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January 5, 2007

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

Subject: **Request for Reconsideration** - Appeal of NTP's response to Chemical Products Corporation's May 31, 2006 Request for Correction of NTP Technical Report 494 - Dr. Allen Dearry response letter dated December 22, 2006.

Dear Madam or Sir;

Chemical Products Corporation (CPC) hereby appeals NTP's December 22, 2006 response to its May 31, 2006 Request for Correction of NTP Technical Report 494 with addenda dated July 13, 2006 and July 17, 2006 (submitted as records were provided to CPC by NIEHS in response to Freedom of Information Act requests). NTP's December 22, 2006 response did not provide CPC with a NIH tracking number, thus this Request for Reconsideration does not reference an NIH tracking number as stipulated in the HHS Information Quality Guidelines for Information Disseminated to the Public.

In its December 22, 2006 response to CPC, NTP states in the second paragraph on page 4, "**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."(emphasis added). In the last paragraph on the prior page (page 3), NTP's response states, "...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." (**Note:** Dr. Smith informed the Technical Reports Review Subcommittee that the aliquot of TR494 test article subjected to the June 2004 mutageinicity assay, upon which the present TR494 is based, had been "stored frozen under argon" during the 7-plus year interval between TR494 animal studies and the mutagenicity assay - thus making decomposition of mutagenic impurities during this period "unlikely"; NIEHS has produced no records in response to Freedom of Information Act requests to show that any portion of lot no. 5893 has been stored frozen under argon).

In spite of the above acknowledgment toward the end of its response that TR494 contains a critical misstatement, the second paragraph on page 1 of NTP's response begins with the incongruous statement, "The NTP does not agree that TR494 contains factual misrepresentations as proposed in the complaint."

We wish to call NTP's attention to the definition of "misrepresentation" in West's Encyclopedia of American Law, published by Thomson Gale, which states in part:

misrepresentation: An assertion or manifestation by words or conduct that is not in accord with the facts. A misrepresentation need not be intentionally false to create liability. A statement made with conscious ignorance or a reckless disregard for the truth can create liability. Nondisclosure of material or important facts by a fiduciary or an expert can result in liability.

Based upon the above legal definition of misrepresentation, CPC submits that Dr. Smith's incorrect characterization of the conditions under which the aliquot of the TR494 test article submitted for mutagenicity assay in June 2004 had been stored for the previous 7-plus years does, indeed, constitute misrepresentation.

CPC further submits that failure by NTP to disclose to the December 9, 2004 Technical Reports Review Subcommittee that a 1999 mutagenicity assay of an aliquot of the TR494 test article, employing NTP's preincubation protocol, determined that the TR494 test article was mutagenic to Salmonella typhimurium strains TA98 and TA100 without metabolic activation, and mutagenic to TA98 with S9 metabolic activation, also constitutes misrepresentation. A copy of the 1999 BioReliance Corporation preincubation mutagenicity assay report (9 pages) is included herewith as Enclosure 1. This assay was submitted to NTP Director Kenneth Olden in a letter dated August 24, 2000; it is referenced in CPC's November 15, 2002 Request for Correction of the 1999 TR494, on page 2, as follows:

“CPC learned that NTP had not actually tested the Anthraquinone sample employed in TR-494 for mutagenicity. CPC obtained a portion of the Anthraquinone retained sample from NTP and submitted it to a respected independent laboratory for mutagenicity testing along with three other samples of Anthraquinone.

Of four samples of Anthraquinone submitted for testing, only the NTP TR-494 sample was mutagenic in Salmonella typhimurium strains TA98 and TA100. CPC submitted this information to Dr. Kenneth Olden along with information about the contents of EPA’s TSCA file for Anthraquinone which describes a sample of Anthraquinone found to be mutagenic; this sample was purified to remove trace nitroanthracene contamination and was found not to be mutagenic on retesting. In

response to these submissions by CPC, Dr. Kenneth Olden acknowledged that the mutagenicity of pure Anthraquinone was in question."

NTP now acknowledges that pure Anthraquinone, CAS # 84-65-1, is not mutagenic to Salmonella typhimurium strains TA98 or TA100. NTP further acknowledges that the TR494 test article is contaminated with about 0.1% of the mutagen, 9-nitroanthracene, as a result of its manufacturing process. Mutagenicity assays of separate aliquots of the TR494 test article by CPC in 1999 and Butterworth et al. in 2000 both found that the TR494 test article was mutagenic to Salmonella typhimurium strains TA98 and TA100 without metabolic activation and was also mutagenic to strain TA98 with S9 metabolic activation.

NTP acknowledges in its December 22, 2006 response to CPC's Request for Correction that NTP's Dr. Smith provided incorrect information to the December 9, 2004 Technical Reports Review Subcommittee (the subcommittee). Dr. Smith stated incorrectly that the TR494 test article aliquot submitted for mutagenicity assay in June 2004 had been "stored frozen under argon" for the 7-plus year interval between the end of the TR494 animal studies and the June 2004 mutagenicity assay. Based upon this factual error, she characterized the possibility of decomposition of mutagenic impurities in that aliquot of the TR494 test article as "unlikely". This incorrect information was relied upon by the December 9, 2004 subcommittee and is incorporated into TR494 on page 20. CPC obtained records through Freedom of Information Act requests documenting that the TR494 aliquot submitted for mutagenicity assay in June 2004 had been stored at room temperature under air (in the absence of an inert atmosphere). No records have been produced to document the existence of an aliquot of the TR494 test article "stored frozen under argon".

NTP states in its response that the factual error concerning the conditions under which the aliquot submitted for mutagenicity assay in June 2004 had been stored will be corrected in TR494, but NTP's response fails to redress the effect this erroneous information had on the December 9, 2004 subcommittee's deliberations. This factual error supported Dr. Smith's assertion to the committee that decomposition of mutagenic impurities in the TR494 test article during the 7-plus year interval between the conduct of the TR494 animal studies and the June 2004 mutagenicity assay was “unlikely”. Thus, the factual error was a critical component of NTP's contention, accepted by the December 9, 2004 subcommittee, that the June 2004 negative mutagenicity assay conclusively demonstrated that mutagenic impurities in the TR494 test article did not confound the results of the TR494 studies conducted in the mid-1990's.

NTP did not disclose to the December 9, 2004 subcommittee that CPC had submitted a positive mutagenicity assay of an aliquot of the TR494 test article in a letter to NTP Director Kenneth Olden dated August 24, 2000; this assay was conducted by BioReliance Corporation using NTP's preincubation protocol. The December 9, 2004 subcommittee, acting on incorrect and incomplete information, vacated the restrictions placed upon the conclusions in TR494 by the February 18, 2004 Technical Reports Review Subcommittee.

CPC hereby appeals the denial of its request that TR494 be withdrawn because the December 9, 2004 peer review which accepted it was flawed. The December 9, 2004 subcommittee relied upon incorrect information to conclude that mutagenic impurities were unlikely to have decomposed during the 7-plus years that had lapsed between administration of the TR494 test article to animals and NTP's negative mutagenicity assay of the TR494 test article in June 2004. Further, the December 9, 2004 subcommittee was not informed of a positive mutagenicity assay of an aliquot of the TR494 test article conducted by BioReliance Corporation in 1999 employing NTP's preincubation protocol.

CPC submits that the December 9, 2004 subcommittee vacated the restriction placed upon the conclusions presented in TR494 by the February 18, 2004 NTP Board of Scientific Counselors Technical Reports Review Subcommittee as a result of it being presented with incorrect and incomplete information. Thus, CPC contends that the December 9, 2004 subcommittee adjudication regarding TR494 is invalid.

The February 18, 2004 NTP Board of Scientific Counselors Technical Reports Review Subcommittee considered NTP's argument that the TR94 studies had not been confounded by the presence of mutagenic impurities in the TR494 test article, even though the TR494 test article contains about 0.1% of the mutagen 9-nitroanthracene as a result of its manufacturing process - nitric acid oxidation of anthracene, and had been determined to be mutagenic to Salmonella typhimurium strains TA98 and TA100. The February 18, 2004 Technical Reports Review Subcommittee did not find NTP's arguments persuasive; it directed that a statement be incorporated into TR494 to unequivocally limit the conclusions presented in TR494 to anthraquinone produced by this single manufacturing process, that is, anthracene-derived anthraquinone. The TR494 approved by the February 18, 2004 Technical Reports Review Subcommittee also contained a discussion of the different manufacturing methods for the production of Anthraquinone: the Friedel-Crafts process and the Diels-Alder process, as well as the oxidation-of-anthracene process by which the TR494 test article was produced.

CPC did not resubmit the 1999 preincubation protocol mutagenicity assay of the TR494 test article in comments to the February 18, 2004 Technical Reports Review Subcommittee because, at that time, NTP conceded that the TR494 test article had been determined to be mutagenic in Salmonella typhimurium strains TA98 and TA100 without metabolic activation and

mutagenic in TA98 with S9 metabolic activation. NTP cited Butterworth et al. (Mutagensis, vol. 16, no. 2 [2001] pages 169-177) as the source for this information; the results of the Butterworth et al. plate incorporation protocol mutagenicity assay are consistent with the results of the CPC preincubation protocol mutagenicity assay, as shown below:

- 1999 BioReliance preincubation protocol assay results submitted to NTP on August 24, 2000 in a letter to NTP Director Kenneth Olden (a copy of the full 9-page BioReliance Corporation report is included herewith as Attachment 3).

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100[a]
0.0	19 ± 6	187 ± 5
10	16 ± 3	181 ± 23
25	20 ± 0	201 ± 37
50	31 ± 1	197 ± 9
100	27 ± 1	193 ± 29
250	61 ± 3	193 ± 25
500	108 ± 6	224 ± 26
1000	127 ± 4	267 ± 18
2500	225 ± 1	409 ± 11
Pos	626 ± 37	621 ± 5

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100
0.0	18 ± 7	143 ± 5
10	13 ± 5	148 ± 2
25	19 ± 2	139 ± 19
50	29 ± 3	139 ± 3
100	20 ± 2	118 ± 16
250	25 ± 8	115 ± 21
500	44 ± 1	115 ± 10
1000	52 ± 8	123 ± 8
2500	115 ± 21	144 ± 22
Pos	840 ± 95	455 ± 28

0.0 = Vehicle plating aliquot of 50 µL

Pos = Positive Control concentrations as specified in Materials and Methods section.
a = Data from Experiment B2

- Butterworth et al. (2001) plate incorporation protocol assay results

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100
0	18 ± 4	89 ± 9
30	20 ± 3	107 ± 14
60	25 ± 10	113 ± 15
125	42 ± 6	113 ± 18
250	62 ± 5	127 ± 18
500	116 ± 16	142 ± 4
1000	213 ± 29	131 ± 21
2000	433 ± 40	220 ± 6
pos.	193 ± 23	617 ± 6

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100
0	30 ± 8	149 ± 4
30	33 ± 4	140 ± 19
60	30 ± 9	138 ± 15
125	32 ± 4	134 ± 3
250	37 ± 4	127 ± 2
500	52 ± 5	130 ± 9
1000	102 ± 11	147 ± 21
2000	162 ± 13	164 ± 22
pos.	373 ± 17	534 ± 81

Thus, CPC saw no benefit in resubmission of the 1999 BioReliance Corporation preincubation mutagenicity assay to the February 18, 2004 Technical Reports Review Subcommittee.

A highly significant limitation was incorporated into TR494 by the February 18, 2004 Technical Reports Review Subcommittee: "The term anthraquinone used in this report refers to anthracene-derived anthraquinone." The title of TR494 was changed to reflect this limitation - "Anthraquinone" in

the title was replaced with “Anthracene-derived Anthraquinone”.

NTP misrepresented its purpose for including TR494 in the agenda for the NTP Board of Scientific Counselors Technical Reports Review Subcommittee meeting on December 9 and 10, 2004. The November 15, 2004 Federal Register Notice announcing the upcoming meeting (69FR12, page 65613 through 65615) states, “the TRR Subcommittee will readdress the title of the Draft NTP Technical Report on Anthraquinone...” and “5. Discussion on Contaminants in NTP Study Materials: Impact on Interpretation of 2-year Bioassays. • Discussion of the Title of Draft NTP Technical Report on Anthraquinone (TR-494).” These are the only references to Anthraquinone or TR494 in the Federal Register Notice announcing the December 9 and 10, 2004 Technical Reports Review Subcommittee meeting and agenda.

NTP presented new mutagenicity assay information generated in June 2004 to the December 9, 2004 subcommittee with the intent of persuading the subcommittee that the TR494 test article was not sufficiently contaminated with mutagenic impurities to have confounded the TR494 studies. Four samples of anthraquinone were shipped from NTP contractor Battelle to BioReliance Corporation for mutagenicity assay on June 2, 2004. These four samples: the TR94 test article produced by nitric acid oxidation of anthracene, two samples reportedly produced by the Diels-Alder manufacturing process, and one sample reportedly produced by the Friedel-Crafts Process, were labeled with lot numbers - the TR494 test article was labeled “Lot No. 5893” the others were labeled “Lot No. GSTU 2517770”, “Lot No. 64005”, and “Lot No. 2Y011”(the Bulk Chemical Shipment Report dated June 22, 2004 containing this information is included herewith as Enclosure 2). The samples were relabeled at BioReliance Corporation and, subsequently, mutagenicity assay results were provided to NTP for Samples A07496, A40147, A54984, and A65343 without reference to the lot numbers which had designated the samples when received

by BioReliance. One of these four samples was mutagenic, the other 3 were not. Documentation available from NTP associating a particular mutagenicity assay result to the TR494 test article, Lot No. 5893, can only be described as deficient. In response to Freedom of Information Act requests for records, NIEHS has produced only an email from an employee at BioReliance to Kristine Witt at NIEHS sent September 16, 2004 (after mutagenicity assay results, associated only with sample numbers, had been provided to NTP on September 3, 2004) saying that another individual at BioReliance had confirmed to him that assignment of sample numbers to the 4 Anthraquinone samples designated with Lot numbers had been done as Witt had described in her email to him dated September 10, 2004. A copy of this chain of emails is included herewith as Enclosure 3.

Had there been an accurate disclosure in the November 15, 2004 Federal Register Notice of NTP's intent to ask the December 9, 2004 subcommittee to make a determination that the TR494 animal studies were not confounded by mutagenic contaminants in the TR494 test article, CPC would have resubmitted the enclosed 1999 BioReliance Corporation mutagenicity assay of an aliquot of the TR494 test article conducted using NTP's preincubation protocol in comments prior to December 9, 2004. NTP suggested to the December 9, 2004 subcommittee that the June 2004 mutagenicity assay should be considered definitive, rather than the Butterworth et al. mutagenicity assay, because the June 2004 mutagenicity assay employed the preincubation protocol. The December 9, 2004 subcommittee was not made aware of the existence of a positive mutagenicity assay conducted using NTP's preincubation protocol, and was also not aware of the deficient chain of custody linking a particular June 2004 mutagenicity assay result to the TR494 test article.

Comparing the mutagenicity assay results of the anthraquinone sample determined to be mutagenic in the NTP 2004 assay with the results of the two

earlier mutagenicity assays of two separate aliquots of the TR494 test article at a dose of 1000 µg per plate (the highest identical dose rate found in all three studies) the following increases in revertants were observed:

	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
CPC	6.7X	2.9X	1.5X	0.9X
Butterworth	11.8X	3.4X	1.5X	1.0X
NTP 2004	8.3X	2.3X	1.0X[a]	1.3X

[a] trend line from 300 µg per plate reading of 0.9X and 3000 µg per plate reading of 1.0X was employed because the 1000 µg per plate reading of 0.5X is an anomaly.

These mutagenicity assay results for one of the 4 samples submitted to BioReliance Corporation in June 2004 are relatively consistent with the mutagenicity assay results on two separate aliquots of the TR494 test article conducted 4 years earlier - aliquots known to have been contaminated with about 0.1% 9-nitroanthracene as a result of its manufacturing process.

In CPC's experience, the color of Anthraquinone powder produced by the different manufacturing processes differs significantly. Of the 4 Anthraquinone samples shipped to BioReliance Corporation on June 2, 2004 by Battelle, we would expect only the TR494 test article to have a golden yellow color. The others would be expected to be described as tan, grey, or brownish.

According to BioReliance Corporation, the physical description of materials it receives for mutagenic assay is routinely recorded twice - once on the sample receipt documentation and again with the raw mutagenicity assay results. Because the TR494 test article should have been observed to be yellow or golden yellow and the other 3 anthraquinone samples should have been observed to be tan, tan-grey, light brown, reddish-brown, or buff in color, the physical descriptions of the 4 Anthraquinone samples recorded by BioReliance Corporation upon sample receipt and again with the mutagenicity assay results should allow definitive identification of one of the mutagenicity assay results as belonging to the TR494 test article (**Note:** the physical

description is provided on the second page of the 1999 BioReliance Corporation mutagenicity assay report enclosed with this letter - "golden yellow powder" - samples of Anthraquinone manufactured by the Friedel-Crafts process submitted to BioReliance at the same time were described as "tan powder").

NIEHS has been unwilling or unable to give CPC access to the BioReliance Corporation physical descriptions of the four Anthraquinone samples subjected to mutagenicity assay in June 2004; a Freedom of Information Act appeal has been submitted by CPC and a copy of this Freedom of Information Act appeal is included herewith as Enclosure 4.

In accordance with the HHS Guidelines for Ensuring the Quality of Information Disseminated to the Public posted at http://aspe.hhs.gov/infoquality/Guidelines/NIHinfo2.shtml#vi1, CPC submits the following information:

- 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 website and web page address (URL), or the presentation, presenter, date and mode of delivery;
  NTP TECHNICAL REPORT ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF ANTHRAQUINONE (CAS NO. 84-65-1) IN F344/N RATS AND B6C3F1 MICE (FEED STUDIES)
  Technical Report 494 dated September 2005
  NIH Publication No. 05-3953

- 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;
  CPC has provided documentation to NTP as detailed above that the December 9, 2004 Technical Reports Review Subcommittee vacated the restrictions placed upon the conclusions presented in TR494 by the February 18, 2004 Technical Reports Review Subcommittee based upon factual errors and incomplete information (including nondisclosure of a highly relevant preincubation mutagenicity assay of the TR494 test article), and in the absence of the requisite public

review and opportunity to provide comments (a result of misrepresentation of NTP's intent with regard to TR494 in the Federal Register Notice announcing the agenda for the December 9 and 10, 2004 Technical Reports Review Subcommittee meeting).

- Suggested recommendations for what corrective action(s) should be taken;

  CPC requests that TR494 be withdrawn and be rewritten to comply with the directives of the February 18, 2004 Technical Reports Review Subcommittee.

- A description of how the person requesting the correction is affected by the information error;

  Through its wholly-owned subsidiary, CPT Pulp and Paper, LLC, CPC markets Anthraquinone aqueous suspensions to the United States paper industry for use as a catalyst in the kraft pulping process to increase the amount of paper pulp produced from a given weight of wood chips. None of the Anthraquinone sold by CPC is anthracene-derived Anthraquinone. The Anthraquinone sold by CPC does not contain mutagenic impurities. Incorrectly identifying Anthraquinone free of mutagenic impurities as a substance which causes cancer in rats and mice, as was done at the December 9, 2004 Technical Reports Review Subcommittee meeting, is believed by CPC to adversely effect use of Anthraquinone in the United States paper industry, even though Anthraquinone is not a component of the paper pulp produced by the Kraft Process employing Anthraquinone as a catalyst.

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

  Contact Information at Chemical Products Corporation:

  - Jerry A. Cook, Technical Director
  - Chemical Products Corporation
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  - P.O. Box 2470
  - Cartersville, GA 30120-1692
  - Telephone 770-382-2144 Ext. 272
  - email - jcook@cpc-us.com

In summary, Chemical Products Corporation once again requests that TR494 be withdrawn because of serious inadequacies in NTP's presentation to the December 9, 2004 Technical Reports Review Subcommittee meeting which vacated restrictions on the conclusions in TR494 adjudicated by the February 18, 2004 Technical Reports Review Subcommittee. CPC respectfully submits that it has conclusively demonstrated the invalidity of the December 9, 2004 Technical Reports Review Subcommittee's actions in vacating the determinations of the February 18, 2004 Technical Reports Review Subcommittee.

As summarized in the first paragraph of NTP's December 22, 2006 response letter, CPC's Request of Correction specifically asked for withdrawal of the NTP Technical Report 494, NIH Publication No. 05-3953 because the conclusions presented in TR494 were accepted by NTP's December 9, 2004 peer review panel based upon untenable assertions concerning NTP's demonstration of the non-mutagenicity of the TR494 test article.

Sincerely,

/s/

Jerry A. Cook
Technical Director

Enclosures:

1. 1999 BioReliance Corporation preincubation mutagenicity assay report
   9 pages
2. Bulk Chemical Shipment Report dated June 22, 2004
   1 page
3. emails to and from Kristine Witt at NIEHS
   3 pages
4. Freedom of Information Act appeal
   7 pages

Enclosure 1

9 pages

FINAL REPORT

Study Title

***Salmonella* Preincubation Mutagenicity Assay**

Test Article

Quinone 1

Sponsor Project Number

5893

Authors

Valentine O. Wagner, III, M.S.
Sean M. Caruthers, B.S.

Study Completion Date

December 22, 1999

Performing Laboratory

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

Laboratory Study Number

AA22JJ.501004.BTL

Sponsor

Chemical Products Corporation
102 Old Mill Road SE
Cartersville, GA 30120

Salmonella Preincubation Mutagenicity Assay

FINAL REPORT

Sponsor:	**Chemical Products Corporation** **102 Old Mill Road SE** **Cartersville, GA 30120**
Authorized Representative:	**Mr. Jerry Cook**
Performing Laboratory:	**BioReliance** **9630 Medical Center Drive** **Rockville, Maryland 20850**
Test Article I.D.:	**Quinone 1**
Sponsor Project No.:	**5893**
BioReliance Study No.:	**AA22JJ.501004.BTL**
Test Article Description:	**golden yellow powder**
Storage Conditions:	**room temperature; protected from exposure to light and moisture**
Test Article Receipt:	**05 October 99**
Study Initiation:	**20 October 99**

Study Director: _____ 22 Dec 1999

Valentine O. Wagner, III, M.S. Date

EXPERIMENTAL DESIGN AND METHODOLOGY

Test System

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98 and TA100 as described by Ames *et al.* (1975). Tester strain TA98 is reverted from auxotrophy to prototrophy by frameshift mutagens. Tester strain TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations.

Experimental Design

The test system was exposed to the test article via the preincubation methodology described by Yahagi *et* al. (1977). The test article was tested at a minimum of eight dose levels along with appropriate vehicle and positive controls with tester strains TA98 and TA100 with and without S9 activation. All dose levels of test article, vehicle controls and positive controls were plated in duplicate.

Plating and Scoring Procedures

In the preincubation method, one-half (0.5) milliliter of S9 or sham mix, 100 μL of tester strain and 50 μL of vehicle or test article were added to 13 X 100 mm glass culture tubes pre-heated to 37$\pm$2°C. After vortexing, these mixtures were incubated with shaking for 60$\pm$2 minutes at 37$\pm$2°C. Following the preincubation, 2.0 mL of selective top agar was added to each tube and the mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test article aliquot was replaced by a 50 μL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37$\pm$2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity. Plates with sufficient test article precipitate to interfere with automated colony counting were counted manually.

Evaluation of Results

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of either one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA98 and TA100 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean vehicle control value.

RESULTS AND DISCUSSION

Solubility

Dimethyl sulfoxide was selected as the solvent of choice based on the Sponsor's request and compatibility with the target cells. Concentrations from 0.20 to 50 mg/mL were delivered to the test system as workable suspensions.

Mutagenicity Assay

The results of the mutagenicity assay are presented in Tables 1 through 4 and summarized in Table 5. These data were generated in Experiments B1 and B2. Precipitate was observed at ≥ 250 μg per plate. No appreciable toxicity was observed.

In Experiment B1, positive responses were observed with tester strain TA98 in the presence (6.4-fold, maximum increase) and absence (11.8-fold, maximum increase) of S9 activation. No positive response was observed with tester strain TA100 in the presence of S9 activation. Due to an unacceptable positive control value, tester strain TA100 in the absence of S9 activation was not evaluated but was retested in Experiment B2.

In Experiment B2, a positive response was observed with tester strain TA100 in the absence (2.2-fold, maximum increase) of S9 activation.

CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the *Salmonella* Preincubation Mutagenicity Assay indicate that under the conditions of this study, **Quinone 1** did cause positive responses with tester strains TA98 and TA100 in the absence of S9 activation and with tester strain TA98 in the presence of Aroclor-induced rat liver S9.

Salmonella Mutagenicity Assay

Table 1

Test Article Id : Quinone 1
Study Number : AA22JJ.501004.BTL
Experiment No : B1
Strain : TA98
Cells Seeded : 2.6 X 10^8
Liver Microsomes : None
Date Plated : 10/26/99
Vehicle : dimethyl sulfoxide (DMSO)
Plating Aliquot : 50 µL
Counted by : hand

Concentration µg per plate	Plate Number	Revertants per plate	Background Code[a]	Average Revertants	Standard Deviation
Vehicle	01	23	1		
	02	14	1	19	6
10	01	18	1		
	02	14	1	16	3
25	01	20	1		
	02	20	1	20	0
50	01	30	1		
	02	32	1	31	1
100	01	28	1		
	02	26	1	27	1
250	01	63	1NP		
	02	59	1NP	61	3
500	01	104	1NP		
	02	112	1NP	108	6
1000	01	130	1NP		
	02	124	1NP	127	4
2500	01	224	1NP		
	02	225	1NP	225	1
Positive Control 2-nitrofluorene 1.0 µg per plate[b]					
	01	652	1		
	02	599	1	626	37

[a]Background bacterial evaluation code
1=Normal 2=Slightly reduced 3=Moderately reduced
4=Extremely reduced 5=Absent 6=Obscured by precipitate
NP=Non-Interfering Precipitate IP=Interfering Precipitate
[b]Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 2

Test Article Id : Quinone 1
Study Number : AA22JJ.501004.BTL Experiment No : B1
Strain : TA98 Cells Seeded : 2.6 X 10^8
Liver Microsomes : Rat liver S9 Date Plated : 10/26/99
Vehicle : dimethyl sulfoxide (DMSO)
Plating Aliquot : 50 µL Counted by : hand

Concentration µg per plate	Plate Number	Revertants per plate	Background Code[a]	Average Revertants	Standard Deviation
Vehicle	01	13	1		
	02	23	1	18	7
10	01	9	1		
	02	16	1	13	5
25	01	20	1		
	02	17	1	19	2
50	01	27	1		
	02	31	1	29	3
100	01	18	1		
	02	21	1	20	2
250	01	31	1NP		
	02	19	1NP	25	8
500	01	44	1NP		
	02	43	1NP	44	1
1000	01	46	1NP		
	02	57	1NP	52	8
2500	01	130	1NP		
	02	100	1NP	115	21
Positive Control 2-aminoanthracene 1.0 µg per plate[b]					
	01	907	1		
	02	773	1	840	95

[a]Background bacterial evaluation code
1=Normal 2=Slightly reduced 3=Moderately reduced
4=Extremely reduced 5=Absent 6=Obscured by precipitate
NP=Non-Interfering Precipitate IP=Interfering Precipitate
[b]Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 3

Test Article Id	: Quinone 1		
Study Number	: AA22JJ.501004.BTL	Experiment No	: B2
Strain	: TA100	Cells Seeded	: 0.8 X 10^8
Liver Microsomes	: None	Date Plated	: 11/23/99
Vehicle	: dimethyl sulfoxide (DMSO)		
Plating Aliquot	: 50 µL	Counted by	: machine

Concentration µg per plate	Plate Number	Revertants per plate	Background Code[a]	Average Revertants	Standard Deviation
Vehicle	01	190	1		
	02	183	1	187	5
10	01	197	1		
	02	165	1	181	23
25	01	175	1		
	02	227	1	201	37
50	01	190	1		
	02	203	1	197	9
100	01	213	1		
	02	172	1	193	29
250	01	211	1NP		
	02	175	1NP	193	25
500	01	242	1NP		
	02	205	1NP	224	26
1000	01	254	1NP		
	02	279	1NP	267	18
2500	01	417	1NP		
	02	401	1NP	409	11
Positive Control sodium azide 1.0 µg per plate					
	01	617	1		
	02	624	1	621	5

[a]Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
4=Extremely reduced 5=Absent 6=Obscured by precipitate
NP=Non-Interfering Precipitate IP=Interfering Precipitate

Salmonella Mutagenicity Assay

Table 4

Test Article Id : Quinone 1
Study Number : AA22JJ.501004.BTL Experiment No : B1
Strain : TA100 Cells Seeded : 0.9 X 10^8
Liver Microsomes : Rat liver S9 Date Plated : 10/26/99
Vehicle : dimethyl sulfoxide (DMSO)
Plating Aliquot : 50 µL Counted by : machine

Concentration µg per plate	Plate Number	Revertants per plate	Background Code[a]	Average Revertants	Standard Deviation
Vehicle	01	146	1		
	02	139	1	143	5
10	01	149	1		
	02	146	1	148	2
25	01	125	1		
	02	152	1	139	19
50	01	137	1		
	02	141	1	139	3
100	01	129	1		
	02	106	1	118	16
250	01	129	1NP		
	02	100	1NP	115	21
500	01	122	1NP		
	02	108	1NP	115	10
1000	01	117	1NP		
	02	129	1NP	123	8
2500	01	159	1NP		
	02	128	1NP	144	22
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	435	1		
	02	474	1	455	28

[a]Background bacterial evaluation code
1=Normal 2=Slightly reduced 3=Moderately reduced
4=Extremely reduced 5=Absent 6=Obscured by precipitate
NP=Non-Interfering Precipitate IP=Interfering Precipitate

Salmonella Mutagenicity Assay
Summary of Results

Table 5

Test Article Id : Quinone 1
Study Number : AA22JJ.501004.BTL Experiment Nos: B1/B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100[a]
0.0	19 ± 6	187 ± 5
10	16 ± 3	181 ± 23
25	20 ± 0	201 ± 37
50	31 ± 1	197 ± 9
100	27 ± 1	193 ± 29
250	61 ± 3	193 ± 25
500	108 ± 6	224 ± 26
1000	127 ± 4	267 ± 18
2500	225 ± 1	409 ± 11
Pos	626 ± 37	621 ± 5

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100
0.0	18 ± 7	143 ± 5
10	13 ± 5	148 ± 2
25	19 ± 2	139 ± 19
50	29 ± 3	139 ± 3
100	20 ± 2	118 ± 16
250	25 ± 8	115 ± 21
500	44 ± 1	115 ± 10
1000	52 ± 8	123 ± 8
2500	115 ± 21	144 ± 22
Pos	840 ± 95	455 ± 28

0.0 = Vehicle plating aliquot of 50 μL
Pos = Positive Control concentrations as specified in Materials and Methods section.
a = Data from Experiment B2

Enclosure 2

1 page

Battelle
The Business of Innovation

BATTELLE-SHIP

Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-CCR
NTP ChemTask No.: CHEM08048
CAS No.: 84-65-1

BULK CHEMICAL SHIPMENT REPORT

ANTHRAQUINONE

9-064-SHIP-379

June 22, 2004

CAS No.: 84-65-1	Amount Shipped: ~2 g of each lot into one 15-mL amber glass bottle each, shipped at ambient temperature
Battelle Task No.: 9-064-SHIP-379	Shipping Date: 6/1/04
NTP ChemTask No.: CHEM08048	Chemical Lot No's.: GSTU 2517770, 64005, 2Y011, & 5893
Program Supported: GTX	Last Analyzed Purity: Lot No. GSTU 2517770 (BCLA, 2/25-5/22/03): ~99.9% Lot No. 64005 (BCLA, 2/25-5/22/03): ~99.8% Lot No. 2Y011 (BCLA, 2/24-5/22/03): ~99.4% Lot No. 5893 (BCR, 11/19-11/20/98): 99.4% relative purity
Shipped To: [redacted] BioReliance Coordinator, Chemical Repository 9630 Medical Center Drive Rockville, MD 20850	Recommended Storage Conditions: Room temperature (~25°C)

This report was prepared by [redacted] and reviewed for accuracy by Melissa Cloud.

Approved By:

For **Donna B. Browning, B.S.**
Task Leader

Approved By:

[redacted]
Discipline Leader, Data Management

Submitted to:

Dr. Cynthia S. Smith
National Institute of Environmental Health Sciences
Mail Drop: EC-06
4401 Commons Building, Suite 100
Research Triangle Park, NC 27709

Enclosure 3

3 pages

Subject: RE: question about aliquot number assignment
Date: Thursday, September 16, 2004 2:26 PM
From: San, Richard <RSan@bioreliance.com>
To: "Witt, Kristine (NIH/NIEHS)" <witt@niehs.nih.gov>
Conversation: question about aliquot number assignment

Hello, Kritine,

[Non-Key Employee] has confirmed that the test article aliquot number assigements are accurate as presented. Also, from a review of the study files, it is noted that we have a Material Safety Data Sheet only for A40147 from Kawasaki Kasei Chemical LTD.

Regards,

Richard

-----Original Message-----
From: Witt, Kristine (NIH/NIEHS) [mailto:witt@niehs.nih.gov]
Sent: Wednesday, September 15, 2004 3:18 PM
To: San, Richard
Subject: RE: question about aliquot number assignment

Hi, Richard.

Have you received word from [Non-Key Employee] about the test article aliquot number assignments? We are having a meeting here Friday morning to review all the new data we've acquired on anthraquinone and try to understand what it means in terms of biological activity for some important commercial compounds. These data are key to that discussion, but without confirmation of the test article identities I'm uncertain as to what the results are telling us. Can you please let [N-Key Emp] know that the need for the information you requested is urgent?

Thanks,
Kristine.

> ----------
> From: San, Richard
> Sent: Friday, September 10, 2004 4:44 PM
> To: Witt, Kristine (NIH/NIEHS)
> Subject: RE: question about aliquot number assignment
>
> Hello, Kristine,

>
> Thanks for your e-mail. I have asked [Non - Key Employee]
> [ ]; who has custody of the test article related documents, to
> confirm the aliquot assignments. As soon as I hear from him, I will
> let you know.
>
> Best regards,
>
> Richard
>
> -----Original Message-----
> From: Witt, Kristine (NIH/NIEHS) [mailto:witt@niehs.nih.gov]
> Sent: Friday, September 10, 2004 9:43 AM
> To: San, Richard
> Subject: FW: question about aliquot number assignment
>
>
> Hello, Richard.
>
> Thank you for sending the preliminary results for the 4 Salmonella
> tests that were recently conducted at BioReliance. The results were
> surprising to me, and therefore, I need to make sure that your aliquot

> assignment matches ours. Can you please confirm that the aliquot
> numbers match the chemical samples described below? If there is a
> discrepancy, please send me your list, matching aliquot with test
> sample.
>
> Regarding the issue of money for the no-cost extension, our contract
> officer is aware of the problem and he is considering an approach to
> resolving the problem.
>
> Thanks for your help in understanding these test results.
>
> Best regards,
> Kristine.
>
> > 4 aliquot numbers were assigned to 4 different samples of
> > anthraquinone. They were:
> >
> > A07496 1. Anthraquinone, Lot #5893. This is from Zeneca Fine
> > Chemicals. The Nitric Acid Oxidation manufacturing process (78.82
> > kg).
> >

> > A40147 2. 9,10-Anthraquinone, Lot #2Y011. This is from
> Kawasaki
> > Kasei Chemical LTD. The Diels-Alder manufacturing process (13.40
> > kg).
> >
> > A65343 3. 9,10-Anthraquinone, Lot #64005. This is from
> > Environmental Biocontrol Intl. The Diels-Alder manufacturing
> > process.
> > (23.20 g).
> >
> > A54984 4. 9,10-Anthraquinone, Lot # GSTU 2517770. This is
> from
> > Environmental Biocontrol Intl. The Friedel-Crafts manufacturing
> > process. (26.70 g).
> >
> > Kristine L. Witt
> > Toxicology Operations Branch
> > Environmental Toxicology Program
> > National Institute of Environmental Health Sciences
> > PO Box 12233, MD EC-32
> > Research Triangle Park, NC 27709
> > phone: 919-541-2761
> > fax: 919-316-4511
> > e-mail: witt@niehs.nih.gov
> >
> >
>
>

Enclosure 4

7 pages

DEPARTMENT OF HEALTH & HUMAN SERVICES Program Support Center

Public Health Service
Freedom of Information Office
Parklawn Building, Room 17-A-46
5600 Fishers Lane
Rockville, MD 20857
PH: 301-443-5252
Fax: 301-443-0925

November 15, 2006

JERRY A. COOK
CHEMICAL PRODUCTS CORPORATION
102 OLD MILL ROAD SE, PO BOX 2470
CARTERSVILLE, GEORGIA 30120

Dear JERRY A. COOK:

This is to acknowledge receipt of your administrative appeal dated 11/7/2006.

Any questions regarding the status of your appeal should be directed to the Public Health Services (PHS) Freedom of Information (FOI) office.

Your appeal has been assigned the following number PHS-2K7-A-029.

Please reference this number on your correspondence.

Sincerely,

PHS Freedom of Information Office

Chemical Products Corporation

102 Old Mill Road SE
P.O. Box 2470
Cartersville, Georgia
30120-1692

Phone: 770-382-2144
Fax: 770-386-6053
e-mail: jcook@cpc-us.com

November 7, 2006

Assistant Secretary for Public Affairs (Media)
Department of Health and Human Services
Room 17A-46, Parklawn Building
5600 Fishers Lane
Rockville, Maryland 20857

Re: FOIA Appeal – FOI Case Nos. 33011 and 33182

Dear Madam or Sir;

Pursuant to 45 CFR Subtitle A, Part 5, Subpart C, Chemical Products Corporation (CPC) hereby appeals the responses it received for (1) FOI Case No. 33011 dated October 11, 2006 and (2) FOI Case No. 33182 dated November 2, 2006. Copies of both of these FOI response letters are enclosed with this letter.

Purpose of the Information

Records obtained through FOI requests have allowed CPC to determine that National Toxicology Program Technical Report 494 (TR494) does not meet the information quality standards required by the NIH Information Quality Guidelines . A significant factual error (critical to the acceptance of the conclusions in TR494 by the December 9. 2004 Technical Reports Review Subcommittee) has been documented. CPC has submitted a Request for Correction of TR494 to NIH and is awaiting a response. The FOI cases being appealed in this letter relate to four samples of Anthraquinone, CAS # 84-65-1, which are the focus of CPC's Request for Correction of TR494. Only by obtaining, and carefully reviewing, the complete records relating to the mutagenicity assay of these four samples performed by NTP contractor BioReliance Laboratories in June and July 2004 can CPC and NIH determine that TR494 does not contain additional significant factual errors.

Background

CPC has requested the records generated by NTP contractor BioReliance Laboratories relating to the Ames mutagenicity assays conducted on the 4 Anthraquinone samples shipped to BioReliance Laboratories by NTP contractor Battelle Columbus Labs on June 2, 2004. These samples were assayed by BioReliance Laboratories in June and July 2004. The samples were shipped from Battelle labeled as lot numbers 64005, "GSTU 2517770", 2Y011, and 5893 according to the Bulk Chemical Shipment report obtained by CPC through a prior FOI request. Mutagenicity assay results were reported by BioReliance Laboratories for samples A07496, A65343, A54984, and A40147.

As a result of FOI Case No. 32925, CPC obtained copies of email correspondence between Richard San at BioReliance and Kristine Witt at NIEHS. These emails demonstrate that uncertainty existed about how the samples designated by lot numbers when shipped to BioReliance were relabeled with sample numbers – Kristine Witt of NIEHS emailed Richard San at BioReliance on September 15, 2004, "Have you received word from [Non-Key Employee] about the test article aliquot number assignments?" CPC has tried unsuccessfully to obtain records to confirm sample number assignments for the lot numbered samples received from Battelle.

One of the ways we hope to confirm the identity of the samples received by BioReliance Laboratories labeled with lot numbers and then relabeled with different sample numbers is through an examination of the physical description of the samples when received and, subsequently, when tested. Anthraquinone powders produced by different processes do not necessarily have the same appearance. An inquiry by CPC to BioReliance Laboratories yielded the following information, "generally the physical description is recorded in the raw data on the dose formulation page and on the test article receipt documentation." and also, "The physical description should be in the report and raw data. It should be in the info NIEHS has on file."

An email from Richard San to Kristine Witt at NIEHS states, "As soon as the entry errors and typographical errors (noted in the attached files) are corrected, the

data will be submitted on diskettes to Miriam Gattis." CPC has not been able to obtain the data submitted on diskettes to Miriam Gattis, which would presumably contain physical descriptions of the samples in the reports and in the raw data, through FOI Case No. 33182; the attached response states, "The procedures for submitting information/data changed and the data were entered directly into a database; therefore, there are no responsive records."

Basis for Appeal

Contrary to the above statement in the final response to FOI Case No. 33182, an email from Richard San at BioReliance to Katherine Witt at NIEHS obtained under FOI Case No. 32925 states, "the data will be submitted on diskettes to Miriam Gattis." We request a copy of the data submitted on diskette to Miriam Gattis because information obtained from BioReliance Laboratories leads us to believe that this data may include test article receipt documentation containing physical descriptions of the samples associated with lot numbers, and may also include raw data in the dose formulation section including physical descriptions of the samples associated with assigned sample numbers.

We appeal the rejection of our request under FOI Case No. 33011 for access to the records stored at BioReliance Laboratories relating to the four Anthraquinone samples described above. The NIEHS final response states, "The FOIA pertains only to records within the government's possession or control and is not a mechanism for requesting non-governmental third parties to provide records." We submit that the records maintained by NTP contractor BioReliance Laboratories are under NIEHS control and are, therefore, subject to the FOIA. Because the records in question are the property of NTP, BioReliance Laboratories will not release them without written authorization from NTP. FOI Case No. 33011 did not ask the government to "request non-governmental third parties to provide records"; rather, FOI Case No. 33011 asks NTP to authorize its contractor BioReliance to release a copy of specific records in its possession belonging to NTP.

In the absence of the data submitted by BioReliance on diskettes to Miriam Gattis, we restate our request that NIEHS authorize and instruct BioReliance Corporation to provide Chemical Products Corporation a copy of all records in its possession related to the 4 Anthraquinone samples designated first as lot numbers 64005, “GSTU 2517770”, 2Y011, and 5893; and later as sample numbers A07496, A65343, A54984, and A40147. Chemical Products Corporation agrees to pay any fees requested by BioReliance for this service.

Thank you for your attention to this matter. If I can answer any questions concerning this request, please telephone me at 770-382-2144 Ext. 272 or 770-714-3806 (cell), or email me at jcook@cpc-us.com.

Sincerely,

/s/

Jerry A. Cook, Technical Director
Chemical Products Corporation

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Phone: 919-541-3411
Fax: 919-541-4395
E-mail: minneman@niehs.nih.gov

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233, MD NH-10
Research Triangle Park, NC 27709-2233

October 11, 2006

Mr. Jerry A. Cook
Technical Director
Chemical Products Corp.
P.O. Box 2470
Cartersville, GA 30120-1692

RE: FOI Case No. 33011

Dear Mr. Cook:

This is a final response to your August 17, 2006, Freedom of Information Act (FOIA) request addressed to me and received in my office on August 23. You requested a copy of the analytical test report(s) and any other communications related to the 4 samples of Anthraquinone, CAS #84-65-1, shipped to BioReliance Corporation on 6/1/04 by NTP contractor Battelle and described by Battelle on the attached Bulk Chemical Shipment report as Lot numbers: GSTU 2517770, 64005, 2Y011, and 5893. Our records indicate that you previously requested these records from NIEHS (FOI Case No. 32925). Our final response in that case was sent to you by letter dated October 10, 2006.

You also requested that NIEHS authorize and instruct BioReliance Corporation to provide Chemical Products Corporation a copy of all records in its possession related to these 4 Anthraquinone samples. The FOIA pertains only to records within the government's possession or control and is not a mechanism for requesting non-governmental third parties to provide records.

Sincerely,

Kim L. Minneman
Freedom of Information Coordinator

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Phone: 919-541-3411
Fax: 919-541-4395
E-mail: minneman@niehs.nih.gov

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233, MD NH-10
Research Triangle Park, NC 27709-2233

November 2, 2006

Mr. Jerry A. Cook
Technical Director
Chemical Products Corporation
P.O. Box 2470
Cartersville, Georgia 30120-1692

RE: FOI Case No. 33182

Dear Mr. Cook:

This is a final response to your October 12, 2006, Freedom of Information Act (FOIA) request addressed to me. In follow-up to FOIA Case No. 32925, you requested a copy of the Test Article Description, which is perhaps shown on the dose formulation page and test article receipt documentation.

The National Toxicology Program (NTP) searched its files and no records responsive to your request for the "Test Article Description" were located. The procedures for submitting information/data changed and the data were entered directly into a database; therefore, there are no responsive records.

While we believe that an adequate search of appropriate files was conducted for the records you requested, you have the right to appeal this determination that no records exist that would be responsive to your request. Should you wish to do so, you must send your appeal within 30 days of receipt of this letter to the Assistant Secretary for Public Affairs (Media), Department of Health and Human Services, Room 17A-46, Parklawn Building, 5600 Fishers Lane, Rockville, Maryland 20857, following the procedures outlined in Subpart C of the enclosed regulations, 45 C.F.R. Part 5. Please mark both the envelope and the appeal letter "FOIA Appeal."

Provisions of the FOIA and Department of Health and Human Services FOIA Regulations allow us to recover part of the cost of responding to your request. Because the cost is below the $25 minimum, there is no charge.

Sincerely,

Kim L. Minneman
Freedom of Information Coordinator

Enclosure:
FOIA Regulations