ASSESSMENT OF CARCINOGENIC RISKS FROM PCBs IN FOOD

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1. Chemical Properties

Polychlorinated biphenyls (PCBs) are a family of compounds produced by the total or partial chlorination of biphenyl. There are ten possible hydrogen bonds which can accommodate chlorine substitution in a biphenyl molecule. A specific chlorobiphenyl molecule may be referred to by naming the positions of the chlorine atoms according to the diagram in Figure 1 (National Institute for Occupational Safety and Health (NIOSH), 1977). In naming a particular molecule, the lowest possible numbers are assigned and the ring with the fewest chlorine substitutions is assigned primed numbers. See Figure 2 for examples of the naming convention.

When two compounds have the same number of chlorine substituents on the biphenyl molecule but the substitutions occur in different locations, the two compounds are called isomers. The class of chlorobiphenyls consisting of one type of isomers is referred to using a prefix denoting the number of chlorine substituents, e.g. pentachlorobiphenyls. There are ten possible chlorobiphenyls and 209 possible individual isomers. The term PCBs is used to refer to a complex mixture of different chlorobiphenyls and their isomers (Nisbet, 1976a).

The synthesis of a pure sample of a specific chlorobiphenyl in the laboratory is quite difficult because the chlorination process of biphenyl naturally forms mixtures of many PCBs. Because some chlorine substitution patterns are more likely than others, some isomers are more likely to occur than others and this in turn causes some of the

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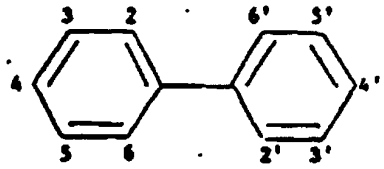
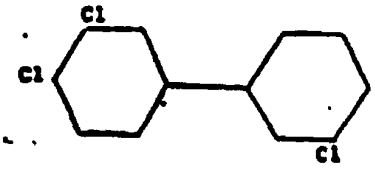
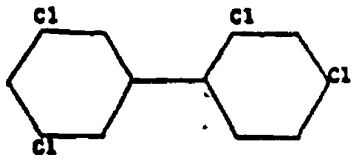


Figure 1: Biphenyl molecule and ring numbering system



3,3',4-trichlorobiphenyl



2',3,4',5-tetrachlorobiphenyl

Figure 2: Examples of the numbering system for chlorobiphenyls

chlorobiphenyls to be more abundant than others. For example, a chlorobiphenyl completely chlorinated on one ring and unchlorinated on the other is not likely to appear (Environmental Protection Agency (EPA), 1976).

PCBs are produced commercially by the chlorination of biphenyl with anhydrous chlorine in the presence of a catalyst, usually iron filings or ferric chloride (EPA, 1976). The chlorination process is terminated at a particular step in order to produce a mixture of chlorinated biphenyls with an average percentage of chlorine. These crude isomeric mixtures are further refined to remove color, traces of hydrogen and the catalyst by a distillation process (EPA, 1976). PCB products made by Monsanto are called by the trade name "Aroclor". Varying kinds of Aroclors are designated by a four-digit number, e.g. Aroclor 1254 or Aroclor 1242. The first two digits refer to the fact that biphenyl is made up of twelve carbon atoms, and the second two digits refer to the approximate percent by weight of chlorine in the mixture. Thus, Aroclor 1254, a mixture of chlorinated biphenyls, would contain by weight, 54% chlorine and 46% carbon, hydrogen and trace elements. Aroclor 1254 would not always contain the exact same amount of a particular compound since commercial mixtures will vary from batch to batch, but the relative amounts of the ten isomer classes will stay the same from batch to batch.

One exception to the usual Aroclor nomenclature is Aroclor 1016, which was introduced in 1971 to gradually replace Aroclor 1242. Aroclor 1016 is comprised mainly of tri- and tetrachlorobiphenyl compounds and contains 41% chlorine by weight. It differs from Aroclor 1242 in that

the latter contains a greater percentage of the more highly chlorinated compounds (NIOSH, 1977).

In Japan other PCB products were marketed under the trade names "Santotherm" and "Kanechors" 300, 400, 500, and 600. In Germany PCB products were marketed under the trade name "Clophens" A50 and A60, and in France under the names "Phenoclors" and "Pyralenes" (NIOSH, 1977).

Studies of the commercial preparations with PCBs have turned up two interesting facts. First, about half of the 209 possible isomers do not occur in the commercial mixtures. In fact, Aroclor 1248 contains less than 50 separable isomers. Second, trace contaminents such as polychloro naphthalenes and polychloro dibenzofurans (PCDF) have be found in some samples of PCBs. The contamination may occur during the manufacturing process (NIOSH, 1977).

PCBs have several physical and chemical properties of both industrial and environmental interest. Even through the basic properties of the different commercial PCB mixcures depend on the particular mixture of chlorobiphenyls in the specific preparations, the mixtures are enough alike that the Aroclors can be used as a basis for discussion of these properties (EPA, 1976). Commercial PCB mixtures can be mobile oils, viscous liquids, sticky resins or crystalline solids at room temperature. Their color ranges from clear to yellow to yellow-green to black. A more complete listing of physical properties is given in EPA (1976), p. 40.

Chemically, PCBs are very stable organic compounds, exhibiting a high heat capacity, low electrical conductivity, a resistance to oxidation, low vapor pressure and excellent dielectric properties.

PCBs are quite stable to long heating and these PCB liquids with four or more chlorine atoms are non-flammable as are their vapors. Although PCBs are not very soluble in water, with this solubility decreasing with increasing clorine content, PCBs are quite soluble in hydrocarbon solvents, lipids and in oils. Also the adsorption rate of PCBs onto plastic, glass, dust particles, silt and sand is high. Because of these "attractive" chemical properties, industry has made extensive use of PCBs, mostly in "closed" or "semi-closed" systems such as electrical transformers and capacitors, and in heat transfer systems (EPA, 1976).

When it became evident in the late 1960's that PCBs had become an environmental pollutant, Monsanto Co. voluntarily phased out production of Aroclors 1232, 1248, 1260, 1262 and 1268 in 1971. Monsanto also began selling PCBs for use in "closed" electrical systems only and, as of 1975, was producing only Aroclors 1221, 1016, 1242 and 1254 (Miebet, 1976a).

2. Food and Environmental Contamination

The particular combination of physical and chemical properties in chlorobiphenyls that led to such a widespread usage of PCBs in industry is also an important factor in the process of PCBs becoming environmental pollutants (EPA, 1976). Some of the more significant properties include low solubility in water, high solubility in lipids and high specific gravity. To determine the impact of PCBs as environmental pollutants and food contaminants, it is necessary to determine first how they are released into the environment and, secondly, how they are dispersed throughout the environment.

7.1 Routes of Contamination and Environmental Distribution

There are basically three ways that PCBs can be released into the environment. First, there is intentional disposal of PCBs and products containing PCBs, mostly into dumps and landfills. Second, there is disposal into water, including losses of industrial fluids and deliberate dumping of industrial wastes. Third, PCBs are released into the air, including vaporization from plasticized products, releases from incomplete burning in dumps or incinerators, and releases from burning scrap products (Kisbec, 1976b).

There are two major methods of transport once the PCBs are in the environment. PCBs are transported aerially in the form of a vapor or because they are trapped on airborne dust particles (EPA, 1976), and eventually return to earth in rain or dustfall. PCBs are transported through water in several ways including the movement of sediment containing PCBs, dumping sewage sludge in the ocean and direct leaks from coastal industries (Kisbec, 1976b). Due to the low solubility of PCBs in water and/or the high specific gravity of PCBs, most of the PCBs discharged into the water will either be absorbed into the sediment at the bottom of the body of water or evaporate into the air. The latter possibility is evidenced by a co-evaporation study on Aroclor solutions, and since each Aroclor is made up of different isomers of several chlorobiphenyls, the vapor pressure or solubility of a particular Aroclor really represents an average over its various isomeric components (EPA, 1976). The former possibility is evidenced in a study of drainage basins in the United States and Puerto Rico where it was apparent that bottom sediments contained much higher concentrations of PCBs than did overlying water (EPA, 1976).

presumably because of the high specific gravity and low solubility in water of PCBs (Dennis, 1976).

PCBs are available for introduction into food chains through the above methods of release into the environment. The PCBs are taken in by biota directly, through absorption, or indirectly, through a food chain. That biological magnification can occur has been observed in the wide ranging accumulation of PCBs within the biota, where extremely large loads are found in the highest members of the food chain (EPA, 1976). It has been demonstrated in the laboratory that fish can accumulate PCBs to concentrations as much as 274,000 times the concentration of PCBs in water (Nisbet, 1976a).

Food may be contaminated by PCBs also through machine leaks, such as heat transfer equipment leaking during pasteurization, contamination of packaging materials (recycled paper) and contamination of animal feed which leads to contamination of meat and poultry. Mothers' milk has also been found to contain PCBs.

Polychlorinated biphenyls are very persistent in the environment. The more highly chlorinated biphenyls are more resistant to degradation, resulting in a "differential persistence" of PCBs. The selective degradation of PCBs has an effect on which of the Aroclors are most persistent in air, water, fish, and in humans. Most of the PCBs found in dry

fallout and in the air resemble Aroclor 1254 (Nisbet, 1976c). In a study done by the U.S. Department of Interior's Geological Survey on PCBs contained in whole water samples and in bottom sediments in the major drainage basins of the United States and Puerto Rico for 1971-1974, the predominant PCB detected was Aroclor 1254 (Dennis, 1976). In other studies though, combinations of PCBs are reported: in samples of both bottom and suspended sediment taken from the upper Chesapeake Bay Aroclors 1254, 1262, 1242 and 1248 were found (Munson, et al., 1976).

A study by Sanborn et al. (1976) on the green sunfish demonstrated that in fish a main difference in metabolism comes between trichlorobiphenyls and tetrachlorobiphenyls, with a relatively efficient storage of tetrachlorobiphenyls. This is also evident in data from the 1970 National Pesticide Residue Monitoring Program in which substantial fractions of Aroclor 1248 occurred in fish taken from the Ohio and Hudson Rivers (Walker, 1976). McDermott, et al. (1976) found that Aroclors 1254 and 1242 were present in Dover sole taken near discharge points of southern California wastewater plants in the ratio of 2:1 while the ratio in the wastewater was about 3:8. This last example would imply that other factors such as the sediment load or solubility of a particular PCB are involved in determining the levels of PCBs in fish. Another factor is the phenomenon of bioaccumulation, the concentration of PCBs in the tissues at much higher levels than in the surrounding water. This is due primarily to the fact that PCBs are highly soluble in lipids and not very soluble in water.

In humans there is differential storage of the more highly chlorinated biphenyls, and the less chlorinated isomers are either metabolized or excreted. Results of studies on residues in human

adipose tissue indicate that the chlorobiphenyl compounds most often found were the penta-, hexa-, and heptachlorobiphenyl compounds, in amounts most comparable to those prevalent in Aroclors 1254 and 1260 (Kutz and Strassman, 1976). But human food monitoring indicates that most humans are exposed to PCBs through diet, and that most PCBs in human food are in fish. This would apparently contradict the fact that substantial quantities of tetrachlorobiphenyls are found in fish. What apparently happens is that humans may be exposed to these less highly chlorinated biphenyls but do not retain them (Nisbet, 1976c).

2.2 Levels in Food from the FDA Total Diet Program

In the FDA Total Diet Program (Johnson and Manske, 1977), a market basket of food representing the basic 2-week diet of a 16-to-19-year-old male is collected in each of several geographic areas. The various foods are prepared in the manner in which they would normally be eaten and are then analyzed for the presence of various substances including PCBs. The lowest concentration at which it is possible to quantify the level of PCBs present is .05 ppm. Levels detected below this level are recorded as "trace". The results of these studies pertaining to PCBs for the fiscal years 71-76 are summarized in Table 1.

Table 1: Total Diet Studies-American teenage male

	Percent of composites containing PCB's								
	Food class composites								
Fiscal Year	Dairy products	Meat, fish & poultry	Grain & cereal products	Potatoes	Legume vegetables	Root vegetables	Garden fruits	Oils, fats & shortening	Sugars and adjuncts
1971		47	13						
1972	6	46	6		6.	3	3	17	6
1973	10	33	17	3				3	
1974		43							3
1975 (1st half)		40							
1976	—	13*							—

* Manske (1979), personal communication

Source: Jelinek and Corneliussen (1976)

(No entries signify zeros)

Table 2: Estimates of daily PCB intakes
(Total Diet Study-teenage male)

	Average daily intake of PCB's[a]	
Fiscal year	Total diet (µg/day)	Meat-fish-poultry food class (µg/day)
1971	15.0	9.5
1972	12.6	9.1
1973	13.1	8.7
1974	8.8	8.8
1975 (1st half)	8.7	8.7
1976	3.3*	3.3*

[a] Lower limit of quantitative reporting = 0.05 ppm with analytical method employed.

* Manske (1979), personal communication

Source: Jelinek and Corneliussen (1976)

The only food class in which PCBs were detected in 1975 or 1976 was meat, fish and poultry and all of the positive observations were trace values. It is likely that these positive finds are all due to PCBs in fish samples.

Jelinek and Corneliussen (1976) estimated the microgram per day intake of PCBs based upon the FDA Total Diet Program. These values which are listed in Table 2, must be viewed as only very crude estimates since, in order to obtain them, numerical values had to be assigned to trace observations. For those levels which were reported as trace, the decision was made to consider them to be at one-half the quantitative lower level of detection, i.e., .025 ppm (Nisbet, 1976a).

2.3 Levels in Fish

Based upon the FDA Total Diet Study (Johnson and Manaka, 1977), it appears that, for the U. S. population as a whole, the most significant exposure to PCBs from food is through fish. It does not seem possible to evaluate adequately at this time the overall distribution of exposures to PCBs through eating fish. The distribution of total fish consumption and consumption of tuna can be calculated from the Seafood Consumption Study (National Marine Fisheries Service, 1976), but the distribution of consumption of other species is not available. Moreover, the concentration of PCBs in fish is highly variable, both between species and within a single species. Fish caught further offshore tend to have smaller amounts of PCBs than estuarine fish, and

freshwater fish caught in areas of high PCB pollution tend to have the highest concentrations of PCBs.

Butler and Schutzmann (1978) reported on PCB residues in juvenile estuarine fish as part of the National Pesticide Monitoring Program. This report is based upon 1524 analyses collected in 144 estuaries nation-wide during the period 1972 to 1976. The annual incidence of PCB residues and the average residue discovered are given in Table 3. These data indicate a possible gradual decline in both the maximum residues observed and average concentrations. This is likely due to the general curtailment in production and use of these compounds. It appears from Table 3, however, that PCBs will continue to contaminate estuarine fish for many years to come.. Table 4 indicates the geographical distribution of PCB residues in estuarine fish. We note that average concentrations vary widely from state to state. Although these data are indicative of geographical distribution and time trends of PCB concentrations, they only represent a lower bound to concentrations of PCBs in fish caught in these waters for human consumption. This is because the fish sampled were generally no more than one year old. Fish caught for human consumption are usually older and consequently more heavily contaminated with PCBs.

Walker (1976) summarized the PCB levels in freshwater fish as determined by the National Fish and Wildlife Monitoring Program.

> Geographically, the higher concentrations appear to be associated with certain river systems having industrial activity . . . . PCB residues expressed as Aroclor 1254 were found in five major river systems in the Atlantic coastal region, with residues exceeding 5 mg/kg. Four of these stations had residues exceeding 10 mg/kg during the last 5 years. Fish in four of the Great Lakes stations had PCB concentrations exceeding the 5 mg/kg level and all stations reported concentrations

Table 3: Annual incidence of ΣDDT, PCB, and dieldrin residues in juvenile whole fish samples, 1972-76

Year	No. of Samples	DDT % Positive	DDT Maximum Residue	DDT Average Residue[1]	PCBs % Positive	PCBs Maximum Residue	PCBs Average Residue[1]	Dieldrin % Positive	Dieldrin Maximum Residue	Dieldrin Geometric X
1972	167	46	1425	63	34	4903	340	7	140	21
1973	453	34	662	38	13	2363	429	6	140	36
1974	380	66	1640	43	29	3078	320	8	143	12
1975	336	34	4083	69	30	7039	460	1	13	14
1976	198	36	2266	56	22	1254	331	0	—	—

[1]Arithmetic average of the geometric means of positive samples from each coastal area.

Source: Butler and Schutzmann (1978)

Table 4: Frequency and average concentration of PCB residues in juvenile estuarine fish by coastal area, 1972-76

Coastal Area	Frequency of Residues, %	Coastal Area	Average Concentration, µg/kg[1]
Connecticut	87	Washington state	1674
Rhode Island	75	Delaware	780
New York	63	Virginia	439
Delaware	52	Virgin Islands	379
Virginia	38	Rhode Island	330
Maryland	36	Connecticut	333
Puerto Rico	32	Georgia	332
California	31	Maryland	306
Florida	30	New York	362
Texas	24	Louisiana	256
Alabama	23	Puerto Rico	345
Virgin Islands	18	Hawaii	344
Washington state	17	North Carolina	343
Hawaii	14	California	379
Oregon	10	Oregon	163
North Carolina	9	South Carolina	162
Georgia	3	Alabama	163
Louisiana	2	Texas	125
South Carolina	1	Florida	23
Alaska	0		
Mississippi	0		

NOTE: Comparisons are limited in that the number of samples, number of sampling stations, periods (years) of sampling, and species of fish differ for each coastal area.

[1]Arithmetic average of geometric means of positive samples in all collection years.

Source: Butler and Schutzmann (1978)

exceeding 0.5 mg/kg. In the Mississippi River system, the Allegheny and Ohio were the hot spots, with seven out of the eight stations reporting residue concentrations in excess of 5 mg/kg. Thirty-one of thirty-five stations in this river system reported residues in excess of .15 mg/kg in the 1970-73 sampling programs. The highest residues, often exceeding 10 mg/kg were found in the Allegheny, Kanasha, Cumberland, Tennessee, and Ohio Rivers along with stations on the Mississippi River at Memphis, Tennessee, and the Missouri River at Herman, Missouri. Other monitoring stations that were found to have residue levels exceeding 5 mg/kg during the sampling periods 1970-73 included: the Willamette River on the Columbia system; the Rouge River in the Pacific coastal drainage; the Sacramento River in California; the Chena River tributary of the Yuken in Alaska; and the Rio Grande, Alabama, and Mississippi Rivers in the Gulf States region. Only in two sample periods of 1972-73 and in the current monitoring samples, which are still yet to be fully analyzed, has there been a downward trend, but this occurs only in these samples where residues are not being detected. The stations where high residues have been noted in the past still remain relatively contaminated with PCB. Unlike the decline of DDT in Great Lakes fishes, PCB concentrations do not show significant changes and may trend upward in salmonids.

An analysis of PCB levels in fresh-water fish in New York State from 1971 through 1975 by the New York State Department of Environmental Conservation (Spagnoli and Skinner, 1977) indicated that Hudson River fish contained the highest known PCB concentrations within the United States. Levels in individual fish were often found to exceed 100 ppm. The highest individual concentration recorded was 538.25 ppm in a large eel.

Lake Michigan is another body of water that has a high level of contamination by PCBs. Table 5 shows data from a three-year study of two species of Lake Michigan fish by the U. S. Fish and Wildlife Service Laboratory (Humphrey, 1977). As shown, the PCB levels in lake trout rose during the three year period and levels in Coho salmon did not change appreciably.

Table 5: PCB contamination in whole fish[a]

Species & Location	Collection Year	Length (inches)	Sample Size	Mean PCB Value (ppm)
Lake Trout near South Haven	1972	20 - 28	9	12.86 ± 4.75
	1973	20 - 28	30	18.93 ± 2.08
	1974	20 - 28	30	22.91 ± 3.75
Coho Salmon near Ludington	1972	20 - 32	10	10.83 ± 2.12
	1973	20 - 32	29	12.17 ± 0.77
	1974	20 - 32	30	10.45 ± 0.92

[a]Data from the Great Lakes Fishery Laboratory, U.S. Bureau of Sport Fisheries and Wildlife.

Source: Humphrey (1977)

2.4 Human Exposure from Consuming Lake Michigan Sport Fish

A two year study (1973-1974) was made under an FDA contract (Humphrey, 1977) of persons who regularly consumed PCB-contaminated Lake Michigan sport fish and randomly selected persons who did not consume such fish. A total of 180 adults participated in the study including 91 who consumed more fish than recommended by the Michigan Department of Public Health (no more than one meal per week or 24 pounds per year), 31 who consumed less than 6 pounds per year, another group who consumed an intermediate amount, and 19 fish eaters who consumed fish primarily from Lake St. Clair which are not as heavily contaminated with PCBs as fish from Lake Michigan. A medical record, a dietary record, and blood specimens were obtained for all participants. Summaries of mean PCB levels of participants arranged by city of residence and level of fish consumption is shown in Table 6. In 1973 those who annually consumed 24 or more pounds of sport fish from Lake Michigan had a mean blood PCB level of 0.073 ppm; persons annually eating 6 pounds or less had a mean level of 0.020 ppm; persons eating no fish had averaged 0.017 ppm; and persons eating 24 or more pounds from Lake St. Clair (who resided in Algonac) had an average blood level of .023 ppm. A comparison of PCB blood levels in persons in the group with highest consumption of Lake Michigan fish to PCB levels in those who ate no fish revealed a highly significant difference in mean PCB levels ($p << .001$). A similar comparison between persons who occasionally ate Lake Michigan fish (1-6 pounds per year) with those who ate no fish also indicated a highly significant difference ($p << .002$). The level of PCBs found in the blood of participants did not change significantly from year to year, nor did it diminish

Table 6: Year to year comparison of mean PCB blood levels for consumers of Lake Michigan fish

City	Mean Total PCB Value (ppm) 1973			Mean Total PCB Value (ppm) 1974		
	Participant Group			Participant Group		
	Control	Intermediate	Exposed	Control	Intermediate	Exposed
Traverse City	0.017	0.026	0.060	0.019	0.038	0.061
Manistee	0.025	0.068	0.109	0.028	0.029	0.120
Ludington	0.021	0.048	0.062	0.024	0.058	0.055
South Haven	0.028	0.036	0.083			
Algonac	-	0.029	0.023			
Group Mean First 5 Cities	0.020	0.044	0.073	0.023	0.041	0.075

Source: Humphrey (1977)

significantly when fish consumption was eliminated for up to nine months. PCB levels measured during the study in cooked Lake Michigan fish are exhibited in Table 7. A comparison with Table 5 reveals that levels in cooked fish appear to be only 1/6 to 1/4 the levels in raw fish. The quantity of PCB ingested from eating Lake Michigan fish averaged 46.5 mg/year and ranged from 14.17 to 114.31 mg/year for the 91 participants eating more than 24 pounds per year. Within this same heavily exposed group, the PCB dose averaged 1.7 ug/kg/day (micrograms PCB per kilogram body weight per day) and ranged from .490 to 3.940 ug/kg/day.

One participant in the study gave birth to a child in January of 1979. A milk specimen from this individual contained 4 ppm PCB (fat basis--the specimen had 2% fat content), whereas a blood specimen collected at the same time contained .033 ppm total PCB.

As a group, the exposed participants had no health problems or medical conditions that could be correlated with PCB blood levels, exposure to Lake Michigan fish, or known symptoms of PCB poisoning.

2.5 PCB Levels in Human Milk

Savage (1979) reported on levels of PCBs in human milk sampled from 1038 individuals residing in 44 different states. Of the 1038 samples, PCBs were detected in all but 9 samples. Trace values were reported for 720 samples and the concentrations in the other 309 samples ranged from a low of 0.3 ppm (fat basis) to 16.92 ppm (fat basis). Eighty-one or 7.8% of the samples contained residues in excess of 2.5 ppm (fat basis) which was the temporary tolerance set by FDA for PCB levels in commercial milk.

Table 7: PCB levels in cooked Lake Michigan fish

LOCATION	FISH SPECIES								
	LAKE TROUT			SALMON			OTHERS*		
	PCB 1254 (ppm)			PCB 1254 (ppm)			PCB 1254 (ppm)		
	1973	1974	1973-74	1973	1974	1973-74	1973	1974	1973-74
Traverse City:									
Mean	2.80	2.22	2.52	2.78	3.49	3.28	1.43	1.11	1.25
No. Samples	11	10	21	3	7	10	6	8	14
Manistee:									
Mean	2.85	2.40	2.60	3.74	2.20	2.86	0.98	0.59	0.78
No. Samples	5	6	11	3	4	7	2	2	4
Ludington:									
Mean	3.36	4.02	3.58	2.50	2.65	2.55	1.06	0.86	0.93
No. Samples	6	3	9	7	4	11	3	1	4
South Haven:									
Mean	4.11			2.51			1.17		
No. Samples	6			6			2		

*Includes: Pike, Whitefish, Smelt, Chubs, Menominee and Perch

Source: Humphrey (1977)

To estimate the average concentration of PCBs in human milk, we took natural logarithms of the reported concentrations in whole milk. Estimates $\hat{\mu}$ and $\hat{\sigma}^2$ of the mean and variance of the log-concentrations of PCBs in whole milk were calculated using the method of Cohen (1961) for estimating the mean and variance of a normal distribution from censored data. We then estimated the mean concentration of PCBs in whole human milk to be $\exp(\hat{\mu} + \hat{\sigma}^2/2) = \exp(-3.4124 + .6337/2) = .0453$ ppm. Since the average percent lipid content in the 309 samples was calculated as 4.497% we estimated the average concentration of PCBs in human milk to be .0453/.04497 = 1.007 ppm (fat basis). By way of contrast, the temporary tolerance set by FDA for infant and junior food is 0.2 ppm. Based upon our use of the log-normal distribution for PCB concentration in whole milk samples, we estimate that approximately 95% of the 1038 human milk samples had PCB concentrations in excess of 0.2 ppm (fat basis). We likewise estimate that the percent of human milk samples having more than 2.5 ppm, 5.0 ppm, 10 ppm is 7%, 1%, .05%, respectively. It would be difficult to estimate precisely the proportion of PCB contamination of human milk that is due to PCBs in food, but most of the contamination is likely due to dietary exposure.

The single measurement of 4 ppm (fat basis) of PCB in human milk of a consumer of Lake Michigan fish in the FDA study (Humphrey, 1977) indicates that consumers of PCB contaminated sport fish may have significantly higher levels of PCBs in their milk than women in general. To corroborate this single measurement, we considered comparisons of PCB levels in human blood plasma and milk made by Polishuk, et al. (1977) in a study involving 29 Israeli women. Average PCB levels measured were .0193 ± .0126 ppm in plasma, and .0442 ± .0412 ppm in whole

milk. Consequently, the concentration of PCBs in whole milk was .0442/.019 = 2.33 times the average concentration in plasma. If this ratio holds for the most heavily exposed group of fish eaters then the average concentration of PCBs in whole milk of humans who consume PCBs from fish at the same level as this heavily exposed group should be about (.073)(2.33) = .170 ppm. If further, we assume the average lipid content of whole milk is 4.497% (Savage, 1979), then the average concentration of PCBs in milk from these heavily exposed individuals should be about .170/.04497 = 3.78 ppm (fat basis). The level of uncertainty associated with this calculated value if fairly high. However, it does agree nicely with the value of 4 ppm measured in the single sample obtained in the FDA study (Humphrey, 1977).

Since high consumers of Lake Michigan fish were purposefully recruited for the study, it is not possible to estimate from the study the number of people in Michigan who consume such high levels of contaminated fish. However, based upon the information in the study, it seems reasonable to assume there is a sizable population of women who consume fairly high levels of PCB contaminated sport fish and who consequently have PCB levels in milk which average about four times (= 4 ppm vs. 1 ppm) the levels in the general population.

3. Carcinogenic Effects

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3.1 Human Data

In the early part of 1968 the accidental contamination of edible rice-bran oil led to an epidemic poisoning of the Japanese families who consumed this oil. The disease later became known as Yusho or rice-oil disease. The contamination of the rice-oil was due to a leak in a heat-exchange unit while the oil was being heated to remove some of its odorous components (NIOSH, 1977). The chief symptoms of Yusho disease were chloracne and eye discharges while other symptoms

included discoloration of the skin, headaches, fatigue, abdominal pain, menstrual changes and liver disturbances. Babies born to mothers who consumed the rice-oil were small-for-date and had temporary skin discoloration. The first symptoms of Yusho disease were registered on June 7, 1968 and 1291 cases had been reported as of May, 1975 (NIOSH, 1977).

The concentration of PCBs in the oil varied according to date of production and method of shipment. For canned oil produced on February 5, 1968, the PCB concentration ranged from 2000 to 3000 ppm, while the bottled oil produced on February 10, 1968 contained 134 ppm and only a trace was detected in samples of oil produced after February 19, 1968 (Cordle, et al., 1978). The main contaminant of the rice-oil was Kanechlor 400, but one important "minor" contaminant was polychlorinated dibenzofuran, present at about 1/200 the concentration of PCBs in the rice-oil (NIOSH, 1977).

An analysis of 146 of the total 1291 reported cases of Yusho disease was done in 1971. It was estimated that the lowest dose producing overt effects was 0.5 g (Cordle, et al., 1978) while the average ingestion producing these same symptoms was 2.0 g and the average volume consumed was 800 ml (NIOSH, 1977). But the question arises as to whether the effects noted are due in fact to the PCBs alone. The ratio of PCBs to PCDFs in the Yusho oil (containing "used" Kanechlor 400) was 200:1 whereas the ratio of PCBs to PCDFs in "unused" (unheated) Kanechlor 400 is 50,000:1. Thus with respect to PCBs the ratio of PCDFs in Yusho oil to PCDFs in rice-oil is 250:1. Also, the toxicity of PCDFs ranges from 200 to 500 times that of PCBs (Cordle, et al., 1978). Thus for equal amounts of rice-oil and pure Kanechlor 400, the

toxicity of the rice-oil would range from 2 to 3.5 times that expected from its PCB content alone.

Because of the uncertainty about the confounding of effects between PCBs and PCDFs, it is difficult to determine from the Yusho data exactly what effect(s) exposure to PCBs alone could have on humans. Careful records of the 1291 Yusho patients have been kept in an effort to determine possible long term effects. At least 9 out of the 29 deaths that occurred as of May, 1975, were attributed to malignant neoplasms (NIOSH, 1977), but a causal relationship between PCBs and cancer can necessarily be inferred because of the high concentration of PCDF in the oil. The Yusho study, nevertheless, had two important results: first, the information established that PCBs can be transferred from mother to fetus and from mother to child through breast feeding, and second, highly chlorinated PCB compounds are excreted more slowly from the body than the less chlorinated ones (NIOSH, 1977).

In a study of chemical workers (Zahm, et al., 1976, 1977), two malignant melanomas were diagnosed in 31 workers heavily exposed to Arochlor 1254 (and also exposed to other chemicals that could possibly cause cancer). It was estimated that .04 malignant melanomas would have been expected from this group of individuals. Among 41 other workers also, but less heavily, exposed to Aroclor 1254, one additional melanoma was diagnosed.

3.2 Data from Lifetime Animal Feeding Experiments

There have been a number of studies on neoplastic effects resulting from feeding PCBs to experimental rats or mice (Ito, 1973, Linder, et al., 1974, Kimbrough, et al., 1973, 1974, 1975, Calandra, 1976, NCI, 1978). Although each of these studies provides some evidence that

PCBs induce neoplastic lesions in rodents, only three of these experiments were of sufficient duration to be classified as lifetime feeding experiments. These three long-term experiments will be summarized below.

3.2.1 The Experiment of Kimbrough et al. (1975) on the Effect of Aroclor 1260 on Female Sherman Rats

Four hundred weanling Sherman strain CDES female rats 21-26 days old were divided randomly into two groups of 200 animals each. One group was maintained as controls and the animals in the other group were fed ad libitum a diet containing 100 ppm Aroclor 1260. Exposure was continued until 6 weeks prior to the termination of the experiment. When the animals were 23 months old all surviving animals were sacrificed and autopsied. A total of 184 dosed rats and 173 controls survived to the end of the experiment. Pathologic findings are summarized in Table E.

Of the three studies discussed in this section, this study provides the most convincing evidence of the carcinogenicity of PCBs. This study involved a relatively large number of animals and the increased incidence of hepatocellular carcinomas in the treated group is highly significant. Following are excerpts from the analysis of this study in the Criteria Document for PCBs (Kimbet, 1976a).

> This was a well-designed and well-conducted study, deviating from recommended protocols in only one respect: its limitation to female rats. . . . Two other limitations may be noted: (a) the rats were not exposed prior to weaning, although the authors . . . had indicated the rats exposed to PCB's during gestation and nursing showed early liver changes at much lower dietary levels; (b) the rats were killed at 23 months, relatively early in comparison to the 26-30 month expected life span. Each of these design features of the experiment, although common practice in carcinogenesis bioassays, is likely to have reduced its sensitivity in demonstrating carcinogenic effects. . . .

Table 8: Incidance and type of liver lesions and tumors of other organs examined histologically

Organ or tissue	Type of lesion	Incidence	
		Controls	Experimental
Liver	Hepatocellular carcinoma	1/173	26/184
	Neoplastic nodule	0/173	116/184
	Foci or areas of cytoplasmic alteration	28/173	182/184
Thyroid gland	Parafollicular cell tumor	37/160	15/156
Adrenal gland	Pheochromocytoma	1/173	1/161
Pituitary gland	Chromophobe carcinoma	41/153	28/139
	Carcinoma	0/153	1/139
Uterus	Endometrial polyp	28/149	28/163
	Adenocarcinoma	0/149	2/163
	Sarcoma of endometrial stroma	3/149	1/163
Urinary bladder	Transitional cell papilloma	1/167	0/169
Mammary gland	Fibroadenoma	11/173*	13/184*
	Adenocarcinoma	3/173*	1/184*
Salivary gland	Fibrosarcoma	1/173*	0/184*
Lung	Adenoma	2/173	2/184
Adipose tissue	Lipoma	0/173*	2/184*
Brain	Glioma	0/173	2/184
Ovary	Granulosa theca cell tumor	5/149	0/163
	Papillary adenoma	1/149	2/163
Hematopoietic system	Granulocytic leukemia	1/173	0/184
	Lymphoma	0/173	2/184
Kidney	Hemangioma	0/173	1/184
Thymus	Thymoma	1/113*	0/131*
Parathyroid gland	Adenoma	0/173	2/184
Skin	Fibroma	0/173*	1/184*

* [illegible] found in other [illegible]

Source: Kimbrough, et al..(1975)

3.2.2 The National Cancer Institute (1978) Bioassay of the Carcinogenic Effect of Aroclor 1254 in Fisher 344 Rats

Groups of 24 Fisher 344 rats of each sex were administered Aroclor 1254 at one of three doses, either 25, 50, or 100 ppm ad libitum for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All animals were 53 ± 2 days of age when placed on study. All surviving animals were sacrificed 104-105 weeks after the animals were placed on study. It was concluded that "under the conditions of this bioassay, Aroclor 1254 was not carcinogenic in Fisher 344 rats; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to the administration of the chemical. In addition, the carcinomas of the gastrointestinal tract may be associated with the administration of Aroclor 1254 in both males and females." The observed incidences of leukemias, lymphomas, and hepatocellular carcinomas are listed in Table 9.

Although Aroclor 1254 was not shown to be carcinogenic in Fischer 344 rats by the NCI Bioassay, it must be kept in mind that this was a relatively small experiment utilizing only 24 animals per dose group. The usual number of animals per group for an NCI bioassay appears to be 50. This study was part of a larger study designed to assess the combined effects of a group of chemicals which is likely the reason this bioassay is smaller than most. Overlooking for the moment the fact that the protocols were different and different Aroclors were tested, the results of the NCI bioassay and the Kimbrough study are not all that inconsistant. A χ^2-test that the risk of hepatocellular carcinoma at 100 ppm was the same for Kimbrough study as for the female rats in the NCI study was barely significant at the .05 level. The corresponding

Table 9: Incidences of leukemias and hepatocellular carcinomas in rats from NCI (1978) Bioassay of Aroclor 1254. Data is reported as no. animals with condition/no. animals examined for condition.

Dose (ppm)	Leukemias		Hepatocellular Carcinomas	
	Male	Female	Male	Female
0	3/24	4/24	0/24	0/24
25	2/24	6/24	0/24	0/24
50	5/24	5/24	1/24	0/24
100	8/24	4/24	2/24	0/24

χ^2-test applied to the Kimbrough study and the male rats in the NCI study was not significant ($p > 0.3$). Thus, because this experiment involved relatively few animals, it is simultaneously consistent with both no carcinogenic effect as well as an effect approaching that observed in the Kimbrough (1975) study.

3.2.3 Industrial Bio-Test Experiment with Charles River Rats

This experiment was described in an unpublished report (Industrial Bio-Test Laboratories, 1971) and a brief summary of results was presented at the National Conference on Polychlorinated Biphenyls (Calandra, 1976). The following comments on the experiment are taken from the EPA Criteria Document on PCBs (Nisbet, 1976a). One thousand Charles River strain albino rats were divided into 10 treatment groups. One hundred rats (50 male and 50 female) served as controls and 100 rats (50 male and 50 female) were included in each of nine exposed groups which were fed diets containing 1, 10, and 100 ppm of Aroclors 1242, 1254 and 1260 respectively. Dosage started when the animals were about 4-6 weeks old and continued for 24 months. The liver slides from this study have been examined twice by the original pathologist, once in the original report (Industrial Bio-Test Laboratories, 1971) and in a later report (Monsanto, 1975). The diagnoses in these two examinations were markedly different. For example, in the animals dosed at 100 ppm the earlier examination diagnosed one hepatoma and two animals with nodular hyperplasia, whereas the later examination diagnosed eleven animals with hepatomas and twenty-eight animals with nodular hyperplasia. A summary of the results of the latter pathological examination as given by Nisbet (1976a) is listed in Table 10.

Table 10: Liver pathology reported in Industrial Bio-Test rat experiment: 1975 re-diagnosis

Dose rate (ppm):	Control	Aroclor 1242			Aroclor 1254			Aroclor 1260		
		1	10	100	1	10	100	1	10	100
No. of animals	23	31	30	20	31	26	27	25	23	27
Vacuolar change	1	7	8	9	8	10	13	5	8	10
Focal hypertrophy	0	2	3	8	3	5	13	3	10	11
Nodular hyperplasia	1	0	2	6	0	3	13	0	9	7
Hepatoma	0	0	0	2	0	0	4	0	0	5
Nuclear hyperplasia	5	3	3	3	6	3	4	6	5	14
Cholangio-hepatoma	0	0	0	1	0	0	2	0	0	2
Hepatocellular necrosis	1	1	1	0	3	1	2	4	2	6

Source: Nisbet (1976a)

For some reason that was not explained, there was unusually high mortality among the rats in the experiment. The numbers of rats were further reduced by interim sacrifices during the course of the experiment. For example, only 6-21 animals out of the initial 100 in each treatment/sex subgroup fed 100 ppm survived to the terminal sacrifice. Even so, the re-diagnosis of the liver pathology (Table 10) indicates a significant tumorigenic effect. The incidence of nodular hyperplasia is significantly elevated in the group fed 10 ppm Aroclor 1260 over the incidence in the control group ($p << .005$, Fisher's exact test). There are a total of 9 hepatomas in the groups fed 100 ppm of one of the three Aroclors, but none in the groups fed lesser concentrations of PCBs.

3.3 Synergistic Effects

The synergistic effects of PCBs acting in conjunction with other chemicals may be quite significant in some instances, although quantitative estimates of these effects at environmental levels of exposure would be quite tenuous.

Ito et al. (1973) reported on experiments in which groups of 12-30 dd male mice were exposed to various levels of PCBs (Kanechlors KC-300, KC-400, KC-500 at 100-500 ppm in the diet) alone or in combination with one of three isomers of BHC (hexachlorocyclohexane β-BHC, δ-BHC, γ-BHC at 50-250 ppm in the diet). In the groups exposed to PCBs alone, only the group exposed to 500 ppm KC-500 developed hepatocellular carcinomas (5 animals out of 12 exposed). In the groups exposed to BHC alone, only the group exposed to 250 ppm α-BHC developed hepatocelluler carcinomas (8 animals out of 30). However, carcinomas

were induced within 24 weeks by joint feeding of PCBs and BHC at levels below those which had no effect when fed independently. The combinations of 100 ppm α-BHC and 250 ppm KC-500 produced 1 hepatocellular carcinoma in 25 animals; 50 ppm α-BHC and 250 ppm KC-500 produced 2 hepatocellular carcinomas in 30 animals, 250 ppm each of δ-BHC and KC-500 produced 6 hepatocelluler carcinomas in 29 animals. The authors concluded that PCBs promoted the induction of tumors by α-BHC and δ-BHC.

Other experiments have produced negative and even antagonistic results. In an experiment with mice (Uchiyama, et al., 1974), PCBs (KC-400) fed in the diet did not appear to accelerate the development of cervical carcinoma induced by methylcholanthrene. In another study in rats (Makiura, 1974), feeding with PCBs for 24 weeks appeared to inhibit the induction of hepatocellular carcinoma by three other carcinogens.

VCM (vinyl chloride) is a known human liver carcinogen and is also a mutagen, but both its carcinogenicity and mutagenicity appear to depend on metabolism in the liver to an active metabolite (Rannug, et al., 1974). The mutagenicity of VCM has been demonstrated only in the presence of liver microsomal preparations, including those stimulated by PCBs (Nisbet, 1976a). This strongly suggests that there may be a synergistic effect between PCBs and VCM in the production of tumors.

4. Quantification of Carcinogenic Risk

4.1 Calculation of Virtually Safe Doses from Different Mathematical Models

To illustrate the computation of virtually safe doses (VSDs) by extrapolation of the results of animal experiments to low dose levels,

we have selected the three data sets listed in Table 11. To this data we have applied three dose response models and related extrapolation procedures; the probit model (Mantel, et al., 1975).

$$P(d) = C + (1-C)\int_{-\infty}^{a+b\log_{10}d} (2\pi)^{-1/2}\exp(-y^2/2)dy \qquad [1]$$

the one-stage or one-hit model (Crump et al., 1977)

$$P(d) = 1-\exp[-(q_0 + q_1 d)], \qquad q_0, q_1 \geq 0, \qquad [2]$$

and the multistage model (Crump, et al., 1977)

$$P(d) = 1-\exp(-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)), \qquad q_0, \ldots, q_k \geq 0 \qquad [3]$$

The gamma multihit model (Rai and Van Ryzin, 1978) was also considered. This model requires at least two positive experimental doses and could not be applied to the first two data sets. When applied to the third data set, the computer algorithm for computing the estimates failed to converge (Van Ryzin, personal communication).

For each of these models, P(d) represents the probability of a particular tumorigmic response in a single animal fed a test substance at a dose-rate d during the course of a lifetime feeding experiment. A discussion of these models and their theoretical rationale is given in the Appendix.

The results of applying these extrapolation procedures to the data sets in Table 11 are summarized in Table 12. In this table are computed maximum likelihood (best-fit) estimates of the dose required to produce extra risks over background risk of 10^{-8}, 10^{-6}, 10^{-5}, respectively. Lower 95% confidence bounds for each of these doses are also computed from the various models. Maximum estimates are not recorded for the method based upon the probit model since this method was not designed to fit data. As explained in the Appendix, the

Table 11: Data sets used for calculating virtually safe doses in Table 12.

Data Set #1--Kimbrough et al. (1975) rat study with Aroclor 1260--Hepatocellular carcinomas

Dietary level (ppm)	No. of animals	No. animals with Hepatocellular carcinomas
0.	173	1
100	184	26

Data Set #2--Kimbrough et al. (1975) rat study with Aroclor 1260--Liver neoplastic nodules

Dietary level (ppm)	No. of animals	No. animals with Neoplastic Nodules
0	173	0
100	184	144

Data Set #3--Industrial Bio-Test rat experiment with Aroclor 1260--Liver neoplastic nodules

Dietary level (ppm)	No. of animals	No. animals with Neoplastic Nodules
0	23	1
1	25	0
10	23	9
100	27	7

Table 12: Virtually Safe Doses computed from data in Table

Data Set	Analytic Method	Maximum likelihood estimates of dose in ppb. corresponding to extra risk of			Virtually safe doses (lower 95% confidence bounds for dose) in ppb. corresponding to extra risk of		
		10^{-8}	10^{-6}	10^{-5}	10^{-8}	10^{-6}	10^{-5}
I	Probit				1.96	14.2	43.6
I	One-Hit and Multistage	.0069	.686	6.86	.0051	.511	5.11
II	Probit				.025	.180	.552
II	One-Hit and Multistage	.00063	.063	.634	.00055	.0551	.551
III	Probit				.178	1.29	3.97
III	One-Hit	.0046	.465	4.65	.00235	.235	2.35
III	Multistage	.0046	.465	4.65	.00205	.205	2.05

parameter b appearing in this model is set equal to unity regardless of the data. This designation precludes the model from providing an adequate fit to most data sets. Likewise, the gamma multihit model is applied only to data set III since this model cannot be applied to data containing only one experimental dose.

These models yield quite different values for the lower confidence bounds on dose. These divergent results are predicted by and, to some extent, explained by the discussion in the Appendix. The probit procedure will typically estimate virtually safe doses larger than those predicted by the multistage models for extra risks below about 10^{-3}. Virtually safe doses based upon the multistage model are identical with those based upon the one-hit model when there is only one positive experimental dose. This is illustrated with data sets I and II. The multistage procedure also yields virtually the same values as the one-hit model for data set III since introduction of the parameters q_2, q_3, etc. into the model cannot improve the fit to this data. However, as explained in the Appendix, the multistage can yield higher values for the VSD then the one-hit model whenever the data exhibit upward curvature and are inconsistent with the one-hit model.

To compare with the VSDs in Table 12, we estimate from Table 2, that the average consumption of PCBs in the United States in 1976 was about 3.3 ng/day ÷ 1557 gm/day = 2.1 ppb of PCB, assuming an average daily food intake of 1557 grams.

4.2 Estimates of Risk from PCBs in Diets of U.S. Adults

Next we shall estimate levels of risk for the general U.S. population and for the population of consumers of fresh water fish. The

risks from consuming human milk in infancy present some special difficulties and will be dealt with separately. The data of Kimbrough et al. (1975) on the increased incidence of hepatocellular carcinomas in rats fed Aroclor 1260 provides the most convincing evidence of carcinogenicity of PCBs. Our estimates of risks to human populations will therefore be based upon this data. The fact that only one dose level of PCBs was used in this experiment means that the one hit is the only one of the models discussed previously that can be utilized. The Mantel et al. (1975) procedure based upon the probit model [1] is an operational procedure for calculating a virtually safe dose. Since the parameter b in the model is not chosen based upon the data, the method should not be used to calculate "best" estimates (e.g., maximum likelihood estimates) or risk at a given dose. Except for the one-hit model the other models require at least two positive experimental doses for their utilization.

In Table 13 are exhibited lifetime risks of cancer based upon extrapolating the Kimbrough data from the experimental doses to PCB exposure levels in humans. This process involved two steps. First, exposure levels in humans were converted to equivalent exposure levels in rats. Results are given for two methods for making this conversion: 1) on a ppm of PCBs in total diet basis and 2) on a mg intake of PCBs per kg body weight per day basis. The next step is to calculate the extra risk of hepatocellular carcinoma at this dose based upon a one-hit model fit to the animal data. The risks exhibited in Table 13 are based upon the one-hit model which fits the animal data best. Upper confidence bounds on these risks are not listed but an upper 95% confidence bound is given by multiplying all corresponding risks in the table by 1.5.

Table 13: Estimates of lifetime extra risk to humans of hepatocellular carcinoma based upon applying a one-hit model [2] to the Kimbrough et al. (1975) rat study.

Human PCB Dosage	Risks calculated from converting human dose to animal dose on the basis of: ppm in diet	mg/kg/day
8.7 ug/day (1975 Total Diet Study, Table 2)	1/123,000	1/288,000
3.3 ug/day (1976 Total Diet Study, Table 2)	1/328,000	1/764,000
127 ug/day (Avg. intake of people consuming more than 24 lbs./yr. Lake Michigan fish, Humphrey (1977)	1/8,000	1/20,000

The estimates of dose from the total diet study (Table 2) are tenuous. The estimates for the years 1975 and 1976 are based upon 8 and 3, respectively, trace findings of PCBs in 20 samples from the meat, fish, and poultry food class and no findings at all in samples from other food classes. The PCB levels in Table 2 were estimated by assuming that .025 ppm PCB (1/2 of .05 ppm, the lower limit of PCB quantification) were present in all food samples in which trace amounts of PCBs were found (Misbet, 1976a).

In computing the risk estimates, no allowance was made for the composition of PCBs in food to which humans are exposed being different from the composition of Aroclor 1260 used in the experiment. As indicated in an earlier section, however, it appears that the higher clorinated biphenyls predominate in the environment and consequently the composition of PCBs in food should not be too different from Aroclor 1260. Also, no allowance was made for the fact that PCB levels in human adipose tissue are likely much greater than corresponding levels in rats for a given level of dietary exposure.

The risk estimates are computed for a hypothetical human population exposed throughout their lifetime to current levels of PCBs in food. In actuality, PCB levels appear to be decreasing.

Based upon the estimates in Table 13 the expected number of extra cases of hepatocellular carcinoma per year resulting from a nationwide exposure at the dietary level detected in the 1976 Total Diet Study (3.3 µg/day) would range between 220,000,000/(738,000 - 70) = 11.1 cases/year and 220,000,000/(764,000 - 70) = 4.1 cases/year.

Using the assumptions of a one-hit model, figures on total catch of Lake Michigan fish (Humphrey, 1977) and estimates of PCB levels in

Lake Michigan fish, we can estimate the total expect number of hepatocellular carcinomas among residents of Michigan from consuming PCBs in Lake Michigan fish. From Table 2 in Humphrey (1977) we estimate that in 1974, Michigan sport fishermen caught 4,065,930 lbs. of lake trout, 6,979,500 lbs. of salmon, and 2,930,220 of other sport fish from Lake Michigan. From Table 7 it is estimated that cooked portions of these fish contained an average of 3.26 ppm, 2.88 ppm, and 1.0 ppm PCBs, respectively. Assuming that 1/3 of the total of weight of these fish were injested by humans, it follows that a total of approximately $[4,065,930 \times 3.26 + 6,979,500 \times 2.88 + 2,930,220 \times 1.16] \times 10^{-6} \times .333$ = 12.28 lbs. of PCBs were injested by humans from eating these fish. Since, according to Table 12 injesting 127 ug/day of PCB ($127 \times 10^{-6} \times 365 \times 70 = 3.2$ gm in a lifetime) would produce on the average about 1/8,000 tumors, 12.28 lbs. = 5575 gms of PCBs would produce (1/8000) × (5575/3.2) = .22 hepatocellular carcinomas/year.

4.3 Estimates of Risk to Breast-Fed Infants

The population of infants whose diet during early life consists wholly or at least in large part of human milk has a much higher exposure to PCBs through food than the general population. As estimated in Section 2.5, human milk in the United States contains an average of 1 ppm (fat basis) PCBs with 7% of the samples containing more than 2.5 ppm (fat basis) PCBs. Further, it was estimated that there is a population of consumers of fresh water fish whose milk samples could contain on the average four times the levels in the general population.

Risks from exposures at such an early age are difficult to quantify. Studies on similar exposure patterns in animals have apparently not been done.

There are a number of animal studies in which various acute effects were detected with 1-10 ppm exposure to PCBs in food. Linder et al. (1974) exposed rats for two generations to 1, 5, 20 and 100 ppm PCBs. Significant increases in liver weights were observed in 21-day-old F_1 male weanlings at the 1 ppm level of Aroclor 1254 and in either sex of F_1 and F_2 weanlings at 5 ppm or higher levels of both Aroclor 1254 and 1260. It must be kept in mind, however, that because of biomagnification, concentrations of PCBs in milk may have been much greater than the levels in feed. Rhesus monkeys are considerably more sensitive to PCBs than rodents: Rhesus monkeys fed 3 ppm PCBs all died after 8 months (McNulty, 1976). Female Rhesus monkeys given 2.5 and 5.0 ppm Aroclor 1248 in their diets developed facial edema, swollen eyelids, erythema, loss of hair and acne within two months (Barsotti, 1976). Following 6 months of PCB exposure the female monkeys were bred to control males. There was a significant reproductive effect of the PCB in the diet as shown in Table 14. The six infant monkeys were permitted to nurse their mothers for 4 months. The mothers' milk contained 0.15-0.40 ppm of PCBs (whole milk basis). Within 2 months focal areas of hyperpigmentation, swollen lips and eyelids, loss of eyelashes, and acne form lesions of the face developed. Within four months, 3 of the 6 infants died due to PCB intoxication. From the data of Savage (1979) it is estimated that approximately 3% of human milk samples in the United States have PCB levels in excess of .15 ppm. (whole milk basis).

Any estimates of carcinogenic risk from exposures to PCBs in human milk on the basis of existing animal data must be regarded as extremely tenuous. Not only does one have to contend with the

Table 14: Modification in reproduction in primates that were exposed to Aroclor 1248 in the diet

	Control	2.5 ppm	5.0 ppm
Total impregnated (no./no. animals)	12/12	8/8	6/8
Resorptions or abortions (no./no. animals)	0/12	3/8	4/8
Stillborn (no./no. animals)	0/12	0/8	1/8
Normal births (no./no. animals)	12/12	5/8	1/8

Source: Allen and Norback (1976)

uncertainties of extrapolation from high doses in animals to low doses in humans, it is also necessary to make assumptions regarding the effect of a time-varying dosage. The effect of dosages early in life on carcinogenicity could be much different from equivalent doses later in life.

To illustrate what such an effect might be, we shall calculate some hypothetical risks of hepatocellular carcinoma by relating the cancer incidence observed in the Kimbrough et al. (1975) study to human PCB exposure levels in human milk. The calculation will be based upon the multistage model of cancer. Peto (1977b) indicates that this model may be particularly applicable to carcinomas. The model is capable of explaining observed high rates of increase of cancer with age without having to specifically posit an aging mechanism. In applying the model we must decide which stages PCBs might effect and the nature of the effect. Let us assume in the absence of evidence to the contrary, that PCBs affect only the first stage and that the effect is to increase the rate at which cells pass through this stage by an amount proportional to the quantity of PCBs present in the diet at the time. This assumption is a "worst case" assumption because with the particular multistage model we have in mind, cells pass through the stages in a prescribed order on their way to becoming cancerous. Consequently, exposures at infancy will have the greatest overall effect if they act upon the first stage.

With such a model, it can be argued that when exposure to PCBs is at a level D from birth to an age which represents an age-fraction f of the normal life span, with no exposure for the remainder of the lifetime, then the extra life-time risk of hepatocellular carcinoma

over risk at zero dose should be approximately of the form

$$P(D,f) = 1-\exp[-\beta D[1-(1-f)^k]] \qquad [5]$$

where k is the number of states in the multistage model. We estimate $\beta = .147 \times 10^{-2}$ from the Kimbrough et al. (1975) data by taking f = 1 and D = 100 ppm. This method of estimating β should tend to underestimate the carcinogenic risk since the rats in this experiment were exposed only after weaning and prior to 21.5 months of age, and were sacrificed at 23 months of age. With β estimated in this fashion we can now calculate theoretical extra lifetime risks of hepatocellular carcinoma from (5) for different values of D, f and k. The results of some such calculations are tabulated in Table 14.

These estimates are based upon unverifiable assumptions and should be regarded as hypothetical. They do illustrate, however, the carcinogenic effect that might result from doses of a carcinogen applied early in life, such as PCBs in human milk. Some of the assumptions made in constructing these estimates were conservative in that other assumptions which are equally reasonable would predict even higher cancer risks. For example, if we had converted dose from rats to humans on a mg/kg/day basis rather than on a ppm basis, the estimated risks would likely be greater. Cells in infants are going through mitosis much more often than cells in adults which might imply that they are at a greater risk per unit time from a carcinogenic insult than cells in adults. However, this possibility was not reflected in the model.

It should perhaps be pointed out that with this model (as well as possibly in real life), even though carcinogenic dose is very early

in life, a cancer eventually resulting from this dose can occur at any age.

The risks tabulated in Table 15 are perhaps more useful for comparative purposes rather than in an absolute sense. By comparing these values with those in Table 13, it appears that the subpopulation of breast-fed infants could be at much higher levels of risk of hepatocelluler carcinoma from PCBs than the U.S. population in general.

Table 15: Theoretical extra lifetime risks of hepatocellular carcinoma in breast-fed infants based upon Kimbrough et al. (1973) rat data.

A k-stage model of cancer is assumed with dose affecting the first stage only (Equation [5]). Conversion of dose from rats to humans is by assuming ppm of PCB in rat diet is equivalent to ppm (fat basis) in human milk.

	Extra risk to infant in general population (D = 1.0 ppm)		Extra risk to offspring of consumers of more than 24 lbs./year of Lake Michigan sport fish (D = 4.0 ppm)	
	Age at Weaning		Age at Weaning	
K	6 months (f=1/140)	12 months (f=1/70)	6 months f=1/140)	12 months f=1/70)
1	1/95000	1/48000	1/24,000	1/12,000
3	1/32000	1/16000	1/8000	1/4000
5	1/19000	1/9600	1/4800	1/2500

APPENDIX

[illegible] AND EVALUATION OF METHODS OF DETERMINING RISKS FROM CHRONIC LOW-LEVEL CARCINOGENIC INSULT

Basic Principles and Considerations

To aid in determining a proper regulatory action regarding [illegible]gen that is present in man's environment, whether it be [illegible] additive, industrial polutant, or otherwise, it is help- [illegible]ave some knowledge about the number of extra cancers [illegible]e likely to be caused by the presence of the carcinogen [illegible] environment. It is also helpful to have some knowledge [illegible] likely change in number of extra cancers that would [illegible]y some projected increase or decrease in the level of [illegible]xposure occuring either as a result of regulatory action [illegible]tion. This kind of information is usually impossible to [illegible] directly from human data. For this reason it is often [illegible]ry to use data from animal feeding experiments to estimate [illegible]isk. This procedure involves two difficult steps, 1) re- [illegible] the animal risk at high doses to doses very near to zero [illegible] relating the animal risk to risk in humans.

[illegible]ypically, animal experiments use on the order of 100 [illegible]s at each experimental dose. If a particular experimental [illegible]uses a lifetime increase in cancer risk of 1/10, this

crease can be measured with a small degree of accuracy using animals. But if the increased cancer risk is less than 1/100 is increase will often not even be detectable by an animal eding experiment. For example, if the true risk is 1/100 it uld require that over 400 animals be tested at that dose in der to be 99% sure of detecting any carcinogenic response at l (i.e., for there to be a probability of .99 that at least one imal gets cancer). If background or spontaneous carcinogenesis present even larger numbers of animals will be required. On e other hand, the extra human risk that we may want to esti- te resulting from environmental exposure is usually (and hope- lly) much smaller than 1/100 for any given chemical, perhaps the order of 1/1,000,000. It is clear that it would not be ctical to conduct an experiment with enough animals to measure ectly an increase in risk this small.

For these reasons the procedure has been developed of nducting lifetime animal feeding experiments using, in addition a control dose of zero, several doses at which the projected tra cancer risk may be 1/10 or larger. This high dose data is en used to estimate the extra risk at a dose where the extra sk may be no larger than, say, 1/1,000,000. An equally import- nt variant to this problem is the calculation of the so-called safe' dose, that is, a dose for which there is some measure of tistical assurance that the extra risk at that dose is no re than, say, 1/1,000,000. These problems are often referred to collectively in the literature as the "low dose extrapolation blem."

Importance of the Mathematical Model

Performing a low-dose extrapolation involves the choice of a mathematical function to model the dose-carcinogenic response relationship and the choice of statistical procedures to apply the mathematical function. The choice for this mathematical function turns out to be extremely crucial to the outcome of low-dose risk estimation. If the assumed relationship between tumor occurrance and dose does not apply in the regions to which the extrapolation is being made, a serious overestimate of the 'safe' dose may result (Mantel and Bryan, 1961, p. 458). Chand and Ko (1974) compared five standard dose response models and observed that they could differ by many orders of magnitude at low dose levels for which extra risks are on the order of 1/100,000,000.

It might be supposed that it should be possible to discriminate among the various potential dose response functions on the basis of experimental data but, unfortunately, two different dose response functions can often fit experimental data equally well but still differ by several orders of magnitude at very low doses. Moreover, even if a particular dose response function were to give a significantly better fit to data than several others this would still not furnish assurance that this function would necessarily correlate in any way with the true dose response at very low doses where it is not feasible to measure the true extra risk directly. As a consequence of the great disparity of dose response functions at low doses it is imperative that the dose response function be selected, neither arbitrarily nor solely on the basis of how well it can be made to

experimental data, but, insofar as is possible, it should reflect known or at least plausible information regarding the biological mechanisms through which a chemical induces or promotes cancer.

What Shape Should be Expected for the Dose Response Curve at Low Doses?

Tumors of so many different types arise in such a diversity of different tissues, their etiology is so little understood, and the agents that cause tumors affect a subject in such diverse ways, that it might seem that no general conclusions can be drawn. However, for a certain broad class of "directly-acting" chemical carcinogens the range of uncertainty associated with the shape of the dose response curve at low doses can be greatly narrowed. As used in this paper, the term "directly-acting carcinogen" encompasses (Guess, Crump and Peto, 1977) carcinogenic agents for which either the agent itself or a metabolite acts directly at the cellular level and produces a hereditable change which eventually leads to the formation of a tumor. Carcinogens which are carcinogenic by reason of their mutagenicity should fall into the category of "directly-acting carcinogens." Accordingly, carcinogens which test positively using the Ames mutagenicity screening test for carcinogenicity are very likely to be directly-acting. (See McCann and Ames, 1976). In a recent study (McCann, Choi, Yamasaki and Ames, 1975) in which about 300 carcinogens and non-carcinogens were tested using the Ames Test, 90% (157 out of 175) of the carcinogens were mutagenic including almost all of the

known human carcinogens. This indicates that the class of directly-acting carcinogens may encompass most of the known carcinogens.

-- A partial solution to the low-dose extrapolation problem for the case of directly-acting chemical carcinogens has been given in Peto (1977), Crump, Hoel, Langley and Peto (1976), and Guess, et al. (1976). The key result is that, at least as long background carcinogenesis is present, we should expect the dose response curve not to be absolutely flat at zero dose. What this means is simply that when risk is plotted against dose response on ordinary linear scales, the tangent line to the dose response curve at zero dose should have a positive slope. When a dose response function has this property we will say it is <u>linear at low dose</u>. This simple property can have far-reaching consequences for low-dose extrapolation. For example, consider the two potential dose response functions 1) $0.1(\frac{99}{999}\, d + \frac{900}{999}\, d^2)$ and 2) $.1d^2$ for the dose interval $0 \leq d \leq 3$. Both of the curves give a risk of 1/10 at a dose of $d = 1$ and are practically indistinguishable at higher doses. However, at a dose of $d = 1/1{,}000$ 1) predicts a risk of 1/100,000 and 2) predicts a risk of 1/10,000,000, a difference of two orders of magnitude. We note also that 1) has a tangent line with a positive slope at $d = 0$ whereas 2) does not.

One explanation of why the dose response function should be linear at low dose when background is present may be found in Crump, et al. (1976) and Peto (1977) and will be briefly outlined here. When background carcinogenesis is present, the cellular mechanism through which the test agent produces cancer should

already be operative in producing background tumors. When this is true the effect of the test agent is to add to an already on going process. The result of this additive effect is illustrat in Figure 1. The dose response curve is for all tumors produce through the mechanism through which the test agent acts. Background carcinogenesis is allowed for in the Figure by an effective background dose d_o. We see that, in this case, the added risk caused by a dose d of the test agent should be expected to increase approximately linearly near d = 0 (i.e., the tangent line at d = 0 will have a positive slope). Implicitly assumed by the way Figure 1 is drawn is the fact that an added dose of a carcinogen acting through this mechanism does not produce a smaller risk. If background carcinogenesis is allowed for as in Figure 1 by positing an effective background dose d_o which is estimated from the data then the wide range of risks obtained using different models effectively disappears (Peto, 1977). We note that the existence of a tangent line with a positive slope at zero dose does not, in itself, imply any lower bound for extra risk at low doses since the slope of the tangent line could possibly be very small.

The evidence given above for a positive slope to the dose response function at zero dose applies particularly to the case in which background carcinogenesis is operative. This does not imply that we expect the dose response curve not to be linear at low dose in the absence of background carcinogenesis. For example, the multistage models of cancer (Armitage and Doll, 1961) are a fairly broad class of models in which it is assumed that

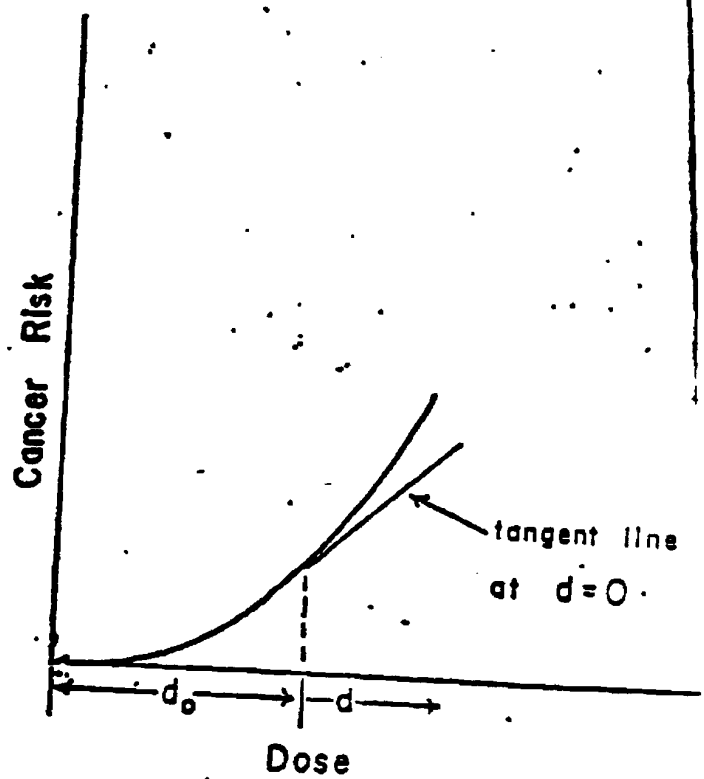

Figure 1. Illustration of why the dose response c
should be linear at low dose in the presence of backgroun
cinogenesis. d_o is the effective background dose and d i
dose of the carcinogen of interest.

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a number of events are required to occur at the cellular level to initiate cancer. Although all models in this class are linear at low dose provided background carcinogenesis is present, a suitable subclass of them are linear at low dose in the absence of background carcinogenesis (Crump, et al., 1976).

Watson (1977) has recently proposed a more specialized model for cancer induction and promotion based upon reversible epigenetic cellular changes. Watson concludes that his model supports the low-dose-linearity hypothesis and states "as suggested by a different argument of Crump, et al. . . . , it is reasonable to assess the risk due to an additional carcinogen at low constant dosage by a linear relation."

The evidence for low-dose-linearity given above applies mainly to directly-acting carcinogens. An indirectly acting carcinogen might be one which causes some gross physiological change such as suppression of ovulation which could predispose the subject to cancer. For such carcinogens the shape of the dose response curve at low dose is highly speculative. There could possibly be a threshold dose below which the agent has no carcinogenic effect at all on an individual. However even if a threshold mechanism is operative, there is likely to be considerable variation in individual thresholds in a large population. Consequently the dose response curve for the entire population could still exhibit a linear trend at risks as low as 1/1,000,000 or lower.

The effects of metabolic activation and detoxification upon carcinogenic dose response have been recently considered by

Cornfield (1977) through a kinetic model that encompasses free toxic substance, metabolite, deactivator, and the interactions of these substances. Only a steady state situation is studied in that variation over time of the concentrations of these agents is not considered. The model predicts a threshold dose below which there is no carcinogenic risk under the assumption that the deactivator is 100% efficient in deactivating the carcinogen. However, in a naturally occurring process it is likely that deactivation would not be perfect and would be less than 100% effective in always combining with 100% of the carcinogen before an amount of the active metabolite reaches a cancer target site. Any of a number of modifications to the model to allow for non-perfect deactivation would rule out a threshold and would lead directly to a model for which carcinogenic response varies linearly with dose at low doses. Cornfield's own modification of perfect deactivation, that of allowing the deactivating reaction to be reversible, leads, as Cornfield points out, to a model which is linear at low dose. This occurs regardless of how slowly the reverse reaction takes place, as long as the possibility is not eliminated entirely. Furthermore, even in the extremely unlikely case of perfect deactivation, an otherwise realistic model should still imply low-dose-linearity since the theoretical time required for perfect deactivation would not be zero and would likely be infinite.

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For most, perhaps all, carcinogens, the mechanisms through which cancer is produced is not sufficiently understood so that the shape of the carcinogenic response curve can be theoretically

predicted with certainty. As pointed out earlier, neither can experiments of sufficient size be conducted that would permit direct experimental investigation of the dose response curve at low dose. We have noted that there are plausible arguments that the dose response curve is linear at low dose for many carcinogens. On the other hand, this author knows of no serious proposal of a mechanism that would lead to a more conservative dose response relationship such as the risk varying approximately as the square root of dose at low dose. In view of these uncertainties it would seem reasonable to base estimates of added risk of cancer upon a mathematical model that encompasses low-dose-linearity unless, of course, the mechanism through which the carcinogen operates is sufficiently understood so that low-dose-linearity can be conclusively ruled out. Once the principle of low-dose-linearity is accepted the problem of estimation of risks at low dose is nearly solved. This is because the disagreement between the upper statistical confidence bounds on risk at low doses based upon a model that incorporates low-dose-linearity and one that does not is typically several orders of magnitude whereas the corresponding disagreement between two reasonable models both of which incorporate low-dose-linearity is usually much less than this.

Specific Methods for Low Dose Risk Estimation

A. Mantel-Bryan

The Mantel-Bryan procedure (Mantel and Bryan, 1961 ; Mantel, Bohidar, Brown, Ciminera and Tukey, 1975) has been selected by the Food and Drug Administration as part of th procedures for evaluating assays for carcinogenic residues edible products of animals. Before the Mantel-Bryan proce

itself is discussed, the "Sensitivity of Method" (SOM) document (Federal Register, Vol. 42, No. 35 - Tuesday, Feb. 22, 1977, p. 10412) will be reviewed briefly so that the role of the Mantel-Bryan procedure in official FDA policy can be understood and appreciated.

The so-called Delaney clause of Section 409 of the Federal Food, Drug and Cosmetic Act of 1958 prohibited the approval of any food additive that "is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of safety of food additivies, to induce cancer in man or animal . . .". This language was interpreted as forbidding FDA to approve the use of a carcinogenic substance as a feed additive for food-producing animals whether or not the compounds might leave any residues in the edible products of the animals. However, in 1962 Congress modified this prohibition as part of the Drug Amendments of 1962. Section 409(c)(3)(A) now reads in part:

> . . . . [N]o additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additivies, to induce cancer in man or animal, except that this proviso shall not apply with respect to the use of a substance as an ingredient of feed for animals which are raised for food production, if the Secretary finds (i) that, under the conditions of use and feeding specified in proposed labeling and reasonably certain to be followed in practice, such additive will not adversely affect the animals for which such feed is intended, and (ii) that no residue of the additive will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsections (f) and (g)) in any edible portion of such animal after slaughter or in any food yielded by or derived from the living animal . . .

Originally, the FDA applied this so-called "DES proviso" on a case-by-case basis without published criteria. However the FDA on February 22, 1977 published the "Sensitivity of Method" document which established objective criteria for acceptance of an assay method for detecting residues of a carcinogenic feed additive and for establishing post-administration withdrawal periods for these additives. Thus the document furnished an operational definition of the no-residue requirement of the DES proviso. This operational definition involves the application of the Mantel-Bryan procedure to animal experimental dose response data. The test-animal species/strains to be utilized are to have "the greatest possible susceptibility to the test compound" while at the same time being "appropriate models for man." (Federal Register, vol. 42, 1977, p. 10418). The Mantel-Bryan procedure is applied to the animal test data to determine residue level, designated S_o, corresponding to a risk of 1,000,000 in test animals (i.e., S_o is the "safe" dose level determined by the Mantel-Bryan procedure). The level S_o is expressed as a fraction of the total diet (i.e., parts per million). This level is adjusted to account for the respective portions of the human diet that is represented by the various meat products containing residues of the carcinogen being tested, transfer from animals to man being made on a fraction of meat diet basis. The resulting dose level S_m is "the level of total residues of carcinogenic concern that can be operationally defined as satisfying the no-residue requirement of the act for edible tissues" (Federal Register, Vol. 42, 1977, p. [illegible]

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The dose level S_m thus represents the upper bound to the lowest limit of reliable measurement that an approved assay method must satisfy.

The Mantel-Bryan procedure is thus an integral part of the "Sensitivity of Method" document. The procedure as originally set forth (Mantel and Bryan, 1961) and "improved" (Mantel, et al, 1975) is for the purpose of conservatively choosing a "safe" dose of a carcinogen, a "safe" dose being defined as one for which it can be expected that, with a given level of statistical assurance (e.g., 99%), the true dose producing a preassigned "safe" level (e.g., 1/1,000,000) of risk will lie above the "safe" dose. In the Mantel-Bryan procedure the mathematical model used for the dose response model is the probit function

$$P(d) = C + (1-C) \int_{-\infty}^{a+b\log_{10}d} (2\pi)^{-1/2} \exp(-x^2/2)\, dx \qquad [A1]$$

where d represents the dose of the carcinogen and P(d) represents the probability of a cancerous response in an animal subjected to a dose d. The parameters in the model are an intercept parameter a, a probit slope parameter b, and C, which represents the probability of a response in untreated animals. The parameter b is not estimated from the data but rather is arbitrarily set equal to 1. This choice is stated as being conservative (Mantel and Bryan, 1961), the argument for this being that typical dose data exhibit a probit slope in the experimentally observable region above 1% incidence that is greater than one. In Mantel and Schneiderman (1975) it was observed that a set of DES data (Gass, G. M., Coats, D., Graham, N., 1964, C3H females) exhibited

a probit slope of 1/2, but the general use of a probit slope of b=1 was still suggested. A slope of b=1 was adopted in the SOM document unless the experimental data exhibit a smaller slope, in which case the smaller value would be used.

With the probit slope parameter fixed at b=1 the remaining parameters, a and C, are estimated from the data and then adjusted so as to produce a higher level of risk as a given dose that corresponds to a upper 99% statistical limit on the true risk at a given dose. The value 99% is the one used in the SOM document but the choice is arbitrary. The safe dose is then determined to be the one producing a given low risk (e.g., 1/1,000,000) based upon the adjusted values of a and C. The risk value of 1/1,000,000 was also adopted in the SOM document.

As pointed out in Mantel, et al. (1975), the Mantel-Bryan procedure rewards larger and better experiments in that the more evidence there is of safety, the higher the calculated safe dose will be. However this advantage should be shared by any extrapolation method that uses reasonable statistical procedures.

Some have considered the Mantel-Bryan procedure as adopted in the SOM document to be too conservative (Federal Register, Vol. 42, 1977, p. 10419) in that it involves three conservative choices (99% statistical assurance, lifetime risk of 1/1,000,000, and probit slope set equal to 1) and that any one of these assumptions alone could provide adequate protection to the public. The first two of these choices are regulatory decisions that would have to be made with any extrapolation procedure. However, the arbitrary selection for the slope parameter seems to be peculiar

to the Mantel-Bryan procedure. To investigate its effect upon the extrapolation procedure a typical fit is presented in figure 2 (from Crump, 1977b) of the Mantel-Bryan probit curve [A1] to experimental carcinogenicity data when the probit slope parameter is fixed at b = 1. As can be readily seen the probit curve typically provides a very poor fit, curving downward even when the trend of the data is toward an increasingly upward curvature. This typically bad fit to data of the probit curve raises serious questions regarding the validity of statistical procedures associated with the Mantel-Bryan method. (See Salsburg, 1977 and Crump, 1977b.)

On the other hand, the Mantel-Bryan procedure utilizing the choice b = 1 was put forth as conservative procedure and it gives that appearance in Figure 1 since the probit curve appears to lie far above the trend of the data at the lowest doses. However as mentioned earlier and also pointed out by Mantel (Mantel and Bryan, 1961, p. 458) a procedure may, while appearing conservative at experimental dose levels, at the same time seriously overestimate the "safe" dose (i.e., be seriously anticonservative) if the assumed dose response relationship does not apply at the low risk levels to which extrapolation is being made. Thus, before the degree of conservation can be evaluated for any procedure, the properties of the dose response curve at very low doses must be evaluated.

As described earlier, there are strong arguments that indicate the dose response curve should be 'linear at low dose' (see p.50 for the definition of this concept) particularly for

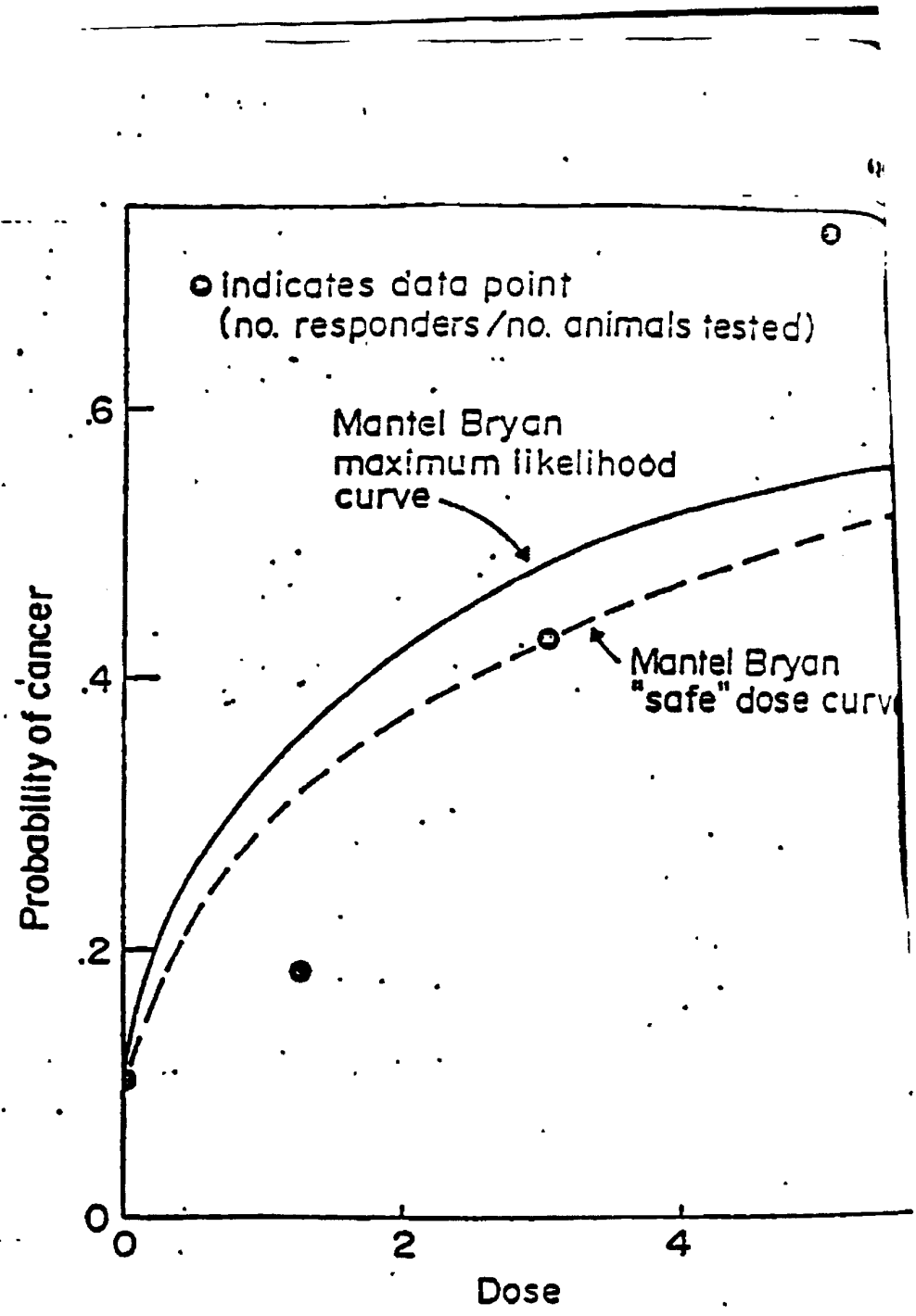


Figure 2. Typical fit of Mantel-Bryan curve to experimental data (from Crump, 1977b).

directly acting carcinogens in the presence of background carcino genesis. This has led Peto (1974) to recommend extrapolation pro cedures using only dose response functions from a class containin only dose response functions which are linear at low dose. At th very least, however, it would seem prudent not to go to the opposite extreme and use a dose response function which rules out linearity at low dose by assumption. However, the Mantel-Bryan procedure, through its use of the probit curve [A] rules out linearity at low dose in favor of a "flatness property" (see Hartley and Sielken, 1977, Mantel, 1977, and Crump, 1977b) at low dose which is anticonservative to the extreme. This property implies that mathematical derivatives of all orders of the probit curve approach zero (through positive values) as the dose approaches zero. This unusual property is most often discussed within the context of mathematical oddities rather than in connection with a scientific investigation. It implies that if the true dose response curve comes from an extremely broad class of functions known as analytic functions and which pervade scientific applications of mathematics, then the probit curve will eventually underestimate the true risk at low doses. Furthermore, at low enough doses, the degree to which the risk will be underestimated will be arbitrarily large (i.e., the ratio of the true risk to the probit estimate will grow arbitrarily large).

It was emphasized earlier that it is important when extrapolating to low doses for the assumed dose response function to incorporate known or at least plausible facts about the mechanisms of carcinogenesis. In neither the original paper

(Mantel and Bryan, 1961) nor in the paper outlining the improved version is biological justification given for the selection of a curve having the above described "flatness property." It should be mentioned at this point that the incorporation of background carcinogenesis into the Mantel-Bryan probit model [A1] using the parameter C implies that the mechanism through which the test carcinogen produces cancer is independent of the mechanisms through which all of the background cancers are produced (Crump et al, 1976). In keeping with the discussion in the last section it would seem more proper to incorporate background into the probit model by positing an effective background dose d_0 which adds to the dose d of the test carcinogen. If background is incorporated in this way the probit curve no longer has the "flatness property" and becomes linear at low dose (Guess, et al, 1977). In fact, with background incorporated in this way, the probit curve assumes a shape similar to the one-hit model, sometimes referred to as the most conservative of all procedures (e.g., Mantel, 1977).

Even though the "flatness property" implies the probit curve should at suitably low doses be anticonservative to the extreme, the "flatness" property holds only for doses approaching zero and the feature of arbitrarily fixing the probit slope at 1 mitigates the anticonservativeness implied by the "flatness" property at any given low dose (although the property itself will hold for all choices of the parameters a, b, c). The cancer risks that are typically extrapolated to are in the risk ranges 1/10,000 to 1/100,000,000. We will examine the outcome of

Mantel-Bryan extrapolations to these risk levels in a later section when we compare several different extrapolation procedures.

B. Linear Extrapolation

The technique for linear extrapolation was recommended by Hoel, Gaylor, Kirschstein, Safiotti and Schneiderman (1975) for use on an interim basis until better procedures could be developed. The procedure is straightforward and is based upon an assumed linear relationship between dose and response at low dose. The procedure utilizes only the data for the group of control animals and a single other dose group, usually either the highest dose that elicits no response or else the lowest dose that elicits some response. In the case there are no cancers in the control animals the 'safe' dose, based upon a maximum risk of 1/1,000,000 and 99% statistical assurance, is calculated as follows: An upper 99% confidence bound is calculated for the cancer risk in the dose group of animals. From this risk and dose one extrapolates back toward zero dose and zero risk using a straight line relationship. The dose corresponding to a risk of 1/1,000,000 on this straight line is taken to be the "safe" dose. If there are cancers in the control animals this procedure is modified to allow for the statistical treatment of the response in the control group while retaining the straight line relationship. When data at other experimental doses are available this method of linear extrapolation has the obvious shortcoming of not fully utilizing the available data.

A linear dose response curve is linear at low dose but the converse is not necessarily true. A curve can be linear at

low dose and still have a high degree of nonlinearity at higher doses.

Linear extrapolation is viewed by some as a very conservative procedure. For example, comments were made during the decision on which extrapolation procedure to incorporate into the SOM document to the effect that linear extrapolation is the most conservative of all procedures. Crump, et al (1976) examined the extent of the conservatism of a linear dose response function when compared with a multistage dose response model (Armitage and Doll, 1961). The multistage model assumes that a cell must go through a number of different stages before cancer is initiated in that cell and the model can encompass a high degree of nonlinearity. It was determined that the maximum possible degree of conservatism of a linear model relative to a multistage model depended rather heavily upon the incidence at the experimental dose relative to the background incidence. (This is consistant with the general relationship between background carcinogenesis and linearity at low dose as discussed earlier.) For example, when the incidence at the experimental dose is four times the incidence at zero dose the extra incidence at low doses derived from the linear dose response differs from the incidence derived from the multistage model by, at most, less than a factor of 2.5 regardless of the number of stages in the multistage process. Thus, when background carcinogenesis is present, the linear dose response curve is not overly conservative relative to the multistage dose response curve. In fact the linear dose response curve is anticonservative when compared to

the one-stage or one-hit models.

Linear extrapolation has long been proposed for use in radiation carcinogenesis (see Brown, J. M., 1976, for a review of the relevant reports). The BEIR (1972) report on radiation risks from the National Academy of Science recommended linear extrapolation as a "best estimate" approach as opposed to a conservative approach. Brown reviewed arguments both for and against linearity and concluded that "linear extrapolation of human data from high doses of low LET radiation cannot be said to overestimate the risk at low doses. In fact, there is some doubt as to whether the risk is not underestimated."

Certainly much remains to be learned about both radiation and chemical carcinogenesis. However, if both radiation and chemicals cause cancer through similar mechanisms then it should be expected that there would also be similarities between the respective carcinogenesis dose response functions. Direct damage to DNA by the carcinogenic agent has been implicated as one cancer initiating mechanism for both radiation and chemicals (Brown, J. M., 1976 and McCann and Ames, 1976). Thus, the findings related to the potential linearity of the dose response function for radiation has implications for chemical carcinogens as well, particularly for "directly-acting" carcinogens.

The results of linear extrapolation will be compared with other methods in a later section.

C. Extrapolation Methods Based Upon the Multistage Model

Two methods of low dose extrapolation which are alternatives to the Mantel-Bryan or linear procedures have recently been proposed independently by Guess, Crump, and Deal (Guess and Crump 1976, 1978 and Crump, Guess and Deal, 1977) and Hartley and Sielkin, 1977. Both of these methods utilize a multistage dose response function of the form

$$P(d) = 1-\exp\{-[q_0 + q_1 d + q_2 d^2 + \cdots + q_k d^k]\} \qquad [$$

where $q_0, q_1, \cdots, q_k$ are all nonnegative parameters to be estimated from the data. This dose response function is general enough to yield a considerably wide range of responses at low dose. On the one hand, if $q_1>0$ and $q_i=0$ for $i\geq 2$ the dose respo function [A2] becomes the one-stage model which yields risks at l doses comparable to what would be obtained with linear extrapolation. On the other hand, the model can produce risks even as low as the probit curve [A1] down to any fixed positive low dose. Thus this model is capable of fitting both highly linear and highly nonlinear dose response relations. Since the model has th property of "linear at low dose" if $q_1>0$ and does not have this property if $q_1=0$, use of this model does away with having to make the arbitrary but crucial decision of having to either assume linear at low dose as in linear extrapolation or else assume a highly nonlinear dose response relationship at low dose as in Mantel-Bryan extrapolation. Thus "safe" doses computed using this model should provide a more realistic measure of the true uncertainty of low dose extrapolation than would "safe" doses based

on either an assumed linear curve shape or an assumed highly nonlinear curve shape.

The dose response relation [A2] contains all of the Armitage and Doll (1961) multistage dose response models as special cases but also contains curves which are much flatter at low dose than any of the multistage curves.

The two extrapolation procedures based upon [A2] have some features which are different. When computing "most likely" estimates the procedure of Guess, Crump and Deal uses an infinite dimensional maximization procedure so that it is not necessary to specify a value of k, the degree of the polynomial in [A2]. However, the two methods differ chiefly in the way the statistical confidence intervals are computed. There have not yet been sufficient comparative calculations made to determine how safe doses may differ using the two approaches. Mantel (1977) has made a critical review of the statistical procedure used by Hartley and Sielkin for calculating the 'safe' dose.

Both the Hartley and Sielkin and the Guess, Crump and Deal (as extended by Crump, 1977) procedures can utilize times at which cancer is detected in the experimental animals rather than just the dichotomous information of whether or not an animal contracted cancer before it died of some other cause or before the termination of the experiment. The utilization of time data in low dose extrapolation is important for at least two reasons: 1) The age at which cancers occur should be important in assessing the magnitude of the harmful effect of a carcinogen upon man (e.g., cancers that occur early in life should be viewed as more

serious than those which occur in extreme old age). 2) In ra animal carcinogenicity experiments the response data at the highest doses lies below the trend of the lower dose data. sometimes appears to be due to the fact that at the highest d some of the animals are being poisoned by the chemical before they have a chance to develop cancer. When this occurs the hi dose data is often just deleted from the analysis. However if the times at which the animals die is properly used the high d data might not appear anomolous. More research needs to be do on the proper utilization of animal time of death data to asse the harmful effects of chemicals to man.

D. The Gamma Multihit Carcinogenesis Dose Response Model

Rai and Van Ryzin (1978) have proposed basing risk estima tion on the gamma multihit model

$$P(d) = C + (1-C)\int_0^{\lambda d} \frac{u^{k-1}e^{-u}}{(k-1)!}\,du \qquad k \geq 0$$

$$= C + (1-C)\left\{1-e^{-\lambda d}\left[1 + \lambda d + \frac{(\lambda d)^2}{2!} + \ldots + \frac{(\lambda d)^{k-1}}{(k-1)!}\right]\right\}$$

$$k = 1,2,\ldots \qquad [A3]$$

where P(d) is the lifetime probability of cancer in a tissue whe subjected to a constant dose-rate d of the carcinogen. This mode is obtained by assuming that cancer due to the carcinogen occurs when the tissue has been hit k times, these hits occurring randomly according to a Poisson distribution. The manner in which background carcinogenesis is incorporated into the model is

equivalent to assuming that the event "cancer occurs due to the action of the carcinogen" is independent of the event "cancer occurs spontaneously." This assumption would not apply, for example, to processes in which the effect of the carcinogen is to speed up the rate at which the "spontaneous" events occur which lead to the background cancers. At low dose rates, the response is approximately given by

$$P(d) = \lambda^k d^k / k!.$$

Consequently, this dose response model is linear at low dose rates when and only when k = 1. Rai and Van Ryzin calculate confidence intervals for added risk at a given dose and for the dose producing a fixed added risk using asymptotic maximum likelihood theory. Although in the theoretical development k must be an integer, in the applications k is allowed to assume any positive value.

Upper statistical confidence intervals on extra risk at a given low dose computed using this procedure can be compared with those computed from the multistage model [A2] by considering two general classes of data.

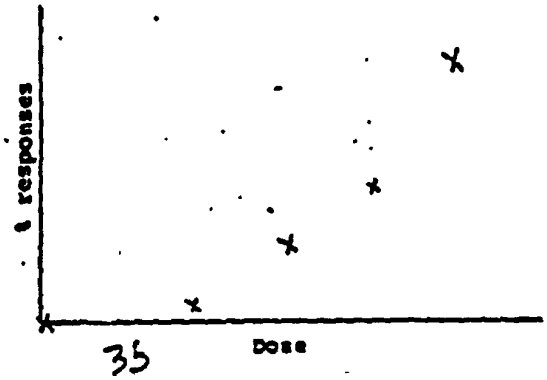
If the data exhibit a general downward curvature as illustrated in Figure 1a, upper confidence bounds on extra risk computed from the gamma multihit model [A3] should generally be greater than or equal to corresponding upper confidence bounds computed from the multistage model [A2]. In certain instances the gamma multihit upper bounds could be much greater than the corresponding multistage upper bounds. This could occur when the data is consistent

with k < 1 in the gamma multihit model (presumably corresp to a fraction of a hit).

On the other hand, if the data exhibit a general upwa curvature as illustrated in Figure 3b the reverse situation hold; gamma multihit upper confidence bounds on extra risk generally be less than or equal to corresponding multistage bounds. Gamma multihit bounds will not in general share th low-dose-linearity of multistage bounds and because of this be smaller than the multistage bounds by orders of magnitud low doses. The reason these large differences can occur is follows. The multistage family of models contains members w are simultaneously linear at low dose and exhibit upward cur at moderate doses. For example, the particular multistage model

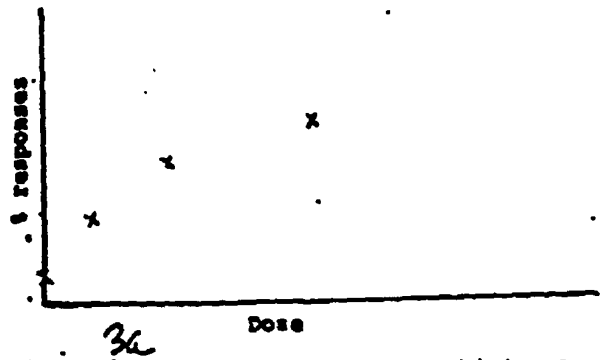
$$1-e^{-(q_1 d + q_2 d^2)} \qquad q_1, q_2 > 0$$

is linear at low dose since $q_1 > 0$ and still can exhibit upwa curvature at moderate doses since $q_2 > 0$. This means there are dose response curves in the multistage class which are both linear at low dose and can adequately describe data of the type exemplified by Figure 3b. On the other hand, this wi generally not be true of gamma multihit models. All dose-respo curves in the gamma multihit class which are linear at low dos must exhibit downward curvature and consequently would generall not be consistent with the data in Figure 3b. When this is tru gamma multihit confidence upper bounds will be sublinear (e.g., quadratic) at low dose and consequently much smaller than the multistage confidence bounds.



35

Figure 3a. Example of Data Exhibiting Upward Curvature



36

Figure 3b. Example of Data Exhibiting Downward Curvature

Confidence bounds based upon the gamma multihit model . be approximately correct whenever this model is the correct A similar statement could be made for the probit model, mult stage model, or any other model to which reasonable statist methods are applied. However, there may be considerable unc tainty as to what the true model may be in a particular situ The multistage model not only reflects some reasonable assum regarding the carcinogenic process which dovetail nicely wit epidemiological data for many cancers (Peto, 1977b), but it al reflects some of the uncertainty with regard to the true mode by virtue of encompassing a relatively large class of dose- response functions. For example, as noted earlier, the multi class contains dose response functions which are linear at lo dose and also exhibit upward curvature at moderate doses. On the other hand, the gamma multihit class is more restrictive this point in that it does not permit such behaviour. Is thi extra restrictiveness of the gamma multihit model justified? To help answer this question, consider the following modifica- tion to this model. Suppose that the hits (phenomenological events which are required to occur in a tissue in order that a cancer appear) can possibly occur spontaneously in the absence of the carcinogen. Part of the effect of the carcinogen would then be to speed up the rates at which the spontaneous hits are occurring. For example, if one of the "hits" is an in- correct base substitution in DNA during mitosis, the carcinoge could speed up the rate at which these "hits" are occurring in individual cells by providing more chances for [illegible]

an incorrect substitution. With this modification to the model, the gamma multihit upper confidence bounds will no longer be sublinear at low dose and will likely be very close to corresponding bounds calculated from the multistage model (A2). On the other hand, versions of the gamma model which incorporate such an assumption as this could still be consistent with data with strong upward curvature as exemplified by Figure 3b. Thus, in order to obtain sublinear upper confidence intervals with the gamma multihit model a modification such as the one described above must be ruled out, not on the basis of data, but by assumption.

By way of summary, confidence intervals based upon the multistage model will always be linear at low dose. Confidence intervals based upon the gamma multihit model may be either "super linear" (corresponding to $k < 1$) or "sublinear" (corresponding to $k > 1$). Superlinearity is achieved by making the model too broad in that a fraction of a hit is allowed which has no biological basis. On the other hand, sublinearity is achieved by making the model possibly too restrictive in that models which are reasonable from a biological viewpoint are ruled out by assumption.

MONS 016203

Comparisons and Discussion

To compare low dose extrapolations using the Mantel-Bryan probit model (A1) with those using the multistage model (A2) we present Figure 4 based upon the same data as Figure 2. In this

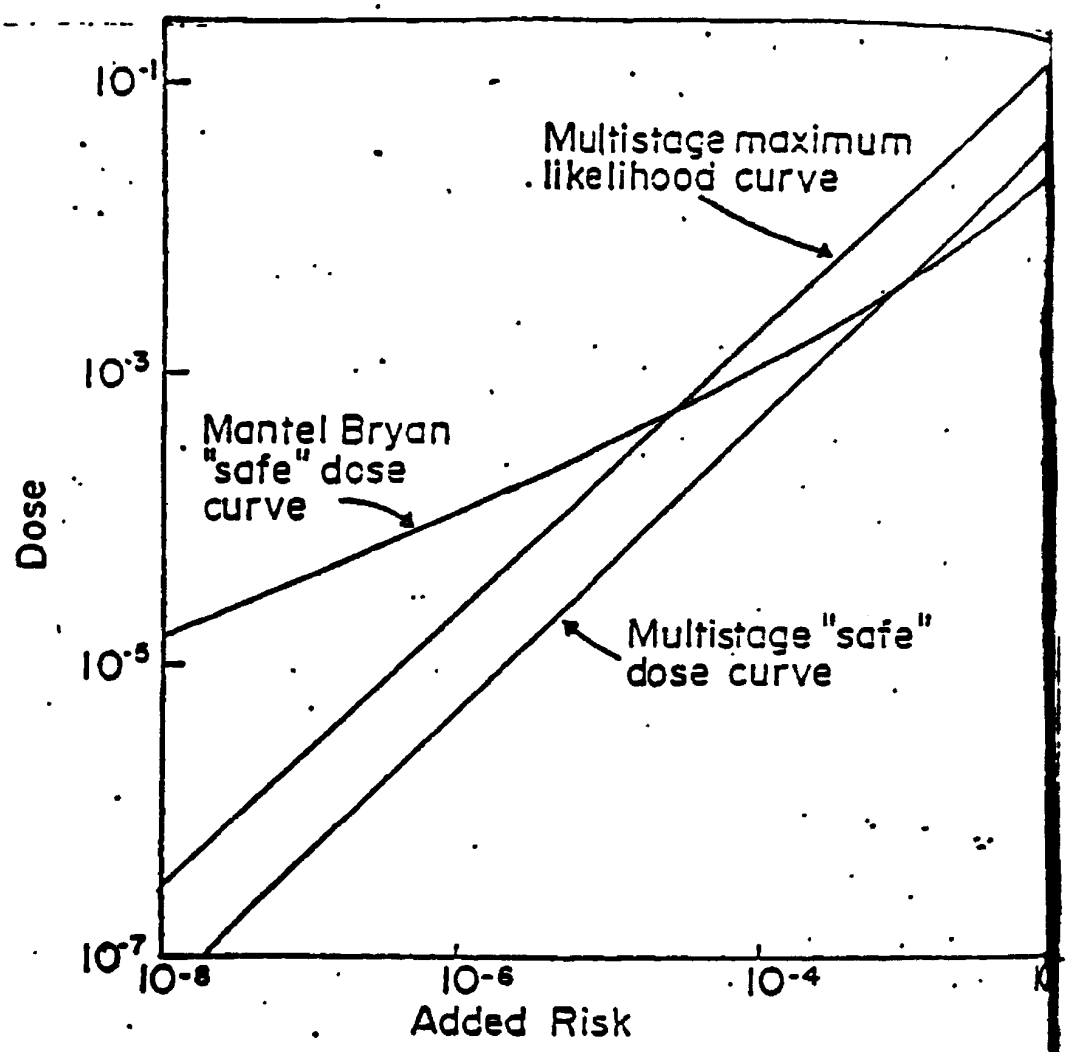


Figure 4. Comparisons of 'safe' doses computed from the Mantel-Bryan procedure and from a procedure based upon the multistage model (from Crump, 1977b).

MONS 016204

Figure are plotted on a log-log scale the Mantel-Bryan 'safe' dos as well as both the multistage 'most likely' curve based upon [A2] and the 'safe' dose based upon [A2] and computed as outlined in Crump, et al. (1977). A 99% statistical assurance was used for both 'safe' dose curves. We note that the Mantel-Bryan 'safe' dose lies above the multistage safe dose curve at values of added risk below 5×10^{-4}. The Mantel-Bryan "safe" dose curve lies above the multistage 'safe' dose curve by a factor greater than 20 for an added risk of 10^{-6} and by a factor greater than 300 for an added risk of 10^{-8}. Because of the 'flatness' property of the Mantel-Bryan probit function [A1] described earlier, the Mantel-Bryan 'safe' dose curve will lie above the multistage safe dose curve by arbitrarily large factors at extreme low doses. Guess, et al. (1977) have compared the Mantel-Bryan 'safe' dose curves to the multistage 'safe' dose curves and found this to be a typical situation. Thus it is clear that if the true dose response curve could be similar to the multistage dose response function [A1] then the Mantel-Bryan procedure could not be justifiably called conservative. (See also Crump 1977 for further discussion of this point.) On the other hand, we have seen that there are quite plausible arguments for the true dose response curve to have the same shape at low dose (linear) as the estimated multistage curve.

We note that both the multistage 'safe' dose curve and 'most likely' curve have a slope 1 in Figure 4 as plotted on the log-log scales which is equivalent to the curves being linear at low dose. The fact that the 'most likely' curve has slope 1 is

due to the fact that with this particular data set the linear coefficient q_1 in (A2) was estimated to be positive. However 'safe' dose curve using the multistage model [A2] will always linear at low dose regardless of whether or not the linear co ficient q_1 is estimated to be positive. This property should shared by any valid statistical procedure based upon a dose re sponse function that does not rule out linearity at low dose b assumption as Mantel-Bryan does. Just as it is not possible t prove statistically the existence of a threshold, it is likewi not possible to rule out the possibility that the true dose response curve is linear at low dose on the basis of statistic analysis. (See Guess, et al. (1977) for a thorough discussion this important point.) The Mantel-Bryan obtains 'safe' dose estimates which are considerably higher than those obtained us the multistage model because it assumes away linearity at low dose, an assumption that we have seen is probably unwarrented that majority of carcinogens which are classified as 'directly acting' carcinogens.

MONS 016206

Since extrapolation based upon a model such as the mult stage model (A2) must always be linear at low dose, the questio arises as to how different the result will be from simple line extrapolation. For some data the difference will be minimal. For example, for the data upon which Figure 2 is based, 'safe doses' computed using the multistage model is almost identical with 'safe' doses based upon linear extrapolation. For some data sets, however, the difference could be considerable. For example, with the Gass, et al. (1964) DES using the [illegible]

mice, the 'safe' dose based upon linear extrapolation is lower than the 'safe' dose based upon the multistage model by a factor of about five and there are doubtless cases where this difference could be greater than an order of magnitude.

REFERENCES

Allen, J. F. and Norback (1976). Pathobiological response of primates to polychlorinated biphenyl exposure. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004.

Armitage, P. and Doll, R. (1961). Stochastic models for carcinogenesis. In Proceedings of the Fourth Berkeley Symposium on Mathematical Statistics and Probability, Vol. 4, pp. 19-38, University of California Press, Berkeley and Los Angeles.

Bahn, A. K., Rosenzweike, I., Herrmann, N., Grover, P., Stellman, J. and O'Leary, K. (1976). Melanoma after exposure to PCB's. New England Journal of Medicine 295, 450.

Bahn, A. K., Grover, P., Rosenzsike, I., O'Leary, K. and Stellman, J. (1977). PCB? and melanoma. New England Journal of Medicine 296, 108.

Barsotti, D. A., Marler, R. J., and Allen, J. R. (1976). Reproductive dysfunctions in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Arochlor 1248). Food and Cosmetics Toxicology.

Bier (1972). The effects on populations of exposures to low levels of ionizing radiation, National Academy of Sciences. National Research Council (Washington, D.C., 1972).

Brown, J. M. (1976). Linearity vs. nonlinearity of dose response for radiation carcinogenesis. Health Physics 31, 231-245.

Butler, P. A. and Schultzmann, R. L. (1978). Residues of Pesticides and PCBs in Estuarine Fish, 1972-1976 - National Pesticide Monitoring Program. Pesticide Monitoring Journal 12, 51-59.

Calandra, J. C. (1976). Summary of toxicological studies on commercial PCB's. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 33-42.

Chand, N. and Neel, D. C. (1974). A comparison of models for determining safe levels of environmental agents. In Reliability and Biometry, ed. F. Prochan and R. J. Serfling, pp. 382-401, Philadelphia: SIAM.

Cohen, A. C. (1961). Tables for maximum likelihood estimates: singly truncated and singly censored samples. Technometrics 3, 535-541.

Cordle, F., Corneliussan, P., Jelinek, C., Huckley, E., Lehman, R., McLaughlin, J., Rhoden, R. and Shapiro, R. (1978). Human exposure to polychlorinated biphenyls and polybrominated biphenyls. Environmental Health Perspectives 24, 157-172.

Cornfield, Jerome (1977). Carcinogenic risk assessment. Science 198: 693-699.

Crump, K. S., Hoel, D. G., Langley, C. H., and Peto, R. (1976). Fundamental carcinogenic processes and their implication for low dose risk assessment. Cancer Research 36:2973-2979.

Crump, K. S., Guess, H. A. and Deal, K. L. (1977). Confidence intervals and tests of hypotheses concerning dose response relations inferred from animal carcinogenicity data. Biometrics 33:437-451.

Crump, K. S. (1977). Response to open query: Theoretical problems in the modified Mantel-Bryan procedure. Biometrics 33:752-755.

Daffer, P. Z., Crump, K. S. and Masterman, M.D. (1979). Asymptotic theory for analyzing dose response survival data with application to the low-dose extrapolation problem (submitted).

Dennis, D. S. (1976). Polychlorinated biphenyls in the surface water and bottom sediments of the major drainage basins of the United States. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 183-194.

Environmental Protection Agency (1976). PCBs in the United States industrial use and environmental distribution, task I. EPA 560/6-76-005.

Food Safety Council (1978). Proposed system for food safety assessment. Food and Cosmetic Toxicology (December).

Gass, G. M., Casts, D. and Graham, K. (1964). Carcinogenic dose-response curve to oral diethylstilbestrol. J. Natl. Cancer Inst. 33: 971-977.

Guess, H. A. and Crump, K.S. (1976). Low-dose rate extrapolation of data from animal carcinogenicity experiments--analysis of a new statistical technique. Mathematical Biosciences 30, 15-36.

Guess, H. A. and Crump, K. S., (1978). Maximum likelihood estimation of dose-response functions subject to absolutely monotonic constraints. Annals of Statistics 6, 101-111.

Guess, H. A., Crump, K. S., and Peto, R., (1977). Uncertainty estimates for low-dose rate extrapolations of animal carcinogenicity data. Cancer Research 37:3475-3483.

Hartley, H. O. and Sielken, R. L. (1977). Estimation of "safe doses" in carcinogenic experiments. Biometrics 33:1-30.

Hoel, D. G., Gaylor, D. W., Kirschstein, R. L., Saffiotti, U. and Schneiderman, M. A. (1975). Estimation of risks of irreversible, delayed toxicity. J. of Toxicology and Environmental Health 1:133-151.

Humphrey, H. E. B. (1977). Evaluation of changes of the level of polychlorinated biphenyls (PCB) in human tissue. Final Report on FDA Contract 233-73-2209.

IARC (1978). Polychlorinated biphenyls and polybrominated biphenyls. IARC (International Agency for Research on Cancer) Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 18; WHO Publications Centre, Albany, N.Y.

Industrial Bio-Test Laboratories, Inc. (1971). Reports to Monsanto Company. Two-year chronic toxicity with Aroclor 1242, 1254, and 1260 in albino rats. Unpublished reports, November 12, 1971. IBT No. B7298.

Ito, N., Nagasaki, H., Arai, M., Makiura, S., Sugihara, S., and Hirao, K. (1973). Histopathologic studies on liver tumorigenesis induced by mice by technical polychlorinated biphenyls and its promoting effect on liver tumors induced by benezene hexachloride. Journal of the National Cancer Institute 51, 1637-1646.

Jelinek, C. and Corneliussen, P. E. (1976). Levels of PCBs in the U.S. food supply. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 147-154.

Johnson, R. D. and Manke, D. D. (1977). Pesticide and Other Chemical Residues in Total Diet Samples (XI). Pesticide Monitoring Journal 11, 116-131.

Kimbrough, R. D., Linder, R. E., Burse, V. W. and Jennings, R. W. (1973). Adenofibrosis in the rat liver, with persistence of polychlorinated biphenyls in adipose tissue. Archives of Environmental Health 27, 390-395.

Kimbrough, R. D. and Linder, R. E. (1974). The induction of adenofibrosis and hepatomas of the liver in mice of the BALB/cJ strain by polychlorinated biphenyls (Aroclor 1254). Journal of the National Cancer Institute 53, 544-552.

Kimbrough, R. D., Squire, R. A., Linder, R. E., Strandburg, J. D., Montali, R. J., and Burse, V. W. (1975). Induction of liver tumors in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. Journal of the National Cancer Institute 55, 1453-1459.

Kutz, F. W. and Strassman, S. C. (1976). Residues of polychlorinated biphenyls in the general population of the United States. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 139-143.

Linder, R. E., Gaines, T. B., and Kimbrough, R. D. (1974). The effect of polychlorinated biphenyls on rat reproduction. Food and Cosmetic Toxicology 12, 63-77.

Makiura, D., Aoe, H., Sugihara, S., Hirao, K. Arai, M., and Ito, N. (1974). Inhibitory effects of polychlorinated biphenyls on liver tumorigenesis in rats treated with 3'-methyl-4-dimethylamino-azobenzene, N-2-fluorenylacetamide, and diethylnitrosamine. Journal of the National Cancer Institute 53, 1253-1257.

Mantel, N. and Bryan, W. R. (1961). Safety testing of carcinogenic agents. J. Natl. Cancer Inst. 27:455-470.

Mantel, N. and Schneiderman, M. (1975). Estimating 'safe' levels, a hazardous undertaking. Cancer Research 35:1379-1386.

Mantel, N., Bahidar, N. R., Brown, C. C., Ciminera, J. L. and Tukey, J. W. (1975). An improved Mantel-Bryan procedure for the "safety" testing of carcinogens. Cancer Research 35:865-872.

Mantel, N. (1977). Aspects of the Hartley-Sielken approach for setting "safe doses" of carcinogens. In Origins of Human Cancer Book C, Human Risk Assessment. H. H. Hiatt, J. D. Watson, and J. A. Winsten (eds.) Cold Springs Harbor, N.Y., Cold Springs Harbor Laboratory, 1397-1401.

McCann, J., Choi, E., Yamasaki, E. and Ames, B. N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72:5135-5139.

McCann, J. and Ames, B. N. (1976). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. Proc. Nat. Acad. Sci. USA 73:950-954.

McDermott, D. J., Young, D. R., and Heesen, T. C. (1976). PCB contamination of southern California marine organisms. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 209-217.

McNulty, W. P. (1976). Use of the rhesus monkey as a model for human toxicity with polychlorinated biphenyls. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.). EPA-560/6-75-004, 332-333.

Monsanto (1975). Industrial Bio-Test Laboratories, Inc. Reports. Histopathological evaluation of additional liver sections. March 24, 1975. Unpublished reports, Monsanto Co., St. Louis, Missouri.

Munson, T. O., Palmer, M. D., and Ferns, J. M. (1976). Transport of chlorinated hydrocarbons in the upper Chesapeake Bay. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 218-219.

National Marine Fisheries Service (1976). Compendium of PCB data. NOAA, U.S. Dept. of Commerce, Washington, D.C.

National Institute for Occupational Safety and Health (1977). Criteria for a recommended standard . . . occupational exposure to polychlorinated biphenyls (PCBs). DHEW (NIOSH) Publication No. 77-225.

NCI (National Cancer Institute) (1978). Bioassay of Aroclor 1254 for possible carcinogenicity. Carcinogenesis Technical Report Series No. 38. CAS No. 27323-18-8 NCI-CG-TR-38.

Nisbet, I. C. T. (1976a). Criteria document for PCBs. Environmental Protection Agency. National Technical Information Service PB-255-397.

Nisbet, I. C. T. (1976b). Introductory remarks, session III. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 137-138.

Nisbet, I. C. T. (1976c). Environmental transport and occurrence of PCBs in 1975. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 254-256.

Peto, R. (1974). (Paper delivered at the NIEHS Wrightsville Beach conference on Low-Dose Risk Estimation).

Peto, R. (1977). The carcinogenic effects of chronic exposure to very low levels of toxic substances. Presented at an NIEHS conference on cancer risk estimation, Pinehurst, N.C., March 10 - 12, 1976. Environmental Health Perspectives.

Peto, R. (1977b). Epidemology, multistage models, and short-term mutagenicity tests. In Origins of Human Cancer Book C, Human Risk Assessment, H. H. Hiatt, J. D. Watson, and J. A. Winsten (eds.) Cold Springs Harbor, N.Y., Cold Springs Harbor Laboratory, 1397-1401.

Polishuk, Z. W., Ron, M., Wasserman, M., Cucos, Simi, Wasserman, Dore, and Lunesch, C. (1977). Organochlorine compounds in human blood plasma and milk. Pesticides Monitoring Journal 10, 121-129.

Rai, K. and Van Ryzin, J. (1978). Risk assessment of toxic substances based on a generalized multihit dose response model. Proc. SIMS Conference, Alta, Utah, June 26-30.

Rannug, U., Johansson, A., Rand, C., and Wachtmeister, C. A. (1974). The mutagenicity of vinyl chloride after metabolic activation. Ambio 3, 194-197.

Salsburg, D. S. (1977). Open query: Theoretical problems in the modified Mantel-Bryan procedure. Biometrics 33:419-421.

Sanborn, J. R., Childers, W. F., and Metcalf, R. L. (1976). Uptake of three polychlorinated biphenyls, DDT and DDE by the green sunfish, Lepomis Cyanellus Raf. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 236-242.

Savage, E. P. (1979). (Personal communication).

Spagnoli, J. J. and Skinner, L. C. (1977). PCB's in fish from selected waters of New York State. Pesticide Monitoring Journal 11, 69-87.

Uchiyama, M., Chiba, T., and Noda, K. (1974). Co-carcinogenic effect of DDT and PCB feedings on methylcholanthrene-induced chemical carcinogenesis. Bulletin of Environmental Contamination and Toxicology 12, 687-693.

Walker, C. R. (1976). The occurrence of PCB in the National Fish and Wildlife Monitoring Program. Proceedings of the National Conference on Polychlorinated Biphenyls (Nov. 19-21, 1975, Chicago, Ill.) EPA-560/6-75-004, 161-176.

Watson, G. S. (1977). Age incidence curves for cancer. Proc. Nat. Acad. Sci. USA 74, 1341-1342.