Before The
Environmental Protection Agency
March 11, 1980
Washington, D.C.

In the matter of:

National Emission Standards for Identifying, Assessing and Regulating Airborne Substances Posing a Risk of Cancer

PLAINTIFF'S EXHIBIT AL-1028

DIRECT TESTIMONY
OF
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1. I reside at 9060 Clayton Road, St. Louis, Missouri 63117. I received my B.A. degree in chemistry from Gustavus Adolphus College, St. Peter, Minnesota in 1938, a Ph.D. degree in biochemistry from St. Louis University School of Medicine, St. Louis, Missouri in 1944 under the direction of Professor E.A. Doisy, Nobel laureate in physiology and medicine (1943), and a M.D. degree from Harvard Medical School, Boston, Massachusetts in 1951. I interned at the Peter Bent Brigham Hospital, Boston, Massachusetts (1951-52). I am a member of the American College of Physicians and am licensed to practice medicine in the states of Pennsylvania and Missouri.

2. I was appointed Instructor in Biochemistry and Nutrition, Harvard School of Public Health and Harvard Medical School in 1946-47, and in 1952 following completion of my medical training, I was appointed Professor of Biochemistry

and Nutrition and Chairman of the Department, Graduate School of Public Health at the University of Pittsburgh with a joint appointment as Lecturer in Medicine in the School of Medicine. While at the University of Pittsburgh, I was Director of the Nutrition Clinic, Falk Clinic, University of Pittsburgh Medical Center from 1953-65 and Director of the Metabolic Unit at St. Margaret's Memorial Hospital, Pittsburgh, Pennsylvania from 1954-60. Part of this research program was the study of toxic chemicals including ethanol, benzene and halogenated hydrocarbons.

3. Since 1965 I have been the Alice A. Doisy Professor of Biochemistry and Chairman of the Department and Professor of Medicine in the Department of Medicine at St. Louis University School of Medicine. In 1967, with the aid of funds from the National Institutes of Health of the United States Public Health Service, the Thailand Government, and the Rockefeller Foundation of New York City, I established and became Director of a Clinical Research Center for the Study of Anemia and Malnutrition in Chiang Mai, Thailand for the decade 1967 to 1977. I had the opportunity, in collaboration with both American and Thai colleagues, to investigate anemias and other metabolic disorders accompanying severe protein calorie malnutrition in children and to devise new therapeutic regimens for that disease.

4. The scope of my research work includes studies of metabolism, endocrinology, toxicology and nutrition in both animals and man. Special emphasis has been given to cardiac

metabolism, the metabolism of tumor tissue, the bioassay of adrenocortical steroids, and the effect of dietary components particularly sulfur-containing amino acids upon the metabolism of the liver with particular attention to lipid metabolism, the secretion of lipoproteins and the biosynthesis and function of ubiquinone and the fat-soluble vitamins. Since 1944 I have published 174 full papers and 29 book chapters or major reviews, and I have edited three books: 1) Perspectives in Biochemistry, Marcel Dekker, 1970; 2) Methods in Medical Research, Volume 12, Yearbook Medical Publishers, 1970; and 3) Protein Calorie Malnutrition, Academic Press, 1975; and a monograph on Environmental Carcinogenesis is in preparation (Marcel Dekker, 1980).

5. I have been an Established Investigator of the American Heart Association, a Guggenheim Fellow twice, (1961-62 Oxford University with Sir Hans Krebs, Professor of Biochemistry and the University of Freiburg in Breisgau, West Germany with Professor Helmut Holzer, 1970-71). I have been a consultant to the U.S. Public Health Service, Division of Research Grants and a member of various Study Sections, Training Committees, and most recently the Program Project Committee of the National Heart and Lung Institute during the period 1953 to 1976. I have been a member of the editorial boards of Nutrition Reviews, the American Journal of Medicine, the American Heart Journal, American Journal on Clinical Nutrition, Circulation Research, Biochemical Medicine, Molecular and Cellular Cardiology and Vitamins and Hormones. I am a member of the Association for the Advancement of Science, the American Association for Cancer

Research, the American Chemical Society, American Institute of Nutrition, the American Society of Biological Chemists, the American Society for Clinical Investigation, the American Society for Clinical Nutrition, the American Society for the Study of Liver Diseases, the Association of American Physicians, New York Academy of Sciences, the Society for Experimental Biology and Medicine, the Royal Society of Health, London, England, and the International Study Group in Cardiac Metabolism.

6. My testimony bears on the criteria for listing hazardous air pollutants, particularly carcinogens, under Section 112 of the Clean Air Act. Carcinogens which have been listed under Section 112 to date include asbestos, beryllium, vinyl chloride and benzene. The mechanisms by which these substances cause cancer is obscure. Unlike the polycyclic hydrocarbons and nitrosamines, there is no evidence that any of these substances react directly with DNA. The burden of my testimony will be to challenge the primary model used by the Environmental Protection Agency (EPA) for estimating carcinogenic risk for <u>all</u> substances namely the linear non-threshold dose-response curve. While this model <u>may</u> be appropriate for primary carcinogens, i.e. those substances demonstrated to react either before, or after activation by cytochrome P-450, with DNA, there are a number of compounds which do not transform cells by this mechanism.

7. As pointed out by Dr. Emmanuel Farber (University of Toronto) in the OSHA hearing in 1977 (Fed. Reg., 1977), "There are thus at least two ways in which chemicals can lead to the development of cancer: by initiating changes in cells and by

permitting selective growth of populations of changed cells." Benzene and ethanol belong to the second class of carcinogens. The latter are cytotoxic agents that cause destruction of some cells and induce proliferation of new cells. U. Saffiotte (NCI) (Fed. Reg., 1980) described this well when he said "The expression of toxic injury, therefore, does not derive for the cells that were orignially hit by the toxic agent, or from their functional products, but rather from the proliferation of a new population of altered cells." This appears to be the basic pattern for benzene, ethanol, and the halogenated hydrocarbons. The target tissue for benzene is the bone marrow where injury, sometimes to the extent of aplastic anemia, is followed by regeneration of bone marrow cells and myelogenous leukemia in a small percent of cases. With ethanol and the halogenated hydrocarbons the principal target tissue is liver, although kidney gastrointestinal tract and brain also are affected. Destruction of liver tissue is followed by regeneration and scarring of liver (cirrhosis) and by hepatoma formation in a small percent of cases.

8. The different etiology of cancers due to cytotoxic agents has implications for regulation. If injury must precede carcinogenesis then the amount of chemical to cause minimum injury is the minimum dose for carcinogenesis. One-hit models are therefore not applicable to this type of carcinogen and the linear non-threshold dose-response curves proposed by the EPA are also not applicable to this type of carcinogen. The evidence to support this reasoning is given in more detail below for benzene and the halogenated hydrocarbons.

9. It is not necessary to produce a somatic mutation to cause cancer. Mintz and Illmensee (1975) have reported that malignant mouse teratocarcinoma (or embryonal carcinoma) cells with a normal modal chromosome number were taken from the "cores" of embryoid bodies grown only in vivo as an ascites tumor for 8 years, and were injected into blastocysts bearing many genetic markers, in order to test the developmental capacities, genetic constitution, and reversibility of malignancy of the core cells. Ninety-three live normal pre- and postnatal animals were obtained. Of 14 thus far analyzed, three were cellular genetic mosaics with substantial contributions of tumor-derived cells in many developmentally unrelated tissues, including some never seen in the solid tumors that form in transplant hosts. The tissues (e.g., immunoglobulins, adult hemoglobin, liver proteins) coded for by strain-type alleles at known loci. In addition, a tumor-contributed color gene, steel, not previously known to be present in the carcinoma cells, was detected from the coat phenotype. Cells derived from the carcinoma, which is of X/Y sex chromosome constitution, also contributed to the germ line and formed reproductively functional sperms, some of which transmitted the steel gene to the progeny. Thus, after almost 200 transplant generations as a highly malignant tumor, embryoid body core cells appear to be developmentally totipotent and able to express, in an orderly sequence in differentiation of somatic and germline tissues, many genes hitherto silent in the tumor of origin. This experimental system of "cycling" teratocarcinoma core cells through mice, in conjunction with experimental mutagenesis of

those cells, may therefore provide a new and useful tool for biochemical, developmental, and genetic analyses of mammalian differentiation.

10. The results also furnish an unequivocal example in animals of a non-mutational basis for transformation to malignancy and of reversal to normalcy. The origin of this tumor from a disorganized embryo suggests that malignancies of some other, more specialized, stem cells might arise comparably through tissue disorganization, leading to developmental aberrations of gene expression rather than changes in gene structure.

11. Metabolism and Toxicology of Benzene in Animals and Man

Benzene (C_6H_6), the parent hydrocarbon of the aromatic group of chemicals, has a density of 0.87 grams per ml and a boiling point of 80.4° C. It is highly volatile and is widely used in the extraction and preparation of benzene derivatives, as a solvent in the rubber and footwear industries, for the preparation and use of inks in the rotogravure industry, as a thinner for paints, as a cleaning agent, as an anti-knock fuel additive. Because of its high volatility, benzene proves a hazard to industrial workers in these plants unless levels are maintained below present standards of benzene exposure in the U.S. of 10 parts per million in the air.

12. Benzene is absorbed principally through the lungs by inhalation and metabolized in the animal body. It is slightly soluble in water and miscible with lipids. It combines with plasma lipoproteins during its transfer from the lungs to various organs in the body. Since benzene is lipophilic, it readily

passes through cell membranes and becomes distributed in the lipid components of the body. Because benzene is a membrane poison, acute toxicity in man results from exposure to 1,000 parts per million or more. Nervous exhaustion, euphoria, headache, cardiovascular collapse, unconsciousness and coma can occur. The acute lethal concentration of benzene is about 20,000 parts per million. At levels of about 100 parts per million in respired air, the standard that prevailed in 1941, chronic toxicity is observed. This is expressed principally as blood dyscrasias in 19-35% of workers exposed to high levels which includes 3.7% aplastic anemia and 0.1-0.2% myeloblastic leukemia (Jandl, 1977). It must be assumed that the toxic agent is benzene itself or a closely related compound since all of the derivatives including the epoxide are less toxic than benzene itself. The liver is the chief organ for detoxification of benzene and competes with other organs for available plasma benzene. The liver lowers the plasma concentration by metabolizing benzene to various congugated and oxidized products. If the concentration in the air drops, the lung contributes to the elimination of the compound from the body through exhalation.

13. Benzene is initially metabolized by the cytochrome P-450 system in liver microsomes. The product is an oxygen derivative, either the epoxide or phenol. The role of the epoxide is not established in the P-450 activation of benzene although it has been postulated by some workers. The primary end product of this mixed function hydroxylation is phenol. Phenol is further detoxified by conversion to either phenyl sulphate

through reaction with 3'-phosphoadenosine 5'-phosphosulfate (PAPS) or reaction with uridine diphosphate glucuronide (UDP-GLc) to yield phenlyglucuronide. These two compounds are water-soluble and are excreted in the urine. It has been shown in many studies that the excretion of phenol and its derivatives parallels the injestion of benzene and is, in fact, a good internal indicator of exposure to the solvent. Benzene can be further hydroxylated to yield either catechol or hydroquinone which are sufficiently soluble to be excreted. Catechol-O-methyl transferase will convert catechol to the O-methyl derivative which is also found in the urine. A small percent of benzene is oxidized further via an oxygenase to cis-cis-muconic acid and then all-trans-muconic acid which in turn can be oxidized to CO_2 and water or excreted in the urine. Finally, benzene is detoxified by condensation with glutathione to yield a thio-ether which is subsequently metabolized by cleavage of glycine and glutamic acid residues and acetylation of the cysteine to yield phenyl mercapturic acid. During exposures of man to benzene levels in the air of 10 parts per million or less, the metabolic detoxification reactions maintain benzene levels sufficiently low in the blood to be below the threshold for any effect on the bone marrow.

14. To my knowledge, there is no case of a human subject exposed to 10 parts per million of benzene in the air or less who has developed any hematologic complications to benzene exposure. In fact, the emergency temporary standards for benzene of 1 p.p.m. promulgated by OSHA on May 3, 1977 was stayed by the

Fifth Circuit Court of Appeals on the grounds that no benefit had been shown to justify the lowering of the standard from 10 to 1 p.p.m. The case is now in the hands of the the U.S. Supreme Court.

15. A threshold exists for benzene. Benzene, if inhaled at an average rate of 30 micrograms per liter of air (10 ppm), the microsomal P-450 system will generate phenol and related molecules at a rate to account for 50% of the benzene ingested. The remaining 50% is exhaled after exposure during the 8 hour working period. There is a great excess of enzymatic capacity to handle benzene ingested at this level and can be further induced by phenobarbital and other drugs. In addition, glutathione (GSH) which is 4 mM in the intestine and 8mM in the liver reacts with these small amounts of benzene to yield phenyl mercapturic acid which is subsequently excreted in the urine. The excess of body GSH over benzene at expected average intakes of 75 mg per day is 100 times, which does not consider renewal from dietary protein. These detoxication reactions are highly competitive reactions which diminish the concentration of benzene before it can, in any significant quantity, reach the nucleus of bone marrow cells.

16. As pointed out earlier, there must be enough benzene in the bone marrow to cause injury before there can be cancer. The primary effect of benzene at concentrations > 100 ppm is to inhibit the differentiation of stem cells and only secondar- in some unknown manner, to cause cancer in a very small percentage of regenerating cells.

17. Metabolism and Toxicology of Perchlorethylene in Animals. Recently, studies on the pharmacokinetics and macromolecular interation of perchlorethylene in rats and mice have been described (Watanabe and Schumann, 1979). The objectives of these studies were to: 1) Understand why the B6C3F1 mouse was sensitive to perchlorethylene tumorigenicity while rats appeared resistant, and 2) Determine whether the tumorigenicity in the mouse involved predominantly a genetic or nongenetic mechanism so appropriate controls could be implemented to protect against any adverse health effects.

18. The dose levels ranged from about 500-1000 mg/kg/day. In male and female mice no dose-response relationship was observed suggesting that the formation of the reactive metabolite from perchlorethylene was saturated at the massive doses administered. The spontaneous or background incidence of hepatocellular carcinomas in untreated and vehicle treated controls range from 0-12%. This indicates that this particular genetically inbred strain of mouse is highly prone to hepatocellular carcinomas without any exogenous stimulus. This will become very important in interpreting the mechanism of perchlorethylene tumorigenicity in this mouse strain.

19. Initial studies were conducted to compare the routes of excretion and extent of metabolism of perchlorethylene in rats and mice. Groups of rats or mice, were exposed to a 10 ppm atmosphere of ^{14}C-labeled perchlorethylene for 6 hours and the percentage of recovered radioactivity excreted by the various routes was determined. In mice, only 12% of the radioactivity was excreted by the lungs as perchlorethylene. This is in contrast to 68%

perchlorethylene excreted in the lungs in rats. Consistent with this pattern suggesting that the mouse metabolizes much more perchlorethylene than the rat is the excretion of urinary metabolites, 62.5% in mice versus only 18.7% in rats. When expressed on a weight basis, the mouse metabolizes 8.5x more perchlorethylene than the rat. If the metabolism of perchlorethylene is associated with the formation of a reactive metabolite, then it would be expected that those reactive metabolites should bind to intracellular macromolecules. Macromolecular binding was determined by exhaustive extraction of the radioactivity in a trichloroacetic acid precipitate of liver tissue. The nonextractable radioactivity was presumed to be covalently bound to liver macromolecules and the data are expressed as μmole equivalents perchlorethylene bound/g liver protein. Since it was important to understand what cirtical macromolecules may be involved if the reactions with perchlorethylene, DNA was isolated and purified to determine if any radioactivity was detected in the pruified liver DNA from mice. No observable radioactivity was detected in the purified liver DNA from mice.

20. These results indicated that the mouse was more susceptible because it metabolized and therefore produced more reactive metabolite than the rat, but even more importantly it did not appear that reaction of perchlorethylene with DNA took place at any significant level. This suggested that perchlorethylene may be promoting the spontaneous incidence of tumors by a nongenetic mechanism. Remember that the B6C3F1 mouse showed a spontaneous incidence of hepatocellular carcinoma in the NCI study of up to 12%. Furthermore, this spontaneous incidence has been reported to be as high

as 40% (NCI bioassay of Proflavin, Jan. 1977). Thus, additional studies were designed to explore whether a nongenetic mechanism was responsible for the tumorigenicity in B6C3F1 mice as reported by the NCI. Male B6C3F1 mice (4/group) were administered 500 mg/kg/day perchlorethylene by oral gavage in corn oil for 12 doses. This dose was the same as the low dose used in the NCI bioassay. To determine whether increased DNA turnover due to toxic injury was occurring the liver, ^{3}H-thymidine (1000 µCi/kg, 20 Ci/mmole) was injected 18 hr following the last dose of perchlorethylene and purified DNA was isolated. Liver weights were recorded and histopathology was also conducted on these mice. The results showed that both absolute and relative liver weights were increased and the DNA turnover indicating increased cellular proliferation (division) was also increased 2x over controls. Consistent with the increased DNA synthesis due to hepatotoxicity, histopathologic examination showed that hepatocytes in perchlorethylene treated mice showed cytoplasmic vacuolization. These data indicate that perchlorethylene is tumorigenic in mice via a nongenetic mechanism. The recurrent tissue damage produced in the liver of mice by daily perchlorethylene administration of 500 or 1000 mg/kg/day for a lifetime (78 weeks) as conducted in the NCI study likely promoted the spontaneous tumor incidence.

21. Metabolism and macromolecular binding is greater in mice than rats which correlates with the greater sensitivity of mice to the induced tumorigenicity. Binding of reactive metabolites of perchloroethylene to liver DNA was not observed in mice indicating that a purely genetic mechanism of tumor formation is unlikely. Mice given only 12 oral doses of perchloroethylene

(500 mg/kg/day) showed increased liver weight, increased DNA turnover and histopathologic alterations of hepatocytes indicating toxic liver injury. If the effects of perchloroethylene are compared to a potent liver carcinogen such as dimethylnitrosamine (DMN at 3 ng/kg) acting via a genetic mechanism, it is found that DNA damage as indicated by moles of alkylated DNA bases/10^6 moles DNA is 1000 vs. <10 for perchlorethylene. DNA turnover was not elevated over control levels following single administration of DMN but it was increased 2x for perchloroethylene. Likewise, histopathology indicative of toxic liver injury was absent for DMN but moderate for perchloroethylene. Thus it was concluded that perchloroethylene is producing tumors in the B6C3F1 mouse by a nongenetic mechanism.

22. Use of the Threshold Concept in Occupational Medicine

The setting of tolerances for noxious compounds in the field of occupational medicine is entirely dependent upon the thresh- concept. The specification of maximum allowable concentrations (MAC) for a variety of compounds in industrial medicine assumes that below the level set there will be no toxicity in workers with long-term exposure to toxic substances. These numbers (MAC) are altered from time to time but the concept underlying the MAC is a threshold concept. One can find threshold limit values (TLV) for many airborne contaminants including benzene in reports of the American Congress of Governmental Industrial Hygienists and in the American National Standards, Federal Register of 1971. The threshold concept has undergirded the determination of standards for safe

drinking water, for limited exposure to ionizing radiations, for tolerances for pesticide residues on foods, and for 50 years of occupational exposures to potentially harmful chemicals.

23. In summary, it is my belief that the standards for identifying, assessing and regulating substances posing a risk of cancer must take into account the basic mechanisms of carcinogenesis to the extent they are known. Estimates of the risk of cancer from polycyclic hydrocarbons and other compounds that are known to derivatize DNA could conceivably follow the recommended EPA linear model or other appropriate models. On the other hand, compounds that operate through cytotoxicity and require injury before a potentially carcinogenic line of cells emerges in the act of repair should be treated by a threshold model for regulatory purposes.

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