dictate that the draft TR494 be withdrawn and revised further prior to peer review.

Revised draft TR494 is intended to address the unfortunate circumstance of a non-mutagenic test compound, Anthraquinone (AQ), having been found to be contaminated with mutagens subsequent to completion of 2-year studies and preparation of the original draft TR494 in 1999. Mutagenic contamination in the TR494 AQ test material is present to the extent that the TR494 Anthraquinone powder employed in 2-year studies is mutagenic in Salmonella typhimurium strains TA98 and TA100 without and with S9 activation. Revision of draft TR494 was to have modified and corrected the pharmacokinetic model previously developed around the incorrect assumption that Anthraquinone was a mutagen, yet this revised draft contains the statement that AQ is a mutagen. The pharmacokinetic model developed to support the conclusions presented in the present draft TR494 appears to be based on the incorrect assumption that AQ is mutagenic; reviewer Medinsky is quoted as commenting in 1999 that it was difficult to adequately evaluate the model because of lack of explanatory text

regarding assumptions underlying the model. Adequate evaluation of the model now appears to be impossible.

Revised draft TR494 now available to the public fails to meet the NIH and OMB Information Quality Guidelines' requirements for Information Quality, particularly transparency and objectivity, in at least 6 critical respects detailed in this letter. These failures, including significant factual errors, preclude the public and peer reviewers from comprehending and evaluating the important issues of scientific judgment engendered in the conclusions presented TR494. CPC requests that TR494 be withdrawn and brought into conformance with NIH's and OMB's Information Quality Guidelines before being resubmitted for public comment and peer review.

Draft TR494 fails to meet the requirements of the OMB's Information Quality Guidelines in the following specific respects:

1. NTP now acknowledges that the pure compound Anthraquinone is not mutagenic in Salmonella typhimurium strains TA98 and TA100 with or without S9 activation, but this information is not objectively and transparently presented. No observed mutagenicity is reported for a sample characterized as 100% Anthraquinone. Yet on page 113 is found, "anthraquinone and most substituted anthraquinones are mutagens", while on page 116 is found, "Based on the questionable mutagenicity of Anthraquinone" and "we have confirmed the nonmutagenicity of pure Anthraquinone". Clear objective presentation of the non-mutagenicity of Anthraquinone is absolutely vital for meaningful evaluation of the conclusions presented in the draft TR494 study; the present draft TR494 is contradictory and, therefore, incomprehensible.

2. NTP now acknowledges that the Anthraquinone powder employed for the TR494 studies was contaminated with a mutagenic contaminant, but again this information is not objectively and transparently presented. Inexplicably, NTP has presented no information concerning the degree of mutagenicity of the TR494 AQ powder even though CPC informed NTP of the results of preincubation mutagenicity assays showing mutagenic activity in a TR494 AQ powder aliquot in early 2000 and NTP committed to a full investigation. Rather than address the strength of the mutagenicity of the TR494 AQ powder sample reported to NTP by CPC in 2000, and Butterworth et al. (2001) who independently presented data showing that a TR494 AQ powder aliquot was mutagenic in Salmonella typhimurium strains TA98 and TA100, NTP has adopted the Butterworth et al. hypothesis that 0.1% contamination of the TR494 AQ powder is the sole source of mutagenic activity in the powder. CPC presented contrary evidence, described herein, to NTP in October 2000.

3. TR494 misrepresents the results of the Gibson et al. (1997) Syrian Hamster embryo cell studies and fails to disclose that Gibson et al. (1997) tested an aliquot of the contaminated, mutagenic TR494 AQ powder.

4. NTP fails to objectively and transparently disclose, or even discuss, that the pattern of tumors reported in NTP494 is consistent with a direct acting mutagen; it is not the pattern seen with nongenotoxic carcinogens, where tumors tend to be induced only in tissues impacted by preceding toxic events [expert opinion reported to CPC by a recognized consultant in this field - see also J. Ashby, R.W. Tennant; 1991; Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP; *Mutation Research*; 257; 229-306; and B. E. Butterworth, R.B. Conolly, K.T. Morgan; 1995; A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments; *Cancer Letters*; 93; 129-146]. Draft TR494 repeats the conclusions presented in 1999 that the tumors are the result of ingestion of the non-mutagenic test substance, Anthraquinone.

5. In draft TR494, NTP readily adopts the hypothesis presented by Butterworth et al. (2001) that all mutagenic activity in the TR494 AQ powder sample is the result of contamination by 0.1% 9-Nitroanthracene. NTP has not fully characterized the contaminants in TR494 AQ powder to independently verify this hypothesis, rather NTP has simply argued against its feasibility. NTP was informed by CPC in late 2000 that a portion of the TR494 AQ powder aliquot furnished to CPC had been purified through dissolution in concentrated sulfuric acid, had subsequently been found to be free of detectable 9-Nitroanthacene, and had nonetheless retained mutagenicity in TA98 in a preincubation mutagenicity assay. A contaminant in the sample definitely survived this purification process; it was not 9-Nitroanthracene. The purified sample was a dark gray color rather than the expected light yellow color of pure AQ. CPC speculates that there may be discrete particulate impurities present in the TR494 AQ powder which may or may not appear in very small aliquots collected for laboratory analysis, but which were present in the larger aliquot CPC subjected to concentrated sulfuric acid dissolution and reprecipitation. Arkion Life Sciences has recently identified several harmful contaminants in an aliquot of the TR494 AQ powder and informed NTP of its findings.

6. NTP has failed to adequately characterize the nature and quantity of the contaminants in the TR494 AQ powder, thus NTP's assertions that contaminants in the TR494 AQ powder cannot account for the observed carcinogenic response are untenable. NTP has added additional analytical data to the current draft TR494 indicating that the TR494 AQ powder may contain up to 0.5% impurities ("Additional purity analysis by high-performance liquid chromatography/ultraviolet detection indicated a purity greater than 99.5%." at page 31). This is not inconsistent with the Arkion Life Sciences information provided to NTP on February 2, 2004 quantifying 0.6% impurities in a TR494 AQ powder aliquot. NTP does not present analytical data identifying the 0.5% impurities; Arkion Life Sciences identifies the impurities as substances that would be expected to impact the results of the 2-year studies.

7. Draft TR494 incorrectly asserts on page 20 that CPC identified the single mutagenic impurity, 9-Nitroanthracene, in its information submissions to NTP. CPC ascertained (1) that NTP was incorrect in classifying AQ as a mutagen based on the extensive published mutagenicity assay data for AQ, (2) that the TR494 AQ powder was mutagenic based upon a preincubation mutagenicity assay commissioned by CPC, and (3) that the TSCA AQ file contained an example of an AQ powder which had been found to be mutagenic as a result of 9-Nitroanthracene contamination. CPC determined that a TR494 AQ powder aliquot was mutagenic in late 1999 and informed NTP of this mutagenicity in early 2000. CPC has repeatedly suggested to NTP that the origin of the TR494 Anthraquinone powder sample, manufactured by the oxidation of anthracene, may have led to contamination by one or more strongly mutagenic contaminants. This was explicitly stated in a letter to Dr. Kenneth Olden dated October 16, 2000 as discussed later in this letter. More than 3 years later, NTP has failed to characterize the extent and origin of the mutagenicity observed in the TR494 AQ powder sample.

7. Even though TR494 states that a 100% Anthraquinone sample showed no mutagenic activity, the “DISCUSSION AND CONCLUSIONS” contains the sentence, “In addition, anthraquinone and most substituted anthraquinones are mutagens.” at page 113. This schizophrenia concerning the mutagenicity of Anthraquinone appears to originate in NTP’s inability to clearly and transparently differentiate between the compound Anthraquinone, CAS# 84-65-1, with the chemical structure shown at the top of page 5 in draft TR494, and the mutagen-containing Anthraquinone powder employed in the TR494 studies.

8. NTP fails to objectively and transparently provide information concerning the mutagenicity of 2-Hydroxyanthraquinone. NTP presents the hypothesis that AQ metabolites contribute to the carcinogenicity observed in the TR494 studies based partially upon the 2-Hydroxyanthraquinone found in collected urine. NTP characterizes 2-Hydroxyanthraquinone as possessing “significant” mutagenic activity, yet Tikkanen et al. (Mutation Research, 116, 297-304 (1983)) reported it was non-mutagenic in TA98 with and without S9 metabolic activation enzymes and non-mutagenic in TA100 without S9 metabolic activation enzymes. Very weak mutagenicity (about two-fold increase in number of revertants) was found in TA100 with S9 metabolic activation enzymes. Tikkanen et al. (1983) also found that 2-hydroxyanthraquinone was not mutagenic in TA2637 in the absence of S9 activation. The discussion on page 28 of draft TR494 simply characterizes Tikkanen et al. (1983) as having found 2-Hydroxyanthraquinone to be a “weak mutagen”. The “Results” section of TR494 Appendix E reports weak mutagenicity in TA 98 without S9 activation and no mutagenicity in TA100. Apparently the reported weak mutagenicity of 2-Hydroxyanthraquinone in TA 98 without S9 activation, a finding contrary to the findings of Tikkanen et al. (1983), is the “significant mutagenicity” employed in NTP’s pharmacokinetic model justify the conclusions presented in TR494.

NIH's and OMB's Information Quality Guidelines require that NTP draft technical reports be factually accurate, transparent, and objective when presented for public comment and subsequent peer review. We respectfully request that the draft TR494 now on the NTP web site be withdrawn and that the scheduled peer review be postponed until factual errors, and transparency and objectivity inadequacies in the report are corrected.

CPC produces Anthraquinone (AQ) aqueous suspensions for use by the North American paper industry as a catalyst in the Kraft pulping process. The Anthraquinone is produced by a Friedel-Crafts process unlike the older oxidation-of-anthracene process that was the origin of the contaminated TR494 AQ powder sample discussed in this letter. CPC's affiliate, Chemical Products Technologies, LLC markets these Anthraquinone aqueous suspensions to the North American paper industry.

Draft TR494 fails to clearly and objectively characterize Anthraquinone (AQ) as a non-mutagenic compound. NTP incorrectly characterized AQ as mutagenic on its web site from early 1999 until late 2003; the Abstract of TR494 available to the public during that period stated, "Anthraquinone was mutagenic in Salmonella typhimurium strains TA98 and TA100 with and without S9 metabolic activation enzymes." In draft TR494, NIH Publication Number 04-3953, NTP acknowledges that the compound Anthraquinone, CAS # 84-65-1, is not mutagenic, but does not present this information in a transparent, objective manner. The present draft TR494 Abstract states, "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 enzymes. Several substituted anthraquinones were also tested in Salmonella, and results showed significant mutagenic activity for 2-hydroxyanthraquinone and 1-, 2-, and 9-nitroanthracene, with and without S9." After presenting an incorrect characterization of the mutagenicity of AQ on its web site for more than 4 years, it is incumbent upon NTP to present the highly significant conclusion that Anthraquinone is not mutagenic in Salmonella typhimurium strains TA98 and TA100 in a clear and objective manner; TR494 fails to do this.

Draft TR494 misrepresents the findings of Gibson et al. (1997) and fails to transparently disclose that Gibson et al. were testing an aliquot of the contaminated Anthraquinone powder sample employed by NTP in the TR494 studies. TR494 at pages 28 and 29 states, ".... and dose-related increases in micronuclei were reported in cultured Syrian hamster embryo cells treated with 3.13 to 25 ug anthraquinone (99% pure)/ml (Gibson et al., 1997)." Gibson et al. did not find dose-related increases in micronuclei; they state in "3.1. Results of in vitro micronucleus assay" at page 65, "All of the chemicals induced a dose-dependent, significant increase in the percentage of MNBC cells at multiple concentrations except anthraquinone and scopolamine hydrobromide which induced weaker responses. Anthraquinone induced a significant increase in the

percentage of MNBC (6.6% compared to 3.2% in control) only at the highest concentration tested, 25 ug/ml, ...". This is a particularly significant misrepresentation because Gibson et al. (1997) were testing an aliquot of the TR494 Anthraquinone powder obtained from NTP, which is now known to contain a mutagenic contaminant.

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 and Draft Technical Report TR-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: In reviewing all of the information available concerning Anthraquinone, including the Draft TR494, NIH Publication Number 99-3953, CPC discovered important discrepancies between the statements in TR-494 and other published information. At least 15 different Anthraquinone samples have been tested for mutagenicity in Salmonella typhimurium and have been determined not to possess mutagenic activity. In Draft TR494, NIH Publication Number 04-3953, NTP now acknowledges that the pure compound, Anthraquinone, is not mutagenic in Salmonella typhimurium strains TA98, TA100, or TA102, with or without rat liver S9 enzymes, however this information is not presented in a clear or transparent fashion. Draft TR494 contains contradictory statements regarding this critically important issue.

NTP had not tested the Anthraquinone powder sample employed in TR-494 for mutagenicity prior to submission of draft TR494 for public comment and peer review in 1999. Apparently NTP still has not tested the TR494 AQ powder sample for mutagenicity. CPC obtained an aliquot of this Anthraquinone sample from NTP and submitted it to a respected independent laboratory, BioReliance Corporation, for mutagenicity testing beginning October 26, 1999. The NTP TR-494 AQ powder sample was found to be mutagenic in Salmonella typhimurium strains TA98 and TA100 without S9 activation and mutagenic in strain TA98 in the presence of S9 activation when preincubation mutagenicity assays were conducted. In early 2000 NTP was informed that the NTP Anthraquinone sample employed for the TR494 studies was the only AQ sample which

displayed mutagenic activity among 4 Anthraquinone samples submitted to BioReliance for preincubation mutagenicity assays. NTP was provided a full copy of the BioReliance test report.

Butterworth et al. (2001) obtained an aliquot of the TR494 AQ powder and presented mutagenicity assay results for the NTP Anthraquinone sample employed in the TR494 studies. The TR494 anthraquinone powder was, once again, found to be mutagenic in Salmonella typhimurium strains TA98 and TA 100.

Amazingly, NTP has released draft TR494, NIH Publication Number 04-3953 without including any preincubation mutagenicity assay data for the contaminated Anthraquinone powder employed in the TR494 studies. There is no evidence that NTP has conducted any mutagenicity evaluation of this TR494 AQ powder even though the strength of the mutagenic activity of this material is vitally important to an objective evaluation of the likely source of the carcinogenicity observed in the TR494 studies.

In draft TR494, NTP has improperly elected to accept the Butterworth et al. (2001) hypothesis that all mutagenic activity in the TR494 sample is the result of contamination with 0.1% 9-Nitroanthracene. NTP has not conducted a proper characterization of the impurities in the TR494 AQ powder and apparently has not independently determined the degree of mutagenic activity exhibited by this test material. NTP was informed that the mutagenicity of the TR494 Anthraquinone sample was not solely related to the presence of 9-Nitroanthracene in a letter from CPC to Dr. Kenneth Olden dated October 16, 2000. That letter, signed by Jerry A. Cook, states in part, "As I described to you in my September 25, 2000 letter, the NTP draft Technical Report 494 Anthraquinone sample has tested positive in strain TA98 after it was purified by dissolution in concentrated sulfuric acid and determined to be free of 9-nitroanthracene.

A portion of our sample of the NTP Anthraquinone test material was "acid purified" by dissolution in concentrated sulfuric acid (the same procedure followed with the Eastman Chemical reagent grade anthraquinone sample described to you in earlier letters) and resubmitted for Ames testing.

The "acid purified" NTP Anthraquinone test material is not a pale yellow color as I had expected based upon my experience with the Eastman Chemical sample. It is a "battleship gray" color. This sample has been analyzed by two laboratories as containing no detectable 9-nitroanthracene."

At the very least, it is incumbent upon NTP to fully characterize the perhaps 0.5% by weight contamination in the TR494 Anthraquinone test material to determine what mutagenic and/or carcinogenic contaminants are present and to fully evaluate and report the degree of mutagenic activity exhibited by the material prior to presenting a draft report for public comment and subsequent peer review.

• Suggested recommendations for what corrective action(s) should be taken:
CPC requests that Draft TR-494, NIH Publication Number 04-3953 be immediately withdrawn from the NTP web site, revised to correct the deficiencies

detailed in this letter, and re-released at a later date for public comment prior to peer review.

• A description of how the person requesting the correction is affected by the information error: - CPC and Chemical Products Technologies, LLC are adversely affected by reduced sales of their Anthraquinone suspension product to the North American paper industry. 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 NTP Draft Report TR-494.

• 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.

The existing draft of TR494 found at http://ehp.niehs.nih.gov/ntp/members/tr494full.pdf fails to transparently and objectively disclose that the contaminated Anthraquinone sample tested by NTP was found to be mutagenic in salmonella typhimurium strains TA98 and TA 100 whereas the compound Anthraquinone, CAS # 84-65-1, itself is not mutagenic in Salmonella typhimurium strains TA98 and TA100 with or without S9 activation. NTP cannot have objectively evaluated the impact of mutagenic contaminants in the TR494 AQ powder because NTP has not objectively characterized what contaminants are present or what degree of mutagenic activity the contaminated TR494 AQ powder exhibits.

In response to information submitted to him by CPC several years ago, Dr. Kenneth Olden initiated laboratory testing to determine the identity of previously unidentified mutagenic contaminants in the NTP Anthraquinone sample 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 NTP Anthraquinone sample, 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 is nowhere to be found in the draft TR494 presented on the NTP web site.

The objectives of NIH’s and OMB's Information Quality Guidelines are not met by provision of a draft technical report lacking objectivity and transparency, and containing factual misrepresentations, for public comment and subsequent peer review. The public and peer reviewers do not have access to the facts

necessary to allow them to make a valid scientific judgment. For this reason, we request that the scheduled peer review of draft TR494 be postponed until the draft TR494 document is brought into compliance with NIH and OMB Information Quality Guidelines.

OMB's Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies state, "'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 draft TR494, NIH Publication Number 04-3953, fails to meet the objectivity requirements of the OMB guidelines and should therefore be immediately withdrawn and revised before undergoing peer review.

If I can answer any questions concerning the contents of this letter, or provide any further information, please telephone me at 770-382-2144 extension 272.

Sincerely,

Jerry A. Cook

Jerry A. Cook
Technical Director

Cc: Dr. John D. Graham, OIRA