CHEMICAL PRODUCTS CORPORATION

February 8, 2004

Dr. [redacted]
NTP Executive Secretary
National Institute of Environmental Health Sciences
PO Box 12233 - MD A3-01
111 T.W. Alexander Dr.
Research Triangle Park, NC 27709

Subject: Comments concerning NTP draft Technical Report 494

Dear Dr [redacted]

This letter comments upon NTP draft Technical Report 494, NIH Publication Number 04-3953. It is submitted by Chemical Products Corporation (CPC). In this letter, CPC intends to summarize vital information lacking from NTP's draft Technical Report 494 (TR494). Chemical Products Corporation (CPC) submitted an Information Quality Request for Correction on February 6, 2004 requesting that NIH withdraw and revise draft NTP Technical Report 494. You have been furnished a copy of that Request for Correction.

NIH's and OMB's Information Quality Guidelines require scientific documents prepared for public review and comment to present information objectively. CPC believes that Tr494 is deficient in this regard and offers the following information in an effort to present an accurate, clear, complete, and unbiased presentation of the scientific issues surrounding the conclusions presented in draft TR494.

The 1999 draft NTP Technical Report 494, NIH Publication Number 99-3953, based its conclusions on its assessment that the test material, Anthraquinone, CAS# 84-65-1, was mutagenic to Salmonella typhimurium strains TA98 and TA100 without and with S9 metabolic activation. A pharmacokinetic model assuming the mutagenicity of Anthraquinone was developed to support the conclusion that there was "clear evidence of carcinogenitic activity of

anthraquinone". The conclusion that "the parent ring system confers carcinogenic potential" was based upon NTP's assessment that the test substance, anthraquinone, was a direct-acting mutagen.

NTP was incorrect in its 1999 assessment of the mutagenicity of Anthraquinone, CAS# 84-65-1. As a result of information submitted by CPC in 2000, NTP initiated a series of mutagenicity tests on the compound Anthraquinone. Draft TR494 now contains the statement on page 116 that "we have confirmed the nonmutagenicity of pure Anthraquinone".

CPC obtained an aliquot of the Anthraquinone test material employed in the TR494 studies and submitted a portion to BioReliance Corporation for preincubation mutagenicity assays in October 1999. NTP had not conducted mutagenicity tests on this material. The Anthraquinone test material employed in the TR494 studies 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 0.5% mutagenic contaminants in the 2500 microgram aliquot of TR494 anthraquinone test material that yielded 225 revertants, 16 revertants/microgram of contaminant were observed in strain TA98 without S9 activation.

CPC informed NTP in 2000 that the TR494 AQ test material was mutagenic while concurrently presenting evidence that the substance anthraquinone was not mutagenic. CPC suggested that the mutagenic contaminants in the TR494 test material probably included 9-Nitroanthracene based upon information CPC found in EPA's TSCA Anthraquinone file. Butterworth et al. (2001) obtained an aliquot of the TR494 test material and repeated the mutagenicity assay at Covance Laboratories, Inc. after learning of CPC's results. Butterworth et al. (2001) also found mutagenic activity in the test material both without and with S9 activation.

The strength of the mutagenic activity observed in the contaminated test material would be expected to be a critical determinant in the attribution of carcinogenic activity to anthraquinone. NTP presents no mutagenicity data on the contaminated test material employed in TR494.

NTP now acknowledges that the test material employed in the TR494 studies was a non-mutagenic compound, Anthraquinone, CAS# 84-65-1, containing a mutagenic contaminant. TR494 acknowledges the presence of 0.1% mutagenic 9-Nitroanthracene in the test material, but also states on page 31 that high-performance liquid chromatography/ultraviolet detection analysis indicates that the TR494 test material may contain up to 0.5% contaminants. An analysis recently performed by Arkion Life Sciences indicates 0.6% contaminants in an aliquot of the TR494 test material; all of the contaminants detected in the Arkion Life Sciences analysis could be expected to confound the results of the TR494 studies. Draft TR494 does not identify any contaminants other than 0.1% 9-NItroanthracene. The quantity and identity of the remaining contaminants in the TR494 test material is uncertain.

CPC dissolved an aliquot of TR494 test material in concentrated sulfuric acid, and then reprecipitated it (the long-standing gravimetric technique for determining Anthraquinone purity). The reprecipitated material contained no detectable 9-Nitroanthracene, yet this refined TR494 test material still retained mutagenic activity. CPC provided this information to NTP in October 2000. CPC found evidence that there were impurities present in the TR494 test material as discrete particulates. These may or may not be uniformly distributed in each aliquot; CPC reported to NTP in late 2000 that the refined aliquot of the NTP test material exhibited a dark gray color as opposed to the pale yellow color of refined Anthraquinone. NTP does not present a comprehensive characterization of the identities and distributions of impurities in the TR494 test material. Draft TR494 cites the hypothesis presented by Butterworth et al. (2001) as evidence

that the TR494 test material contained no mutagenic contaminants other than 0.1% 9-Nitroanthracene.

The pattern of tumors reported in draft TR494 appears to be consistent with a direct acting mutagen; it does not appear to be the pattern seen with nongenotoxic carcinogens, where tumors tend to be induced only in tissues where there are preceding toxic events [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 Lett.; 93; 129-146]. NTP apparently considered the reported pattern of tumors to be consistent with a direct acting mutagen in 1999 when it incorrectly believed that Anthraquinone was mutagenic. Draft TR494 does not present a rationale of why the pattern of tumors reported in TR494 were considered to be consistent with the action of a direct acting mutagen in 1999 and are now considered to be consistent with the action of a nonmutagenic test material.

Draft TR494 now bases its conclusion of "clear evidence of carcinogenic activity of anthraquinone" on the weak mutagenicity reported for the compound 2-Hydroxyanthraquinone found in collected rat urine samples. Mouse urine was not analyzed for the presence of this substance. NTP reports that 1-Hydroxyanthraquinone is nonmutagenic. Draft TR494 reports that 2-Hydroxyanthraquinone is weakly mutagenic only in TA98 without S9 activation, however 2-Hydroxyanthraquinone has been reported elsewhere to exhibit no mutagenic activity in Salmonella typhimurium strain TA98 without or with S9 metabolic activation [L. Tikkanen, T. Matsushima, S. Natori; 1983; Mutagenicity of anthraquinones in the Salmonella preincubation test; *Mutation Research*; 116; 297-304]. The Tikkanen et al. (1983) result is not presented in detail in draft TR494 and is only referenced by the statement on page 27, "Hydroxylation, up to a maximum of four substitutions, also appears to enhance mutagenic potential

(Tikkanen et al., 1983; Matsushima et al., 1986)", and the statement on page 116, "2-hydroxyanthraquinone has been reported to be a bacterial mutagen (Tikkanen et al., 1983)". If NTP is to hypothesize that the tumors reported in TR494 are the result of the mutagenic activity of 2-Hydroxyanthraquinone, NTP should present all of the information available about the mutagenicity of this substance.

Draft TR494 incorrectly reports that Gibson et al. (1997) found dose-related increases in micronuclei in cultured Syrian Hamster embryo cells, but fails to disclose that the material tested was an aliquot of the contaminated, mutagenic TR494 test material. In fact, Gibson et al. (1997) report that Anthraquinone induced a significant increase in the percentage of micronucleated cells (6.6% compared to 3.2% in control) only at the highest concentration tested, 25 ug/ml.

A "Catch 22" scenario has evolved regarding draft TR494. A pharmacological model was developed prior to release of the 1999 version of TR494, NIH Publication Number 99-3953, based upon NTP's assumption that Anthraquinone was mutagenic without and with metabolic activation, NTP's assumption that Anthraquinone was the only toxicologically significant compound in the TR494 test material, and NTP's conclusion that "carcinogenic activity conferred by the parent ring structure" could be logically inferred based upon the tumors observed in the 2-year TR494 studies. Now the first 2 of these assumptions are known to be incorrect and the third has been reduced to conjecture. A pharmacokinetic model based upon these assumptions is not valid, yet it appears that the model constructed in 1999 based upon the conclusion that there was "clear evidence of carcinogenic activity of anthraquinone" is being employed in 2004 to justify the conclusion that nonmutagenic anthraquinone has yielded "clear evidence of carcinogenic activity". Conclusions regarding the carcinogenic activity of anthraquinone derived from the 1999 pharmacokinetic model are untenable.

CPC commissioned mutagenicity assays in 1999 and 2000, CPC supplied

information to NTP in three letters in 2000, CPC submitted an Information Quality Request for Correction to NIH in 2002, and CPC submitted an Information Quality Request for Reconsideration in 2003. As a result, NTP initiated further studies and analyses, resulting in this "revised and corrected" TR494, NIH Publication Number 04-3953. NTP now recognizes that the compound Anthraquinone is not mutagenic. In evaluating metabolites found in rat urine, NTP found no mutagenic activity in 1-Hydroxyanthraquinone. NTP found weak mutagenic activity in 2-Hydroxyanthraquinone only in TA98 without S9 metabolic activation; this finding of mutagenicity in TA98 contradicts the findings of Tikkanen et al. (1983) who found no mutagenic activity in TA98 with or without S9 metabolic activation. Tikkanen et al. (1983) also accurately identified the nonmutagenicity of Anthraquinone in the same paper.

The Information Quality Request for Correction submitted to NIH on February 6, 2004 asks that the draft TR494 now presented on NTP's website be withdrawn and revised further to bring it into compliance with NIH's and OMB's Information Quality Guidelines before being resubmitted to the public for review and comment. CPC believes that the public and reviewers cannot adequately evaluate the conclusions presented in draft TR494 because some vital information is not clearly presented, and other vital information is completely absent from the document.

Thank you for providing me the opportunity to comment on this draft NTP technical report.

Sincerely,

[redacted]

[redacted]
Technical Director