NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS' MEETING
JULY 26, 1984

Peer Review of the Data from the Chronic Carcinogenesis Animal Bioassay of D & C Red No. 33 By the Technical Reports Review Subcommittee and Panel of Experts

Summary Minutes

The National Toxicology Program (NTP) Board of Scientific Counselors Technical Reports Review Subcommittee and ad hoc Panel of Experts (the Panel) met at 10:30 a.m. on July 26, 1984, in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. This open meeting was held at the request of the Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA), for the purpose of providing independent peer review of the data from the chronic carcinogenesis bioassay of D & C Red No. 33 in Charles River CD-1 mice and Charles River Sprague-Dawley rats. The bioassay was sponsored by the Pharmaceutical Manufacturers' Association (PMA), conducted by the International Research and Development Corporation (IRDC), and submitted to the FDA in support of permanent listing of D & C Red No. 33 (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda). In advance of the meeting, the peer reviewers were provided by the CFSAN with a data package consisting of an introduction, materials and methods, Division of Pathology reports, results, conclusions, references, and appendices. Also in advance of the meeting, the Panel was supplied with a package from the PMA that included written critiques of the data by their expert consultants plus supporting material. Dr. Curtis Harper, member of the Subcommittee, chaired the meeting. Peer reviewers are listed in Attachment 2.

Dr. Harper read the charge to the Peer Review Panel as stated in a letter from Dr. Sanford Miller, Director, CFSAN, to Dr. David P. Rall, Director, NTP. The guidance of the Panel was sought to answer two questions: (1) "Do the results of the long-term feeding studies of D & C Red No. 33 in CD-1 (Charles River) mice and Sprague-Dawley (Charles River) rats indicate a possible carcinogenic effect that could be attributed to exposure to this color additive?; and (2) In particular, do the splenic changes in rats constitute evidence of neoplastic potential?" Dr. W. G. Flamm, FDA said, the FDA has the responsibility within the meaning of the Federal Food, Drug and Cosmetic Act to establish the safety of Red No. 33. Safety within the meaning of the Act is reasonable certainty of no harm. He asked the Panel to give their opinion as to whether the burden has been met establishing safety, or, whether the evidence suggests a potential for induction of neoplasia. He also asked the Panel to consider the possible need for further testing. He stated there were two reasons for concern despite the fact that the splenic lesions were not statistically significant, either by pair-wise comparisions or by dose-related trend analyses. First, the lesions are very rare in Sprague-Dawley rats. Secondly, comparisions made between Sprague-Dawley rats and Fischer 344 rats have indicated the latter species was much more sensitive to induction of splenic neoplasms by other aromatic amines.

Toxicology Review: Dr. Benjamin Jackson, CFSAN, presented an overview of the chemistry and toxicology of D & C Red No. 33 (Red No. 33) (Attachment 3). Included were summaries of toxicity results in acute, prechronic and two-year feeding studies in rats and dogs. The only consistent findings across studies were anemia and splenic changes such as enlargement, congestion and discoloration. In three generation rat reproduction studies and evaluations for teratologic effects in rats and rabbits, there were no effects that could be attrbuted to treatment. In genotoxicity testing, no effects were observed in the Salmonella plate test and in Drosophila while in E. coli, Red No. 33 gave a positive result directly but not after metabolic activation. Dr. Jackson described the most recent long-term feeding studies in rodents (control and 2.0% in rats; control, 0.1, 1.0, and 5.0% in mice). The only findings of note other than reduced survival at the highest dose (5.0% in mice and 2.0% in rats) were anemias and spleen changes.

Pathology Overview: Dr. Ronald Moch, CFSAN, discussed the findings in the spleens of male and female rats, showing histopathologic slides of the various lesions and giving special emphasis to the areas of capsular and parenchymal fibrosis, and vacuolated areas believed to be infiltrated fat. Based on the Division of Pathology's recent review, the following conclusions were drawn: (1) The treatment-related splenic lesions which included splenic parenchymal fibrosis, vacuolated cells compatible with fatty change, capsular fibrosis, fibrosarcoma, fibroma and capsular hemangioma were noted in both male and female rats; (2) Three male animals in the treated group--that is, the 2% D & C Red No. 33--were diagnosed with fibrosarcoma of the spleen. Of the three animals, two of the animals had fibrosarcomas which appeared to be of intrasplenic origin and were in association with parenchymal fibrosis. One of the animals had a fibrosarcoma which appeared to be arising from the splenic capsule and was in association with capsular fibrosis. One female rat in the treated group had an intrasplenic fibroma as well as parenchymal fibrosis; (3) Some of the observed splenic lesions--that is, splenic parenchymal fibrosis, capsular fibrosis and vacuolar change compatible with fatty change--are not usually seen in untreated control rats. They were not seen in control animals of the current study; (4) Splenic fibrosarcoma has been and is generally considered to be a rare tumor in rats; (5) While the majority of the lesions observed in this study were non-neoplastic, the apparent occurrence of fibrosarcomas in association with or blending with areas of splenic or capsular fibrosis suggests fibrosis as a possible precursoral lesion to the development of neoplasia. Dr. Jackson then presented the results of the statistical analyses performed by the Division of Mathematics on the incidences of the various lesions as shown in the last two pages of Attachment 3. The only lesions for which statistical significance (PF0.05) was demonstrated were parenchymal fibrosis in males and females, and capsular fibrosis in males. Discussion by the Panel members cen tered on whether splenic lesions were observed in an earlier two-year feeding study in rats (control, 0.025, 0.05, and 0.2%). Dr. Jackson said no spleen lesions were reported for the 0.2% dose, and spleens from the lower doses were not examined unless a gross lesion was observed. Dr. Tannenbaum commented that in trying to establish a precursor relationship knowledge of lesions at lower doses would be most helpful. Dr. Friess said the same sort of information on spleen loading with hemosiderin would have been useful.

Discussion From the Floor: There followed presentations by three consultants representing the PMA (Attachment 4). Mr. Charles Cleveland, Director of Scientific Services, PMA, said that the PMA consultants would confine their

remarks to a discussion of the splenic changes and their interpretation. Dr. Louis Van Petten, Norwich Eaton Pharmaceuticals, Inc., noted that since 1966 a series of studies on Red No. 33 have been submitted to the FDA in support of a petition to permanently list the color. He summarized the findings. He proposed that the dose (2%) used in the rat study exceeded the maximum tolerated dose (MTD) and created a nonphysiologic condition in treated rats as evidenced by serious depression in body weight gain and resulting in marked hematologic effects as early as 90 days on test. Dr. Van Petten contended that many of the splenic lesions reflected borderline hematopoietic exhaustion secondary to hemolytic anemia. He concluded that the anemia, but not the subsequent splenic pathology, was a direct compound effect and that carcinogenesis was not demonstrated.

Dr. William Carlton, Purdue University, reported that after a pathologic review of the spleen microslides he had concluded that Red N. 33 was not oncogenic in this study. His diagnoses of the lesions varied only in small degree from those reported by the sponsor's pathologists and the FDA pathologists. He agreed with Dr. Van Petten that the splenic changes represented a stress response to development of the anemia. He stated that there is the possibility that splenic fibrosis is a precursor lesion of sarcoma; however, the correlation had not been established in this case. In response to a question by Dr. Beliczky, he reiterated that there may or may not be a precursor relationship.

Discussion by the Peer Review Panel: Dr. Ward, pathology consultant to the Panel, said that based on weight gain effects the MTD was achieved. In his review of the spleen microslides, he agreed with the diagnoses of the FDA, the original pathologist, and Dr. Carlton with only minor exceptions. Based on the few tumors, he could not conclude that Red No. 33 was a carcinogen for the spleen. However, he noted that the nonneoplastic and neoplastic lesions observed were similar to those reported to be induced in higher incidences by other chemically related aromatic amines. Dr. Ward suggested that in view of variable rates of adrenal gland tumors there be sectioning and examination of adrenals not previously examined.

Dr. Tannenbaum, a principal reviewer, criticized the use of the dye mixture rather than the pure chemical as the test agent. Dr. Flamm responded that a certified batch of the commercial dye was used as that is what is regulated and what must be shown to be safe. Dr. Tannenbaum felt there was no evidence of carcinogenicity from feeding Red No. 33, and, further, the study was either inadequate or inadequately evaluated to determine whether the splenic lesions were preneoplastic. The toxicity of the compound along with lack of either dose-response or time-response data preclude such a determination. He opined that the postulated differences between F344 and Sprague-Dawley rats in susceptibility to induction of splenic lesions by azo dyes may relate to differences in gut flora, which could result in differences in reduction of the azo dyes by bacterial azo reductases to the free aromatic amines which are the toxicants, e.g., aniline in this case. Dr. Flamm asked whether the questions as relating to the nonneoplastic lesions were substantial enough to prompt another study. Dr. Tannenbaum replied that the lack of response at the 0.2% level in the previous study cast doubt that there was a progression with dose, and rather, the incidence of lesions at the 2% level reflected toxicity; thus, a new study done with an intermediate dose would be of doubtful value.

Dr. Van Ryzin, a second principal reviewer, stated that one study rather than two would have been preferable, and the lack of histopathologic evaluation of

the midlevel doses in the first study left no way to do an adequate dose response. The obvious exceeding of the MTD in animals receiving 2% Red No. 33 confounded comparison of the two rat studies. Among many comments, Dr. Van Ryzin asked that analysis of the data on hemolytic anemia be included in some systematic way, and he said a better more unified statistical methodology section was needed. He asked that there be more discussion of the adrenal tumors observed at the 0.2% level in the first study. Dr. Jackson replied that the adrenal tumors were discounted since they did not appear in the animals receiving 2%.

Dr. Kociba, a third principal reviewer, commented that the MTD was either achieved or exceeded at the 2% diet level. He said that data such as splenic weights, hematologic data including methemoglobin levels, body weight data, and statistical evaluations should have been tied together in a concise way to evaluate any dose-response relationships. With regard to the greater susceptibility of F344 rats to induction of splenic lesions by azo dyes, he cautioned that the high spontaneous incidence of leukemia in this species might be a confounding or obscuring factor. Based on his brief examination of the microslides, Dr. Kociba said he had no basic disagreements with the diagnoses of the other pathologists. He concluded that the data was insufficient to establish a precursor relationship between nonneoplastic and neoplastic lesions, and the low incidence rates for the latter suggested insufficiency for showing a carcinogenic response to Red No. 33. Dr. Jones also had examined the spleen microslides and said that he had no basic disagreement with the diagnoses. He called for studies to determine whether there is a correlation between the nonneoplastic and neoplastic lesions.

There was considerable discussion centered on what constitutes a maximum tolerated dose (MTD) and whether a MTD had been achieved or exceeded at the 2% level in rats. Dr. Friess stated that although the 2% dose clearly exceeded the MTD, it was still a valid study providing information on tumorigenesis and nonneoplastic effects. If one accepts the validity then there is the question to be decided whether additional studies should be done, and what these might be.

Dr. Harper said there appeared to be two issues to be decided by the Panel which would be presented as statements. The first would be a statement of the evidence as agreed on. The second would deal with whether or not further studies should be recommended. After further discussion, the Panel members agreed that there should be two statements relating to the evidence, one for the neoplastic lesions and one for the nonneoplastic lesions. With regard to types of studies that should be recommended, Drs. Kociba and Ward proposed that a study on the whole family of aromatic azo compounds and amines seeking for the mechanism of their splenic effects would be most useful while performing another conventional bioassay would be less useful.

Conclusions and Recommendations Approved by the Peer Review Panel: At the request of the Chair, Dr. Kociba formulated three draft statements. These were reworked by the various Panel members. In revising the conclusion pertaining to treatment-related nonneoplastic effects, Dr. Friess requested inclusion of aromatic nitro compounds along with aromatic azo compounds and amines as the nitro compounds are biotransformed, especially in the intestines, to aromatic amines. The two revised conclusions and one recommendation were approved unanimously by the Panel. They are as follows:

(1) Quantitatively, the low incidence rates for primary mesenchymal neoplasms of the spleen in male and female Charles River CD-1 rats

given long-term dietary administration of 2% D & C Red No. 33 could not be considered sufficient to be categorized as a demonstrated carcinogenic response to chemical treatment.

(2) Qualitatively, there appears to be treatment-related nonneoplastic target organ (spleen) toxic responses which are similar to those previously described for certain other aromatic azo compounds, aromatic nitro compounds, and amines.

(3) Further research is necessary and should be directed toward developing understanding of the mechanisms of toxic action of this particular family of compounds in the spleen of rats.

ATTACHMENT 1

Food and Drug Administration

[Docket No. 76N-0462]

American Cyanamid Co.; Refusal To Approve Supplemental New Animal Drug Application; Availability of the Commissioner's Decision

AGENCY: Food and Drug Administration.

ACTION: Notice of availability of Commissioner's decision.

SUMMARY: The Food and Drug Administration (FDA) is announcing that the Commissioner of Food and Drugs has issued his final decision concerning a supplemental new animal drug application (NDA) for Proban Cythioate Oral Liquid, 1.6% ("Proban") submitted by the American Cyanamid Co. The Commissioner has determined that the supplemental NADA for Proban should not be approved. The decision therefore affirms the initial decision of the Administrative Law Judge, which held that the submission seeking Proban's approval failed to satisfy the Federal Food, Drug, and Cosmetic Act's safety and effectiveness requirements for new animal drugs. Differences between the Commissioner's and the Administrative Law Judge's opinions are specifically identified in the Commissioner's decision.

DATE: Effective June 20, 1984.

ADDRESS: The Commissioner's decision, including the final order, and all other documents related to the decision, may be seen in the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857, between 9 a.m. and 4 p.m., Monday through Friday.

FOR FURTHER INFORMATION CONTACT: Theodore E. Herman, Regulations Policy Staff (HFC-10), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-34880.

SUPPLEMENTARY INFORMATION: This notice is issued in accordance with 21 CFR 12.130(e).

Dated: June 21, 1984.

William F. Randolph,

Acting Associate Commissioner for Regulatory Affairs.

[FR Doc. 84-17040 Filed 6-22-84; 11:05 am]

BILLING CODE 4160-01-M

[Docket No. 84C-0192]

DOW Corning Ophthalmics, Inc.; Filing of Color Additive Petition

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing that Dow Corning Ophthalmics, Inc., has filed a color additive petition proposing that the color additive regulations be amended to provide for the safe use of 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one for coloring silicone resin contact lenses.

FOR FURTHER INFORMATION CONTACT: Andrew D. Laumbach, Center for Food Safety and Applied Nutrition (formerly Bureau of Foods) (HFF-334), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-472-5690.

SUPPLEMENTARY INFORMATION: Under the Federal Food, Drug, and Cosmetic Act (sec. 706(b)(1), 74 Stat. 399-402 as amended (21 U.S.C. 376(b)(1))), notice is given that a color additive petition (CAP 4C0180) has been filed by Dow Corning Ophthalmics, Inc., P.O. Box 1767, Midland, MI 48640, proposing that the color additive regulations be amended to provide for the safe use of 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one for coloring silicone resin contact lenses.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding may be seen in the Dockets Managment Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857, between 9 a.m. and 4 p.m., Monday through Friday.

Dated: June 20, 1984.

Richard J. Ronk,

Acting Director, Center for Food Safety and Applied Nutrition.

[FR Doc. 84-17044 Filed 6-26-84; 8:45 am]

BILLING CODE 4160-01-M

[Docket No. 84F-0216]

Halssen & Lyon; Filing of Food Additive Petition

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing that Halssen & Lyon has filed a petition proposing that the food additive regulations be amended to provide for the safe use of ethyl acetate as a solvent in the decaffeination of tea.

FOR FURTHER INFORMATION CONTACT: Partricia J. McLaughlin, Center for Food Safety and Applied Nutrition (HFF-334), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-472-5690.

SUPPLEMENTARY INFORMATION: Under the Federal Food, Drug, and Cosmetic Act (sec. 409(b)(5), 72 Stat. 1786 (21 U.S.C. 348(b)(5))), notice is given that a petition (FAP 4A3804) has been filed by Halssen & Lyon, c/o Pine Consultants, Inc., 1905 Pine St., Philadelphia, PA 19103, proposing that § 173.228 *Ethyl acetate* (21 CFR 173.228) be amended to provide for the safe use of ethly acetate as a solvent in the decaffeination of tea.

The potential environmental impact of this action is being reviewed. If the agency finds that an environmental impact statement is not required and this petition results in a regulation, the notice of availability of the agency's finding of no significant impact and the evidence supporting that finding will be published with the regulation in the Federal Register in accordance with 21 CFR 25.40(c) (proposed December 11, 1979; 44 FR 71742).

Dated: June 20, 1984

Richard J. Ronk,

Acting Director, Center for Food Safety and Applied Nutrition.

[FR Doc. 84-17045 Filed 6-25-84; 8:45 am]

BILLING CODE 4160-01-M

Public Health Service

National Toxicology Program Board of Scientific Counselors' Meeting

Pursuant to Pub. Law 92-463, notice is hereby given of the meeting of the National Toxicology Program Board of Scientific Counselors, U.S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, on July 26, 1984.

The meeting will be open to the public from 10:30 a.m. until adjournment for the purpose of providing peer review of the data from the chronic carcinogenesis bioassay of D & C Red No. 33 in Charles River CD-1 mice and Charles River Sprague Dawley rats. The bioassay was sponsored by the Pharmaceutical Manufacturers Association, conducted by International Research and Development Corporation, and submitted to the Food and Drug Administration (FDA) in support of permanent listing of D & C Red No. 33. The review will be conducted by the Technical Reports Review Subcommittee of the Board in conjunction with an *ad hoc* panel of experts.

The meeting will commence with a brief overview of the studies. This will be followed with presentations by scientific staff from the Center for Food

Safety and Applied Nutrition, FDA, concerning the pathology findings. Sufficient time will be allowed for public comment.

The Executive Secretary, Dr. Larry G. Hart, Office of the Director, National Toxicology Program, P.O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919) 541-3971, FTS 629-3971, will furnish program information prior to the meeting and summary minutes subsequent to the meeting.

Dated: June 20, 1984.

David P. Rall,

Director, National Toxicology Program.

[FR Doc. 84-17046 Filed 6-26-84; 8:45 am]

BILLING CODE 4140-01-M

National Toxicology Program Board of Scientific Counselors; Meeting

Pursuant to Pub. L. 92-463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U.S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, on July 27, 1984.

The meeting will be open to the public from 9:00 a.m. until adjournment. The primary agenda item is the completion of peer review on draft technical reports of long-term toxicology and carcinogenesis studies from the National Toxicology Program. Reviews will be conducted by the Technical Reports Review Subcommittee of the Board in conjunction with an *ad hoc* panel of experts.

Draft technical reports on the following chemicals (listed alphabetically with Chemical Abstracts Service registry numbers, routes of administration, and NTP chemical managers for each study) are scheduled to be peer reviewed on July 27. Since NTP policy requires that a data audit be performed with a summary of the audit report included in the appendix of the technical report prior to peer review, there is the possibility that not all of the technical reports listed below will be reviewed at this meeting.

Chemical (CAS Registry No.)	Route	Chemical manager (telephone No.)
Asbestos, Chrysotile (12001-29-5).	Feed	Dr. E. E. McConnell (919-541-3267).
Benzene (72-43-2)	Gavage	Dr. J. E. Huff (919-541-3780).
2-Chloroethanol (107-07-3).	Dermal	Dr. D. Goldman (202-382-7635).
1,3-Dichloropropene (Telone II) (542-75-6).	Gavage	Dr. R. Yang (919-541-2947).
Dimethyl Hydrogen Phosphite (868-85-9).	Gavage	Dr. J. Dunnick (919-541-4811).
HC Blue No. 2 (33229-34-4).	Feed	Dr. J. Mennear (919-541-4178).

In addition, the technical report on the toxicology and carcinogenesis studies of 1,1,1-trichloroethane (methylchloroform) (CAS No. 71-55-6) is being revised. If completed in time for the meeting a re-review will be done. This report was reviewed and approved by the Panel in February 1983. However, the subsequent data audit resulted in sufficient changes such that the NTP considers another peer review necessary

The Executive Secretary, Dr. Larry G. Hart, Office of the Director, National Toxicology Program, P.O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919-541-3971), FTS (629-3971), will furnish final agenda, rosters of subcommittee and panel members, and other program information prior to the meeting, and summary minutes subsequent to the meeting.

Dated: June 20, 1984.

David P. Rall, M.D., PhD.,

Director, National Toxicology Program.

[FR Doc. 84-17047 Filed 6-26-84; 8:45 am]

BILLING CODE 4140-01-M

DEPARTMENT OF THE INTERIOR

Bureau of Land Management

Canon City, Colorado, District Advisory Council; Meeting

AGENCY: Bureau of Land Management, Interior.

ACTION: Canon City District Advisory Council Meeting.

SUMMARY: Notice is hereby given in accordance with Pub. L. 94-579 that a meeting of the Canon City District Advisory Council will be held on Thursday and Friday, July 19 and 20, 1984.

The Council will meet from 1 p.m. to 5 p.m. on July 19 at the Chaffee County Bank Building, 146 G Street, Salida, Colorado. On July 20th from 8 a.m. to 1 p.m. a field trip to view recreation and other uses of public lands along the Arkansas River is planned.

The meeting agenda will include:

1. The Northeast Resource Area Draft Resource Management Plan and Environmental Impact Statement.
2. Briefing on Cooperative Management Agreements.
3. Public presentations to the Council (open invitation).

The meeting is open to the public, transportation for the field trip is the responsibility of the individual. Persons interested may make oral presentations to the Council between 1:30 p.m. Thursday July 19, or they may file written statements for the Council's consideration. The District Manager may limit the length of oral presentations depending on the number of people wishing to speak.

ADDRESS: Anyone wishing to make a presentation to the Council orally or in writing should notify the District Manager, Bureau of Land Management, 3080 East Main (P.O. Box 311), Canon City, Colorado 81212 by July 18, 1984.

SUPPLEMENTARY INFORMATION: Summary minutes of the meeting will be available for public inspection and reproduction during regular working hours at the District Office approximately 30 days following the meeting.

FOR FURTHER INFORMATION CONTACT: Glen Wallace, (303) 275-0631.

Donnie R. Sparks,

District Manager.

[FR Doc. 84-17024 Filed 6-26-84; 8:45 am]

BILLING CODE 4310-JB-M

[C-35468 and C-36846]

Realty Action; Noncompetitive Sale of Public Lands in Chaffee County, Colorado

AGENCY: Bureau of Land Management, Interior.

ACTION: Notice of Realty Action C-35468 and C-36846. Noncompetitive Sale of Public Lands in Chaffee County, Colorado.

SUMMARY: The following described land has been examined and identified as suitable for disposal by sale under Section 203 of the Federal Land Policy and Management Act of 1976 (90 Stat. 2750, 43 U.S.C. 1713) at no less than the appraised fair market value:

Serial No.	Parcel	Legal description	Acreage	Appraised value
C-35468	368B	Sixth Principal Meridian, Colorado. T. 11 S., R. 79 W., sec. 31, lots 59 (0.79), 60 (0.34), 61 (0.64), 70 (2.65)	4.42	$6,200
C-36846	368A	T. 12 S., R. 79 W., sec. 34, SW¼SW¼	20.00	$34,850

Parcel 368B is being offered to Walter Maass and Parcel 368A to Franklin

ATTACHMENT 2

AGENDA

Board of Scientific Counselors
National Toxicology Program

July 26, 1984
10:30 a.m.

Conference Center, Building 101
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

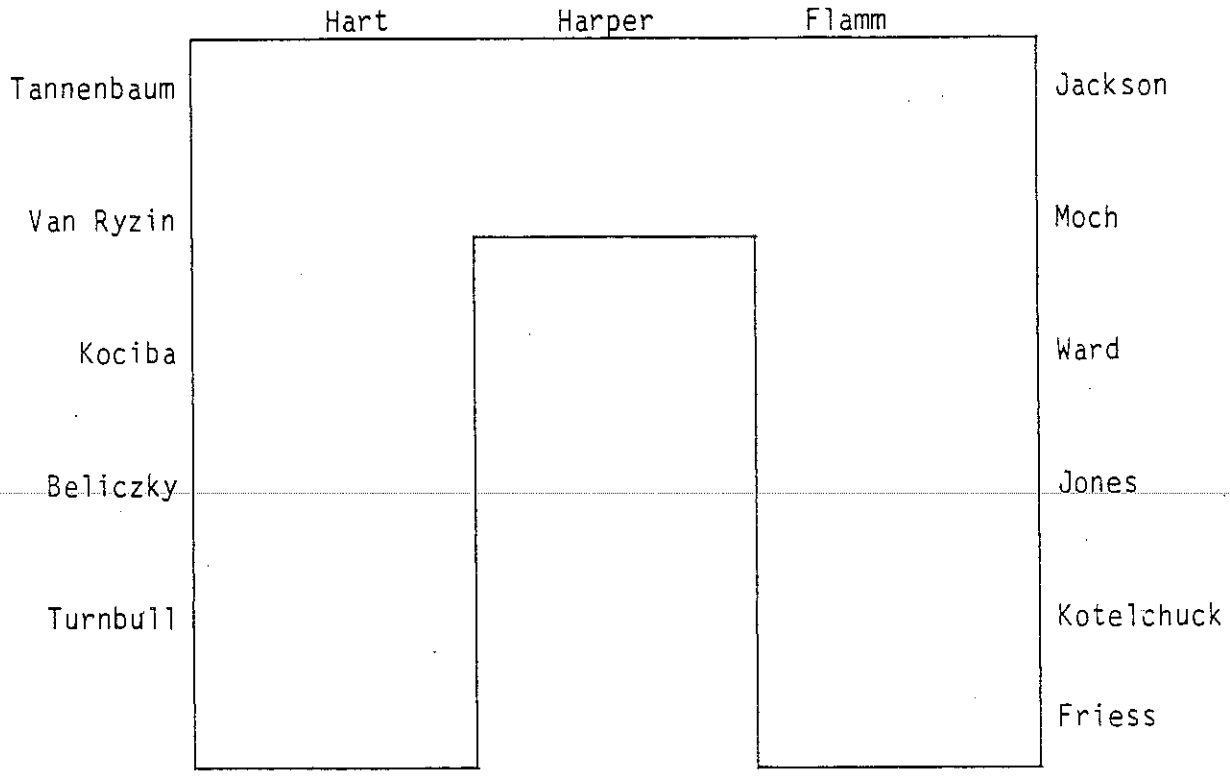
Peer Review of the Data from the Chronic
Carcinogenesis Animal Bioassay of D & C Red No. 33
By the Technical Report Review Subcommittee and Panel of Experts

Overview	Dr. W. G. Flamm, Associate Director for Toxicological Sciences, Center for Food Safety and Applied Nutrition, FDA
Discussion of Toxicology	Dr. B. A. Jackson, Chief, Color and Cosmetics Evaluation Branch, Division of Toxicology, Center for Food Safety and Applied Nutrition, FDA
Discussion of Pathology	Dr. R. W. Moch, Director, Division of Pathology, Center for Food Safety and Applied Nutrition, FDA
Public Comments	
Peer Review Comments and Discussion of Data from Bioassay of D & C Red No. 33	Dr. J. Ward Dr. S. Tannenbaum Dr. J. Van Ryzin Dr. R. Kociba
Conclusions	Peer Review Panel

NTP PEER REVIEW OF THE DATA ON D&C RED NO. 33

Conference Center, Building 101
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

July 26, 1984



NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE AND PANEL OF EXPERTS

July 26, 1984

Subcommittee Members

Dr. Curtis Harper (Chairman)
Associate Professor of Pharmacology
School of Medicine
University of North Carolina
Chapel Hill, NC 27514

Panel Members

Dr. Louis S. Beliczky
Director of Industrial Hygiene
Department of Industrial Hygiene
United Rubber Workers
International Union
87 South High Street
Akron, OH 44308

Dr. Seymour L. Friess
1901 N. Fort Meyer Drive
Suite 204
Arlington, VA 22209

Dr. Thomas C. Jones
Professor, Comparative Pathology
New England Regional Primate Research
Center
Harvard Medical School
One Pine Hill Drive
Southborough, MA 01772

Dr. Richard J. Kociba
Dow Chemical USA
Building 1803
Midland, MI 48640

Panel Members (Cont'd)

Dr. David Kotelchuck
United Electrical, Radio and Machine
Workers of America
Research Department
11 East 51st Street
New York, NY 10022

Dr. Steven R. Tannenbaum
Professor
Massachusetts Institute of Technology
Department of Nutrition & Food Science
Room 56-309
77 Massachusetts Avenue
Cambridge, MA 02139

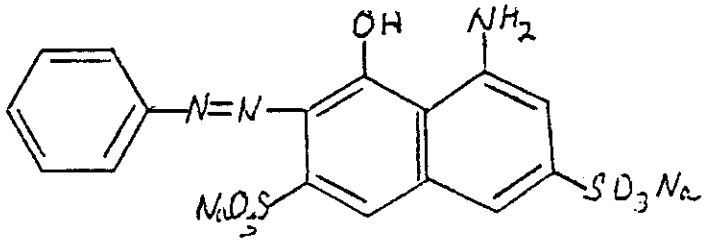
Dr. Bruce W. Turnbull
Professor and
Associate Director
Cornell University
College of Engineering
321 Upson Hall
Ithaca, NY 14853

Dr. John R. Van Ryzin
Columbia University
Division of Biostatistics
School of Public Health
600 West 168th Street
New York, NY 10032

Expert Pathology Consultant

Dr. Jerrold Ward
National Cancer Institute
Frederick Cancer Research Facility
Bldg. 538
Frederick, Maryland 21701

D&C RED NO. 33



CHEMICAL NAME: Disodium salt of 8-amino-2-phenylazo-1-naphthol-3,6-disulfonic acid

SYNONYMS: Acid Fuchsine D, Fast Acid Fuchsine B, Naphthalene Red B

D&C RED NO. 33
ACUTE AND SHORT-TERM ORAL TOXICITY STUDIES

SPECIES	DOSAGE	FINDINGS
RAT	3160 mg/kg (highest single dose)	NO DEATHS
	0.1, 0.2, 0.6, 1.3 and 3.0% DIET (6 wks.)	ENLARGEMENT AND CONGESTION OF SPLEEN HEMOSIDEROSIS
	0.25,0.5,1.0,2.0 and 5.0% DIET (90 days)	ENLARGEMENT OF SPLEEN ANEMIA
DOG	1000mg/kg (highest single dose)	NO DEATHS
	Up to 3.0% DIET (3 months)	DISCOLORATION OF SPLEEN, KIDNEY, BILE AND PERITONEAL FAT

D&C RED NO. 33

LONG-TERM ORAL TOXICITY STUDIES

SPECIES	DURATION	CONCENTRATION IN DIET	FINDINGS
RAT	2 YEARS	0.05, 0.2, 0.5 and 2.0%	ANEMIA (2.0%) ENLARGEMENT OF SPLEEN (0.5 and 2.0%)
DOG	2 YEARS	0.05, 0.4 and 2.0%	ANEMIA (0.4 and 2.0%)

D&C RED NO. 33
REPRODUCTION/TERATOLOGY STUDIES

STUDY	SPECIES	DOSAGES	FINDINGS
REPRODUCTION (3-GENERATION)	RAT	0.25, 2.5, 7.5 and 25 mg/kg/day (DIET)	NONE ATTRIBUTED TO TREATMENT
TERATOLOGY	RAT	2.5, 7.5 and 25 mg/kg/day (GAVAGE)	NONE ATTRIBUTED TO TREATMENT
	RABBIT	2.5, 7.5 and 25 mg/kg/day (GAVAGE)	NONE ATTRIBUTED TO TREATMENT

D&C RED NO. 33
GENOTOXICITY

TEST SYSTEM	Results
AMES/SALMONELLA	NEGATIVE (400 ug/plate)
E. COLI: FLUCTUATION TEST	POSITIVE (10mg/ml)
"REC" ASSAY	POSITIVE (10mg/ml
DROSOPHILA	NEGATIVE

D&C RED NO. 33
RECENT LONG-TERM ORAL TOXICITY STUDIES

SPECIES	DURATION (MOS.)	CONCENTRATION IN DIET	FINDINGS
MOUSE	24 MOS.*	0.1, 1.0 and 5.0%	DECREASED SURVIVAL (5.0%) NO CARCINOGENIC EFFECT ANEMIA (all levels) INCREASED SPLEEN WT.
RAT	28-29	.025, 0.05, 0.2 and 2.0%	DECREASED SURVIVAL (2.0%) ANEMIA (2.0% FEMALES) SPLEEN ENLARGEMENT SPLEEN CHANGES

*except high dose

D&C RED NO. 33

EXPERIMENTAL DESIGN FOR RAT
LONG-TERM FEEDING STUDY
(IN UTERO)

TREATMENT	EXPECTED DOSAGE (mg/kg/day)*	NO. MALES	NO. FEMALES
IA CONTROL	0	70	70
IB CONTROL	0	70	70
II 0.0255% DIET	12.5	70	70
III 0.05% DIET	25	70	70
IV 0.2% DIET	100	70	70
IC CONTROL	0	70	70
V 2.0% DIET	1000	70	70

*Based on expected daily food intake of 20 grams for 400g rat.

D&C RED NO. 33
ANALYSIS OF SPENIC FINDINGS
OF DIVISION OF PATHOLOGY[a]

FINDING	MALES CONTROL	2% DIET	p-VALUE	FEMALES CONTROL	2% DIET	p-VALUE
Fibrosis, Parenchymal	0/56	21/52	0.0001	0/54	10/46	0.002
Fibrosis, Capsular	0/56	13/52	0.0001	0/54	4/46	0.057
Vacuolated Cells	0/56	4/52	0.070	0/54	0/46	-
Fibrosarcoma	0/56	3/52	0.140	0/54	0/46	-
Fibroma	0.56	0.52	-	0/54	1/46	0.50
Hemangio-sarcoma	1/56	0/52	0.50	0/54	0/46	-
Hemangioma	0/56	1/52	0.406	0/54	0/46	-

a) Statistical analysis conducted by Division of Mathematics, Center for Food Safety and Applied Nutrition

D&C RED NO. 33
PREVALENCE ANALYSIS OF SPLENIC FINDINGS[a]

FINDING	MALES CONTROL	2% DIET	p-VALUE	FEMALES CONTROL	2% DIET	p-VALUE
Fibrosis, Parenchymal	0/56	21/51	0.0001	0/65	12/57	0.0004
Fibrosis, Capsular	0/56	13/50	0.0001	0/44	4/42	0.064
Vacuolated Cells	0/44	4/36	0.052	0/12	0/15	-
Fibrosarcoma	0/44	3/36	0.110	0/12	0/15	-
Fibroma	-	-	-	0/12	1/15	0.500
Hemangio-sarcoma	1/20	0/12	0.5	0/12	0/15	-
Hemangioma	0/30	1/37	0.406	0/12	0/15	-

a) Statistical analysis conducted by Division of Mathematics, Center for Food Safety and Applied Nutrition

Statement of the
Pharmaceutical Manufacturers Association
Before the
Technical Reports Review Subcommittee
Board of Scientific Counselors
National Toxicology Program
July 26, 1984

My name is Charles B. Cleveland, and I am Director of Scientific Services for the Pharmaceutical Manufacturers Association (PMA). The PMA is a non-profit trade association representing 129 companies that develop, manufacture and market prescription drugs, medical devices and diagnostic products. PMA is the primary petitioner requesting the permanent listing of provisionally listed D&C Red No. 33. PMA sponsored the most recent chronic carcinogenesis bioassay studies which are the subject of this peer review.

Accompanying me are William Carlton, Ph.D. D.V.M., Professor of Veterinary Pathology and Toxicology at Purdue University, and Louis E. Van Petten, D.V.M., Associate Director of Professional and Regulatory Affairs Division, Norwich Eaton Pharmaceuticals, Inc. Dr. Carlton was retained as a consultant to PMA and was requested to review and prepare a report on the spleen microslides from the current bioassay study in rats. Dr. Van Petten is a member of PMA's Color Additive Committee and will assist in this presentation and in responding to questions raised by the panel.

Our comments today will be restricted to those issues raised by FDA in correspondence with NTP. Specifically this involves:

Memorandum of Conferences of the FDA Cancer Assessment Committee (CAC) meetings on August 19, September 2, and December 21, 1982 (attachment A) which concluded that "...D&C Red No. 33 is not carcinogenic to rats and mice under the conditions of studies described."

Memorandum of Conference of the CAC meeting of November 10, 1983 (attachment B) which states that although the Committee still concurs in the earlier CAC judgement, it is concerned that "...unique histopathological changes occuring in the rat spleen may well reflect potential carcinogenicity to the spleen". Further, the CAC report states that the FDA Division of Pathology "...has found in its preliminary review of the D&C Red No. 33 splenic microslides at least two additional neoplastic lesions which are currently awaiting further microscopic confirmations."

Preneoplastic Splenic Lesions Manuscript (abstract-attachment C) which proposes that: "In view of the rarity of fibrosarcoma of the spleen as a spontaneous neoplasm in Fischer 344 rats (18), when it occurs following the chronic administration of moderate-high dose levels of an aniline-related aromatic amine in association with splenic lesions of the type described in this report (even if the

number of sarcomas is small and not statistically significant) the reviewing pathologist and toxicologist should maintain a high level of suspicion that the observed sarcomas were in fact induced by the test chemical."

FDA Letter of March 30, 1984 (attachment D) to Dr. Rall which states: "In particular do the splenic changes in rats constitute evidence of neoplastic potential."

In our presentation, we do not propose to review any of the other findings in the studies; we will confine ourselves to a discussion of the splenic changes and their interpretation.

At this time Dr. Van Petten will continue with our presentation.

Statement of

Louis E. Van Petten, DVM

By way of background the panel should be aware that since 1966 a series of studies on D&C Red No. 33 have been submitted to FDA in support of the petition to permanently list the color. These include acute, subacute and chronic studies in rats and dogs; repeated dermal application in mice and rabbits; teratogenic studies in rabbits and rats; mutagenicity tests; and multigeneration reproduction studies in rats. (see attachment E for a list of studies and the date submitted to FDA) These studies are summarized in FDA's "Data Package" (previously submitted to subcommittee) beginning on Page 51.

An earlier chronic feeding study was conducted by Hazelton Laboratories and submitted to FDA on 12-19-67. The following are some of the pertinent findings in the study as reported by FDA in 1968.

Groups of the Charles River Albino rats, were fed D&C Red No. 33 in the diet at levels of 0.05, 0.2, 0.5, and 2.0% for up to 2 years. A consistent tendency toward decreased hematocrit and hemoglobin values and increased reticulocyte counts was seen in male and female rats, statistically significant at 2.0%, less so at 0.5% and in males at the 0.2% level, at various intervals. At one interval (terminal) the reticulocyte count in 0.05%-male rats also was significantly elevated, while readings at other intervals, although giving an impression of slight elevation appeared essentially normal. Spleen weights were significantly enlarged at 0.5% and 2.0% in both males and females. Microscopic pathology revealed effects consistent with gross pathology. Effects in the spleen, liver, and kidney at the 2.0% level, were manifested by cytotoxic alterations in the hepatic parenchyma, pigment deposition, splenomegaly, slightly increased thyroid activity, and staining without definite morphologic alterations in the adrenal and stomach. The dose related hemolytic anemia seen at 2.0% and 0.5% and scattered and minimal at 0.2% was also morphologically evident as manifested by pigment deposition (possibly hemosiderosis) of liver, spleen, and/or kidneys and abnormal and increased erythropoiesis in liver, spleen, adrenal and/or bone marrow.

In 1976, FDA published a notice in the Federal Register (41 FR 41860 - 9/23-76) which stated that "...when judged by

contemporary standards for toxicological studies, the studies conducted in support of the petitions...were deficient." In particular FDA requested new life-time studies in mice and rats with the latter conducted with in utero exposure. PMA disagreed with the need to conduct additional studies and did not agree that in-utero exposure represented contemporary standards. Nevertheless the studies were initiated.

The dietary levels in the initial in utero rat study (0, 0.025, 0.05, and 0.2%) were based on the earlier chronic feeding study in rats which established an effect level of 0.2% and a "questionable" no effect level at 0.05%. The low dose was approximately 260 fold greater than the estimated maximum ingestion from both lipstick and drug use.

Subsequent to the start of the in utero rat study, and at the request of FDA, a new high dose in utero study was begun with a dietary level of 2%. In our view this 10 fold increase above the effect level determined by a previous chronic study exceeded the Maximum Tolerated Dose (MTD) and created a nonphysiologic condition in the treated rats. From the beginning of the 2% in utero study the treated animals were seriously compromised. The high dose caused serious depression in body weight from birth (see Table 1).

Table 1 Cumulative Mean Body Weights (Relative to Controls) Fed Diets Containing 2% D&C Red No. 33

Mean Body Weights (Grams)

Dose	Postpartum Day					On Study			
	0	4	14	21		Initial		Final	
				M	F	M	F	M	F
0	6.9	12.4	32.2	50.3	47.9	172	145	774	559
2%	6.4	10.4	25.4	35.5	34.3	119	105	637	387
%Diff.*	-7.2	-16.1	-21.1	-29.4	-28.4	-30.8	-27.6	-17.7	-30.8

*All Significantly different from control group mean, $p<0.01$

As stated in the draft Office of Science and Technology Policy (OSTP) document, "Chemical Carcinogens; Review of the Science and Its Associated Principles" (49 FR 21635; May 22, 1984), "There should be reasonable scientific certainty that the dose used meets the objectives of maximally enhancing the sensitivity of the test without introducing qualitative distortions in the results." Further, "both the National Cancer Advisory Board Subcommittee (NCAB) (1977) and the Interagency Regulatory Liaison Group (IRLG) (1979) cautioned against the use of a dose so high that it produced "unwanted toxic side effects" (IRLG) or "unphysiologic conditions [which] may in themselves enhance tumor formation (NCAB)." With regard to dose selection the draft OSTP document states, "This maximum tolerated dose (MTD) is ordinarily determined in a 90-day study; and was

initially based on a weight gain decrement, i.e., the highest dose that will produce a slight depression in body weight (approximately 10-12%) if administered over a lifetime. NTP has more recently suggested refinement of MTD selection on the basis of a broader range of biological information. Other signs of toxicity that may be used to establish the MTD include primarily gross and microscopic pathology, but alterations in serum enzyme levels, hematological effect or other physiological, biochemical or pharmacological indices of abnormality may be useful." (49 FR 21633)

We believe that distortions have occured in the 2% in utero rat study because of excessively high doses. Yet even at this dose level there is no statistically significant increase in splenic tumors.

In our opinion:

1. Under the conditions of this experiment, 2.0% D&C Red No. 33 shortens the life span of red blood cells evidenced by decreases in RBC, HcT and Hb, increases in reticulocyte counts, and hemosiderosis of the liver, kidney and spleen. These findings were observed as early as 90 days on test (hematology) and at the 12 month interim sacrifice (hemosiderosis).

2. Histopathology at twelve months and at term revealed increased cellularity of the bone marrow in dosed rats; a natural defensive response.

3. Splenomegaly appeared predominately in the treated group due to congestion and increased hematopoietic activity; also a natural defensive response.

4. Splenic stromal fibrosis (14/53 males, 11/52 females) observed in treated animals is indicative of borderline hematopoietic exhaustion. Additionally, fibrosarcoma (1/53 males) and fibroma (1/53 male, 1/52 females) may be a sequellae to the fibrosis and not directly due to D&C Red No. 33.

5. The mesothelial hyperplasia can be attributed to small healed spleenic ruptures due to the splenomegaly, increased fragility of the organ and to frequent handling the rats.

6. Hemangioma is a uncommon primary tumor of the spleen and in our opinion is an incidental finding.

7. Dosing of D&C Red No. 33 at 2.0% of the diet in the rat produces a hemolytic anemia to which the hematopoietic system responds but never quite recovers. The gross and histopathology observed in the spleen is a sequellae to this problem, probably accelerated by the aging process in these animals. In other words the anemia, but not the subsequent splenic pathology, is a direct compound effect. For this reason, we believe that carcinogenesis was not demonstrated when D&C Red No. 33 was administered to the laboratory rat at extremely high doses over prolonged periods of time.

Statement of

Dr. William Carlton, DVM, Ph.D.

After review of the spleen microslides I have concluded that D&C Red No. 33 was not oncogenic in this study with this species and strain of rat (see attachment F for complete report).

With regard to FDA's question of "evidence of neoplastic potential",

splenic alterations in rats associated with the feeding of D&C Red #33 have not been studied and comments on the pathogenesis of these pathoanatomic changes would be speculative at best. However, plausible proposals for their development seem possible.

The splenic alterations apparently arise through "stress" placed upon the hematopoietic tissue by compound-induced decrease in erythrocyte numbers and increase in leukocyte numbers, a process that begins early in the feeding period (detected by 3 months). The process of decreased erythrocyte numbers may be hemolytic in nature due to an increased residence time in an atypically congested spleen (congestion induced by an unknown mechanism or by irritation of vascular structures by the test compound or metabolites released from lysed erythrocytes) or by a specific hemolytic effect of the test compound (production of methemoglobinemia). The continued stress of erythrocyte loss results in stimulation of hematopoiesis in the spleen and bone marrow and the deposition of increased amounts of hemosiderin pigment in the spleen and liver. It is possible that continuous engorgement of the spleen by blood results in capsular fibrosis as the pressure may be a stimulus to fibroplasia or the pressure may produce microfractures of the capsule that heal by fibrosis. The presence of pigment and hematopoietic cells within the capsule of some spleens is suggestive of such a possiblity. Expansive pressure or points of microfracture may be the stimulus for the production of the papillary formations of mesothelial cell hyperplasia.

With time, the hematopoietic tissue of the spleen can no longer respond by proliferation and focal areas of the splenic hematopoietic parenchyma become exhausted. Some spleens from treated rats contained variable size foci composed only of splenic stroma without hematopoietic tissue and without fibrosis. This proposition is also supported by decreased extramedullary hematopoiesis in the spleens of treated rats at the terminal sacrifice. Hematopoietic tissue exhaustion becomes a stimulus for fibrous tissue proliferation resulting in foci of parenchymal fibrosis. Certain of the multi-potential mesenchymal cells of the splenic stroma may differentiate into lipocytes producing the foci of parenchymal steatosis.

Thus, this proposal would make the splenic alterations secondary to a stress on the hematopoietic tissue and the test compound would have no direct "splenotoxic" effects.

Such a scenario, in our view, is more acceptable than the schema proposed by Weinberger, et al; we have the following comments on his manuscript.

(1) The proposal that "normal splenic cells were injured by the preferential accumulation of aniline HCl and related compounds" stimulates questions of

What is a "normal splenic cell"?

erythrocyte, granulocyte, megakaryocyte?

reticulum cell, fibroblast, endothelial cell?

pericyte, smooth muscle cell, fibroblast?

(2) If the erythrocyte is indeed the target cell of aniline HCl toxicity, then the spleen is an "innocent bystander" because of its role in the removal of aged and abnormal erythrocytes.

(3) What "critical splenic macromolecules" do these compounds or metabolites react with?

DNA, RNA, protein, glycoproteins?

and of what cell or cells?

I am unaware of the existence of splenic macromolecules which are not components of cells or blood serum. If this reaction is confined to the spleen which functions in the removal of damaged or aged erythrocytes, then the reaction would be concentration dependent and unlikely to occur at more reasonable exposure likely in human populations.

(4) The proposal that the "first injurious event" was "diffuse vascular injury" also raises questions. First, the authors must have used the term diffuse in reference to the spleen and not the body as a whole. We have no reports of vascular congestion as a common alteration in other organs after rats are exposed to these test materials. "Vascular injury" is nonspecific. Are the authors suggesting damage to endothelial cells, or pericytes or smooth muscle cells or to disruption of mechanisms that control the flow of blood into and out of the spleen?

(5) The authors propose that hemorrhage in the spleen may be the trigger for fibrosis. This may be so, but seems unlikely. Hemorrhage is difficult to detect and evaluate in the spleen, especially so in a congested one. If present, hemorrhage would indicate endothelial cell damage as we have no evidence of dysfunction of coagulation in treated rats. No microscopic evidence of endothelial cell damage has been presented, but this may not be necessary for hemorrhage by diapedesis. Since hemorrhages were not found in other organs, we must assume that this lesion, if induced

in the spleen by the test compounds, must be dose dependent as concentration great enough to produce the lesion was achieved only in the spleen via breakdown of erythrocytes containing the compound or metabolites. If this be so, then the alterations produced in the spleen of rats by large doses of these compounds would have little or no relevance to the human situation with possible exposure to very much lower doses.

(6) If splenic fibrosis is indeed the key lesion in the genesis of splenic sarcomas, then the situation is hopeful because not only its incidence but its progression into neoplasms are dose dependent. Thus, male and female rats fed 0.1% D&C Red #9 had 26 and 37% incidence of splenic fibrosis respectively, but no malignant fibroblastic neoplasms were observed. These data provide us with two conclusions: (1) fibrosis can develop at fairly high incidence without the development of neoplasms and (2) "transformation" into neoplasms depends upon dose. Because of the possibility of low exposure of humans, the data obtained with such high doses in rats may not be relevant.

(7) These observations, like so many others, of splenic alterations and neoplasms in rats given these chemicals are open to various interpretations. Thus, splenic fibrosis and splenic "fatty metamorphosis" may or may not be significant in the development of splenic sarcomas. The evidence for fibrosis to have a key role in the genesis of sarcomas must be greater than just their occurrence within the same organ. Thus, for D&C Red #9, it would have been impossible for there not to have been a possible association between the presence of fibrosis and sarcoma, because the incidence of fibrosis was 100%. But, there was a 100% incidence of hemosiderosis

as well in the female rats. From what we know about the mechanisms involved in the carcinogenesis process and those involved in the induction of fibrosis, it appears likely that many are different and unrelated. Thus, it seems likely that the pathogenesis of the two lesions are different and the occurrence of one would likely be independent of the other, although both could be present in the same organ.

It is not real evidence to propose that splenic fibrosis is the precursoral lesion for splenic fibrosarcoma because the cells are "strikingly similar" (histologically) in the two lesions. This should not come as a surprise to anyone. That fibroblasts have histologic features in common whether in an area of fibrosis or in a fibrosarcoma is the basis of the histogenetic classification of neoplasms. Tumor cells must have a reasonable histologic similarity to non-neoplastic cells of the same cell type in order for a specific tumor diagnosis to be made.

FDA raises the question that perhaps the splenic lesions seen were related to those resulting in studies on aniline and D&C Red #9 and that if D&C Red 33 had been tested in Fischer 344 rats the outcome might well be positive.

Even if this speculative process has validity, Bus et al (see attachment G) reports on studies, in part using C14-aniline, that support the hypothesis that splenic injury may be secondary to erthrocyte damage. Bus states:

"Although the precise mechanisms by which aniline or nitrobenzene produce spleen toxicity have not been defined in these sutides, they do support hypothesis that splenic injury may be an event secondary to erythocyte damage. A clear implication of these findings, therefore, is that splenic injury in man, and in particular, the potential deveopment of cancer, may not occur at the low levels of chemical exposure encountered in the workplace or general environment that are insufficient to cause erythrocyte damage. "