DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

December 22, 2006

Jerry A. Cook
Technical Director
Chemical Products Corporation
P. O. Box 2470
Cartersville, Georgia 30120

Dear Mr. Cook:

I am responding to the Request for Correction of Information filed by Chemical Products Corporation (CPC) on May 31, 2006, and amended on July 13 and July 17 under the auspices of the National Institutes of Health's *Guidelines for Ensuring the Quality of Information Disseminated to the Public*. You specifically ask for withdrawal of the National Toxicology Program (NTP) Technical Report 494, NIH Publication No. 05-3953 because you believe the document contains "undocumented genetic toxicology data" and because "the conclusions presented in TR494 were accepted by NTP's peer review panel based upon untenable assertions concerning the non-mutagenicity of the TR494 test article." I will respond to concerns you raise about factual information in TR494.

The NTP does not agree that TR494 contains factual misrepresentations as proposed in the complaint. The Methods and Materials section for TR494 identifies the source of the anthraquinone used in the NTP 2-year studies as lot no. 5893. The NTP conducted follow-up genetic toxicology studies on a sample from lot no. 5893 as well as samples of anthraquinone produced by other processes. Appendix E, which contains the results from these follow-up studies, identifies the source of each sample noting that Sample A07496 is from lot no. 5893. Also, in response to Freedom of Information Act requests by CPC filed on March 28, 2006 and July 19, 2006, the NTP sent you records documenting shipment of lot no. 5893 to BioReliance Corporation for genetic toxicology testing and verifying Sample A07496 as an aliquot from lot no. 5893 (Enclosures 1 and 2, respectively). The results for Sample A07496 presented in Table E3 clearly show no mutagenicity in the preincubation mutagenicity assay in *Salmonella typhimurium* strains TA98, TA100, and TA1537 with or without S9 metabolic activation.

In the amended complaint of July 13, you question (1) the reporting in TR494 of purity for the anthraquinone used in the NTP studies (test article lot no. 5893) and in the genetic toxicology studies in Appendix E and (2) information about the purity analyses provided by Dr. Cynthia Smith at the 2004 meeting of the NTP Board of Scientific Counselors Technical Reports Review Subcommittee. With regard to the first issue, the amended complaint states that "the reported last analyzed purity (BCR, 11/19-11/20/98)" of lot no. 5893 anthraquinone noted on the Bulk Chemical Shipment Report for June 22, 2004 (Enclosure 1) "as 99.4% relative purity conflicts with the reported 99.8% purity of the test article in TR494." These measurements are not inconsistent, but represent results from different analytical approaches used by the NTP for determining purity, which I will describe briefly below. A summary of the purity analyses for the anthraquinone test article lot no. 5893 is provided in the Methods and Materials section of TR494 (page 27) and is explained in more detail in Appendix J of the report (pages 305-316).

As explained in Appendix J, the contract laboratories for these NTP studies conducted in-depth purity analyses using several methods at different times. Purity analysis of the anthraquinone test article lot no. 5893 by gas chromatography with flame ionization detection (GC-FID) was conducted in 1995 prior to initiation of the NTP studies by the study laboratory and again in 2002 by the analytical laboratory following their completion. The purity in each case was determined to be 99.9% using the "area %" approach.[1] It indicated a single impurity of 0.1%, tentatively identified (in 2002) as 9-nitroanthracene. In 2002, purity analysis of the anthraquinone test article lot no. 5893 was conducted by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) using the area % approach and purity was determined as 99.5%. Usually the NTP accepts differences in purity analysis by two techniques if they are within the limits of analytical variability, which for chromatographic methods is generally within a half percent; however, because of concern raised by you and others about the purity of the anthraquinone used in the TR494 studies, the NTP had additional analyses of lot no. 5893 performed in 2004 using the method of standard addition[2] to reconcile any differences in the purity values. The method of standard addition, which is the most accurate method for quantitative analyses of this type and whose results are independent of the analytical instrument used to generate the data, gave an absolute purity of lot no. 5893 for

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[1] The "area %" technique compares the percentage of anthraquinone in its analyte peak relative to the total sum of all peak areas in the chromatogram. The purity value reported is absolute, not relative, because no internal standard is used and no comparison is made to a reference sample.

[2] The method of standard addition measures the concentration of each impurity in the sample against an authentic standard solution of that substance. The NTP identified each impurity in the TR494 anthraquinone test article lot no. 5893, purchased an authentic sample of the purest available quality of each impurity, prepared standard solutions of it in the analytical concentration range found in the test article to construct a calibration curve, calibrated each analytical instrument for each authentic sample of each component of the anthraquinone test article lot no. 5893, and then assayed the test article by both GC-FID and HPLC-UV. The concentration of each impurity in the analyzed solution of test article was calculated from the area versus concentration of the standard calibration curve for its authentic sample.

anthraquinone by GC-FID and HPLC-UV as 99.85% and 99.83%, respectively, and 9-nitroanthrocene was quantitatively determined to be present at 0.09% and 0.11%, respectively; other impurities were present at 0.05% or less. As a further check to elucidate the difference between results obtained by GC-FID and HPLC-UV, the extinction coefficients of anthraquinone and the impurities were measured and differences were found that are believed to account for the lower area % purity value determined in 2002 by HPLC-UV versus GC-FID. The NTP reported these results at the December 2004 meeting of the NTP Board of Scientific Counselors Technical Reports Review Subcommittee.

In addition to the detailed purity analysis conducted by the analytical chemistry laboratory, over the course of the NTP studies the study laboratory conducted periodic checks of the purity of the anthraquinone test article lot no. 5893. This analysis, which could be done using the area % approach or the relative purity approach,[3] is used during the "in life" portion of studies to detect trends in purity over time. The relative purity approach is less precise than the area % approach, is only useful to detect trends, and is not a stand-alone method for determining absolute purity; differences of half a percent or so are not taken as a change in bulk purity unless successive analyses indicate a consistent trend. The purity measurement from 11/19-20/98 of 99.4% recorded on the Bulk Chemical Shipment Report is simply, as stated, the last recorded purity analysis for the test article at that time. The difference in the purity of 99.4% determined by GC-FID using the *relative purity approach* versus the 99.8% purity by GC-FID and HPLC-UV reported for the *method of standard addition* simply represents the inherent analytical variability of the methods used and varying rigor of the different approaches used to determine purity of lot no. 5893 and not changes in the condition of the bulk material.

With regard to the second issue, the amended July 13 complaint brings to our attention a comment made by Dr. Smith during her presentation to the NTP Board of Scientific Counselors Technical Reports Review Subcommittee on December 9, 2004.

> *Dr. Klaunig asked if the samples assayed were the original test material and if any degradation might have occurred during the interval. Dr. Smith replied that this was the same material used in the animal studies, and it was stored frozen under argon, so degradation was unlikely.*

In follow-up, it appears that Dr. Smith mistakenly replied that the sample assayed in the genetic toxicology testing was from the archived sample of anthraquinone lot no. 5893, which was maintained frozen, instead of the archived bulk material of lot no. 5893 from the NTP 2-year studies, which was stored at room temperature in an amber glass bottle.

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[3] The relative purity technique calculates the percentage of anthraquinone in its analyte peak as determined by GC-FID compared to a frozen reference sample of lot no. 5893 after correcting for mass of the material and the internal standard in the samples.

As you correctly point out, the Bulk Chemical Shipment Reports for sample A07496 of lot no. 5893 used in the genetic toxicology studies (June 22, 2004, Enclosure 1) and lot no. 5893 used in the NTP 2-year studies (February 2, 1999, Enclosure 3) show storage in amber glass bottles at room temperature (approximately 25^0C) as the recommended storage conditions. The Methods and Materials section of TR494 (page 27) also notes that the anthraquinone used in the 2-year NTP studies was "stored at room temperature, protected from light, in amber glass bottles with Teflon-lined caps."

The NTP appreciates you bringing this misstatement to our attention and we will amend the minutes for the December 2004 meeting and the information contained on page 20 of TR494 to correct Dr. Smith's response to Dr. Klaunig as noted below. The NTP will post the erratum on the NTP website both with the minutes and with electronic copies of TR494 and include it as an insert in all printed reports.

> *Dr. Klaunig asked if the samples assayed were the original test material and if any degradation might have occurred during the interval. Further examination of the shipment information for the sample from the 2-year bioassay sent to BioReliance Corporation for genetic toxicology testing in Salmonella showed that it was from archived bulk material. Following completion of the bioassay, this material was stored as received at room temperature (approximately 25^0C), protected from light and without inert gas headspace. Results from purity analysis of this material upon receipt, throughout the study, and at the end of the study showed no degradation.*

Finally, I would like to address an additional issue you raise in the amended July 17 complaint regarding storage of anthraquinone. You sent us further information relating to this issue on October 31, 2006, which we reviewed and considered. TR494 states the conditions for storage of the anthraquinone test article lot no. 5893, "[t]o ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles with Teflon-lined caps" (page 27). You are correct that three separate aliquots of lot no. 5893 were received by Battelle-Columbus Chemistry Support Service for the NTP and one of these (Chemical Storage Report: Anthraquinone 4-064-CS-41 February 2, 1999) is the aliquot from which the sample was taken and shipped to BioReliance Corporation for genetic toxicology testing (Enclosure 1). The purity analyses described above and in Appendix J of TR494 were all conducted on aliquots of the anthraquinone test article lot no. 5893 stored at room temperature. Each of these analyses conducted at different times over a 10-year period gave purity values for the anthraquinone test article lot no. 5893 that are in agreement and do not show evidence of degradation of the bulk test article: 1995: 99.9% by GC-FID using the area % approach, 2002: 99.9% by GC-FID using the area % approach; 2002: 99.5% by HPLC-UV using the area % approach, and 2004: 99.8% by both GC-FID and HPLC-UV using the method of standard of addition.

In conclusion, I believe that the information in TR494 is accurate to the best of our knowledge. We will amend the minutes for the December 2004 meeting and the information contained on page 20 of TR494 to correct Dr. Smith's response to Dr. Klaunig to note that the sample assayed for genetic toxicity was from the anthraquinone test article that had been stored at room temperature. The NTP will post this erratum on the NTP website both with the minutes and with electronic copies of TR494 and include it as an insert in all printed reports.

I would like to let you know that you may appeal our agency's decision either in writing or electronically within 30 days of receiving this response. Your request should state the reasons for your appeal. It does not need to reference tracking numbers. The request may be sent electronically to InfoQuality@od.nih.gov or in hard copy to the Associate Director for Communications, Office of the Director, National Institutes of Health, Building 1, Room 344, 9000 Rockville Pike, Bethesda, Maryland 20892. If the appeal is sent in hard copy, please clearly mark the appeal and outside envelop with the phrase "Information Quality Appeal."

Sincerely,

/s/

Allen Dearry, Ph.D.
Interim Associate Director

Enclosures