Chemicals Studied and Evaluated in Long-Term Carcinogenesis Bioassays by Both the Ramazzini Foundation and the National Toxicology Program

In Tribute to Cesare Maltoni and David Rall

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ABSTRACT: The Ramazzini Foundation (RF) in Bentivoglio, Italy and the National Toxicology Program (NTP) in Research Triangle Park, North Carolina have carried out several hundred chemical carcinogenesis bioassays: 200 by RF and 500 by NTP. Of these, 21 have been evaluated by both laboratories. The 14 chemicals for which both laboratories have designed, conducted, and reported bioassay results are: acrylonitrile, benzene, chlorine, diesel fuel, ethylbenzene, methylene chloride (dichloromethane), propylene, styrene, styrene oxide, toluene, trichloroethylene, trichlorofluoromethane, vinylidene chloride, and xylenes. The other seven chemicals (two are fibers) were evaluated by both laboratories, but results have not yet been published. Results of these 14 interlaboratory studies were compared both to explore consistency of carcinogenic responses and to identify possible factors that may reveal reasons for any differences observed. Individual carcinogenesis results from each laboratory were duplicated and complementary. Of the 14 chemicals compared, 11 (80%) were either carcinogenic (9 chemicals) or noncarcinogenic (2 chemicals) in both studies. Eight of the paired chemicals had at least one carcinogenic target site in common. The other three were carcinogenic in one laboratory but not in the other. Possible explanations for these differences include dose, method of administration, duration of follow-up, and whether or not total tumors are counted. The collaboration between these two pioneering bioassay laboratory programs contributes greatly to our understanding of chemical carcinogenesis and results in better protection of workers and the general population from chemical diseases, especially cancers.

KEYWORDS: bioassay; chemical carcinogens; hazard identification; long-term tests; Maltoni; National Toxicology Program; Rall; Ramazzini Foundation

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Ann. N.Y. Acad. Sci. 982: 208–230 (2002).

TRIBUTE

Cesare Maltoni and David P. Rall were extraordinary and compassionate physicians as well as steadfast and innovative experimentalists, a grand combination that showed in their unrelenting empathy for workers and their deep understanding of the value of experimental testing and research to better protect and advance public health worldwide. They led their respective programs (the Ramazzini Foundation and the National Toxicology Program) to the forefront of the public and occupational health field, thereby helping to reduce exposure to toxic and carcinogenic chemicals. Their combined strength and uncompromising attitude directed to the betterment of health of the individual stands as a model for all of us. These two giants in the field of public health are sorely missed. This paper is dedicated to my two long-time friends, mentors, colleagues, coworkers, and defenders and champions of public health and primary prevention of diseases, especially cancers.

INTRODUCTION

Long-term chemical carcinogenesis bioassays are the cornerstone for primary prevention and for protection of the worker and the general public from chemically and occupationally associated cancers.[1–73] The two largest, longest existing, and most well-established bioassay programs in the world are the Ramazzini Foundation (RF)[1–19] and the National Toxicology Program (NTP).[20–73] More than 700 chemicals or agents have been tested for carcinogenic activity by these two programs: roughly 200 by RF and 500 by NTP. Twenty-one chemicals have been tested by both laboratories; 14 have been reported by both (TABLE 1). The results of the tests of these 14 chemicals make up the primary aspect of this paper.

TABLE 1. Chemicals evaluated in long-term carcinogenesis bioassays by both RF and NTP[a]

1. Asbestos	12. Styrene oxide[b,113,114]
2. Acrylonitrile[b,74–79]	13. Tetrachloroethylene
3. Benzene[b,80–93]	14. Toluene[b,118–122]
4. Chlorine[b,94–97]	15. 1,1,1-Trichloroethane
5. Diesel fuel[b,98–100]	16. Trichloroethylene[b,123–126]
6. Dichloroethane	17. Trichlorofluoromethane[b,127,128]
7. Ethylbenzene[b,101–103]	18. Vinylidene chloride[b,129–134]
8. Methylene chloride[b,104–106]	19. Vitamin C
9. Nitrilotriacetic acid	20. Wollastonite
10. Propylene[b,107–111]	21. Xylenes[b,136–138]
11. Styrene[b,112,115–117]	

[a]Available published paper on each of these 21 chemicals are listed in the REFERENCES section.
[b]Studies by RF and NTP form the basis for the evaluations and comparisons in this paper. Cited are chemical-specific and other relevant references published by RF and NTP.

CARCINOGENESIS BIOASSAYS

Experimental long-term chemical carcinogenesis bioassays are designed and carried out to identify potential carcinogenic effects for humans.[1,2,5,10,14,15] Carcinogenesis results in rodents, mainly rats and mice, have been shown to be a consistent and reliable indicator and predictor of human cancer risk.[22–24,26–29,35,38–42] All known human carcinogens that have been evaluated adequately in animal bioassays are also carcinogenic in animal bioassay studies. Of the nearly 100 recognized human carcinogens, about one-third were shown first to be carcinogenic in experimental animals.[27,28] Hence, for chemicals discovered to be carcinogenic to laboratory animals, prudent public health policy suggests strongly that eliminating exposures to these carcinogens would reduce or eliminate certain environmentally associated cancers.[2,20,23,24,27–29]

Findings from carcinogenesis bioassays are used for establishing and setting occupational exposure standards; for developing primary prevention strategies by national and international regulatory agencies and other organizations; for carcinogen evaluation organizations like the U.S. Congress–mandated Report on Carcinogens, and among others the California Environmental Protection Agency's PROP 65 program, and the International Agency for Research on Cancer's Monographs Programme; and for formulating and promulgating policy decisions by environmental and occupation research and regulatory agencies.

PERSPECTIVE

For many years, a key to primary cancer prevention was to identify known animal and human carcinogens and to either eliminate or drastically reduce exposures to these carcinogens. In more recent years this long-accepted *prima facie* evidence has been challenged, largely by vested industries and other parties, often using speculative mechanisms of carcinogenesis or modes of action purported to be rodent specific and irrelevant to humans. Many of these arguments have settled on what has been described as "modes of action," rather than "mechanisms," alleging that the "modes" of carcinogenicity in animals are not or will not be the same in humans and that therefore a particularly chemical would *de facto* be safe for human exposure. This results in a lowering of the estimated relative hazards and risks from exposures to rodent carcinogens and thus raises the potential cancer risks of humans exposed to these chemicals.

EXPERIMENTAL DESIGN COMPARISONS

Overall, the designs for long-term bioassays are similar between the RF and NTP laboratories.[1–73] That is, treatment of animals, laboratory characteristics and Good Laboratory Practices, and pathology assessment and reporting (TABLE 2; see Belpoggi *et al.*, Soffritti *et al.*, and Bucher, these proceedings, for more extensive descriptions and details).[1,2,5,27,28,42,47,52] However, there are several important differences between the two laboratories.

TABLE 2. General experimental bioassay designs and conditions typically used by RF and NTP

	RF	NTP
Species/Strain		
Rats	Sprague-Dawley	Fischer 344/N
	Wistar	
Mice	Swiss, RF/J	B6C3F1
Numbers per Group		
	50–100	50–60
Routes and Exposures	**MTD + 2–3 doses and controls**	**MTD + 2–3 doses and controls**
Gavage	4 per week [M,T,Th, F]	5 per week [M,T,W,Th,F]
Vehicle	olive oil	corn oil
Inhalation	7 h/d–5 d/wk	6 h/d–5 d/wk
Feed	continuous	continuous
Age at Start		
	6–8 weeks	6–8 weeks
Duration of Exposures and Experiments		
	52–104 weeks exposure, continue with no chemical exposure for natural lifetime	104 weeks
Necropsy/Histopathology		
	complete	complete
Tissue Preservation		
	70% ethyl alcohol	10% formalin

NOTE: For more details about experimental designs and laboratory conditions see the papers in this volume by Belpoggi *et al.*, by Bucher, and by Soffritti *et al.*

Strain of Rat

RF typically uses Sprague-Dawley rats in their bioassays, whereas the NTP uses Fischer 344/N rats. NTP usually uses B6C3F1 mice; RF generally uses rats only (sometimes Wistar instead of, or in addition to, Sprague-Dawley), but occasionally uses mice of Swiss and RF/J strains. Almost without exception, RF and NTP use both sexes of whatever strains are chosen for the long-term studies, and both routinely use 50–60 animals per sex, per group of control and exposed animals.

Exposures

RF exposes animals via inhalation for 7 hours per day and NTP for 6 hours per day; both for 5 days per week. For oral intubation, there are two differences: RF uses virgin olive oil as a vehicle for chemical administration, while the NTP uses corn oil. RF intubates chemical/oil mixtures 4 days a week and NTP does so for 5 days per week.

Duration

This is a key and major difference between these two laboratories. RF exposes animals for 52 to 104 weeks and then allows the animals to live their natural life without any additional exposures. NTP terminates their bioassays at 104 weeks. On

TABLE 3. Routes of exposure for the 14 chemicals evaluated by RF and NTP for carcinogenic activities

Chemical	RF	NTP	S/D
1. Acrylonitrile	inhalation, gavage	inhalation	S
2. Benzene	inhalation, gavage	gavage	S
3. Chlorine	drinking water	drinking water	S
4. Diesel fuel	gavage	skin	D
5. Ethylbenzene	gavage	inhalation	D
6. Methylene chloride	inhalation, gavage	inhalation	S
7. Propylene	inhalation	inhalation	S
8. Styrene	inhalation, gavage, injection	gavage	S
9. Styrene oxide	gavage	gavage	S
10. Toluene	gavage	inhalation	D
11. Trichloroethylene	inhalation	gavage	D
12. Trichlorofluoromethane	inhalation	gavage	D
13. Vinylidene chloride	inhalation	gavage	D
14. Xylenes	gavage	gavage	S

TOTALS: RF for four chemicals used more than one route of exposure; for eight chemicals the two laboratories used the same route, and for six they used different routes; of the 33 routes, 16 were gavage, 13 inhalation, 2 drinking water, 1 skin (dermal), 1 injection.

S/D = Same or Different route of exposure comparisons; S was used if one of the routes for the same chemical was the same.

occasion, each laboratory alters its standard protocol. For example, parent generations are exposed during conception, gestation, and lactation; and exposures continue for the offspring or both parents and offspring.

Routes of Exposure for the 14 Chemicals

For eight chemicals both RF and NTP used the same route of exposure, whereas for another six they used different routes of exposure (TABLE 3). In four cases, RF used multiple routes. Gavage (oral intubation) was the most common route, with inhalation following close behind, and drinking water, skin, and injection used rarely.

Abbreviated Tumor Analysis Results by Chemical

A summary of "plus" or "minus" results for each of the 14 chemicals evaluated and published by both the RF and the NTP are shown in TABLE 4. These simplified designations (+,–) are expanded on in the following chemical-specific text details. For 11 (80%) of the 14 chemicals there is positive or negative concordance. An outline of the comparative organ sites is given in TABLE 5 for each of the 14 chemicals. The most common collective organ sites for carcinogenesis for these 14 chemicals are: total malignant tumors, 10; mammary gland, 8; liver and lung, 6; forestomach and leukemia, 5; head, 4; and kidney, skin, testes, and Zymbal glands, 3.

Generally, NTP does not report total malignant tumors as an indication of carcinogenesis. For this paper, the total malignant tumors "site" is listed for benzene,

TABLE 4. Abbreviated results on chemicals evaluated by RF and NTP

Chemical	+ or – carcinogenicity results		Concordance
	RF	NTP	
1. Acrylonitrile	+	+	Y
2. Benzene	+	+	Y
3. Chlorine	+	+	Y
4. Diesel fuel	+	+/–	Y?
5. Ethylbenzene	+	+	Y
6. Methylene chloride	+	+	Y
7. Propylene	–	–	Y
8. Styrene	+	+/–[a]	Y
9. Styrene oxide	+	+	Y
10. Toluene	+	–	N?
11. Trichloroethylene	+	+	Y
12. Trichlorofluoromethane	–	–	Y
13. Vinylidene chloride	+[b]	–[c]	N?
14. Xylenes	+	–	N?
		Totals	11Y, 3N

NOTE: + = Positive chemical-related carcinogenic response in one or more target organs; – = no evidence of carcinogenic activity related to chemical exposure; Y = correlation of similar positive or no evidence of carcinogenicity responses from both laboratories; N = noncorrelation of carcinogenic responses between both laboratories; ? = questionable correlation or noncorrelation between the two laboratories (explanations given in the text for the particular chemical).

[a]Positive studies reported in literature: Cruzan *et al.* (see ref. in text).

[b]Embyro exposures from 12 days + 104 weeks.

[c]NTP studies considered basically inadequate because exposure concentrations were low.

whereas the other NTP chemicals did not show significant increases in total tumors compared to controls. Total tumors data (benign, malignant, combined) are listed in each NTP technical report, but no statistical comparisons are made routinely. Comparing target sites for the tested and reported 14 chemicals shows eight having at least one target organ in common or both bioassays exhibiting no carcinogenic responses; six chemicals do not show target organ concordance. Comparative carcinogenic findings by the RF and the NTP for each of the 14 chemicals are summarized alphabetically by chemical.

Acrylonitrile

Acrylonitrile[74–79] induced tumors of the forestomach and harderian glands when administered orally to mice (NTP); neoplasms of the ovary and lung in female mice may have been related to administration of acrylonitrile. Nonneoplastic lesions of the forestomach and harderian gland in males and of the forestomach and ovary in females were associated with exposure to acrylonitrile. Via inhalation (RF), in rats,

TABLE 5. Simplified organ/tissue site carcinogenesis results

Chemical	Organ/tissue site carcinogenesis[a]		Site Concordance[b]
	RF	NTP	
1. Acrylonitrile	Br, MG, Z, L, An, T	FS, HG, Ov?, Lu?	N
2. Benzene	Mult	Mult	Y
3. Chlorine	Leuk	Leuk?	Y
4. Diesel fuel	T, He, Ut/Vg	Skin?	N
5. Ethylbenzene	T, He	K, Te, Lu, L	N
6. Methylene chloride	Lu, T?, MG?	MG, Lu, L	Y
7. Propylene	none	none	Y
8. Styrene	T, MG, Lu?	Lung?	Y
9. Styrene oxide	FS	FS	Y
10. Toluene	T, MG,He, Leuk	none	N
11. Trichloroethylene	Te, K	K, L, Te	Y
12. Trichlorofluoromethane	none	none	Y
13. Vinylidene chloride	T, Leuk	none?	N
14. Xylenes	T, MG, He, Leuk	none	N
		Correlative totals	8Y, 6N

[a]Organs/tissues listed alphabetically, including benzene listed here as multiple (see TABLE 7): An = angiosarcomas, extrahepatic, 1; Br = brain, 1; FS = forestomach, 5; He = tumors of the head, 4 (often includes combined total tumors of Zymbal gland, ear duct, nasal cavities, oral cavity); HG = harderian gland, 2; K = kidney, 3; L = liver, 6; Leuk = leukemia, 5; Lu = lung 6; lymphoma, 3; MG = mammary gland, 8; Mult = multiple organ/tissue sites [TABLE 6]; oral, 2; Ov = ovary, 2; preputial gland, 1; skin, 3; T = total malignant tumors, 10; Te = testis, 3; Ut/Vg = uterus/vagina, 2; Z = Zymbal gland, 3; ? = questionable response.

[b]Concordance: Y = at least one chemical-specific carcinogenic target organ is the same between the two laboratories; N = no chemical-specific target organ is the same.

acrylonitrile caused tumors of the brain, mammary and Zymbal glands, liver, extrahepatic angiosarcomas, and total malignancies (TABLE 6).

Even though there were 10 individual tumor sites, none were overlapping among rats and mice. Conversely, for four tumor sites there was concordance in both sexes within a species.

In contrast to mice, no tumors in rats were observed when acrylonitrile was given by gavage (RF). The use of only a single low dose (5 mg/kg) and a 52-week exposure duration (RF) are in contrast with the use of doses of 2.5, 10.0, and 20 mg/kg and 104 weeks of exposure (NTP study) and likely account for the lack of carcinogenesis found in the RF study.

A structurally related chemical, methacrylonitrile, given by gavage did not induce any tumors in rats or mice (NTP). However, in male and female rats, methacrylonitrile administration caused significant increases in the incidences of nonneoplastic lesions of the nose and liver; these chronic toxic lesions did not lead to tumors.

TABLE 6. Acrylonitrile: organ/tissue site tumors from the RF and NTP in six experiments using one strain of rats and one strain of mice

	Sprague-Dawley (RF) Rat				B6C3F1 (NTP) Mice		
	Inhalation		Gavage		Gavage		Tumor sites
	M	F	M	F	M	F	
Brain	+	+	–	–	–	–	2
Angiosarcoma	+	+	–	–	–	–	2
Forestomach	–	–	–	–	+	+	2
Harderian gland	–	–	–	–	+	+	2
Mammary glands	–	+	–	–	–	–	1
Zymbal gland	+	–	–	–	–	–	1
Liver	+	–	–	–	–	–	1
Lung	–	–	–	–	–	+?	1
Ovary	–	–	–	–	–	+?	1
All malignant tumors	+	+	–	–	–	–	2
Number of tumor sites	5	4	0	0	2	4	15

NOTE: + = positive chemical-related carcinogenic response in those target organs; +? = questionable or marginal positive carcinogenic response; – = no evidence of carcinogenic activity related to chemical exposure.

Benzene

Benzene[80–93] induced multisite and multispecies/strains carcinogenic effects in both sexes in both RF and NTP studies.

There were 10 tumor sites in the RF bioassays and 11 tumors sites in the NTP studies. In both studies, using different strains of rats and mice, there were 8 sites and "total malignant tumors" in common (TABLE 7) and 13 unique sites. The most responsive strains in these experiments were the B6C3F1 mice with 10 sites of carcinogenic activity and Sprague-Dawley rats with 8 sites. Fischer rats had three positive tumor sites. All strains showed increases in total malignant tumors. Tumors of the Zymbal gland were the first and most consistent response from benzene exposure.[88] This tumor site has been criticized because humans do not have an exact replica, but there are modified sebaceous glands of the ear in humans.[90] The second tumor site in prevalence was mammary glands. In three of the experiments benzene induced lung tumors; and, in two others, benzene caused tumors of the skin even though in all these cases benzene was given by gavage.

Chlorine

Chlorine[94–97] showed leukemogenic effects in female Sprague Dawley rats (RF) and in female Fischer rats (NTP) when given by drinking water. No carcinogenic effects were observed in male rats or in mice. Chloraminated drinking water also caused marginal increases in leukemia in female rats (NTP).

TABLE 7. Benzene: organ/tissue site tumors from RF and NTP in seven experiments using three strains of rats and three strains of mice

Strain Species	Sprague-Dawley rats		Wistar rats	Fisher[a] rats	Swiss mice	RF/J mice	B6C3F1[a] mice	
Route	Gavage	Inhalation	Gavage	Gavage	Gavage	Gavage	Gavage	Total
Zymbal gland	+	+	+	+	+	–		6
Mammary gland	[+]	[+]	–	–	+	+	+	5
Oral	+	+	+	+	–	–	–	4
Lung	–	–	–	–	+	+	+	3
Nasal cavities	+	[+]	+	–	–	–	–	3
Lymphoma	[+]	–	–	–	–	+	+	3
Liver	+	[+]	–	–	–	–	+	3
Forestomach	+	–	–	–	–	–	[+]	2
Skin	+	–	–	+	–	–	–	2
Uterus	–	–	–	–	–	–	+	1
Ovary	–	–	–	–	–	–	+	1
Harderian	–	–	–	–	–	–	+	1
Preputial gland	–	–	–	–	–	–	+	1
All malignancies	+	+	+	+	+	+	+	7
Total Sites	9	6	4	4	4	4	4	11

[a] = the two strains utilized in the NTP studies. + = a positive carcinogenic response; [+] = marginally increased carcinogenic response; – = no significant carcinogenic activity; sites listed in order of prevalence of responses per organ/tissue.

Diesel Fuel

Diesel fuel,[98–100] or, as in the NTP dermal studies, marine diesel fuel at doses of 250 and 500 mg/kg resulted in dose-related incidences of squamous cell neoplasms of the skin (primarily carcinomas), providing equivocal evidence of carcinogenicity for male and female B6C3F1 mice. The sensitivity of detecting systemic carcinogenicity in female mice dosed with marine diesel fuel was reduced by poor survival. Two-year NTP dermal studies of JP-5 navy fuel at doses of 250 and 500 mg/kg provided no evidence of carcinogenicity for male and female B6C3F1 mice. RF studies included unleaded gasoline, leaded gasoline, gasoil (diesel), kerosene, and several solvents therein; only the first three are mentioned here. All exposures were via gavage, four days per week, at exposures of 0, 500, and 800 mg/kg. Carcinogenic effects of all three of these gasolines included increases in total malignant tumors, mammary gland (except gasoil), head, and uterus/vaginal with unusual neurinosarcomas. Total tumors in the low-dose female mice (but not in male mice) were doubled in the NTP JP-5 navy fuel study. Conversely in the diesel fuel study, female but not male mice controls had a doubling of total tumors over those in exposed animals, but there was poor survival among treated animals.

Ethylbenzene

Ethylbenzene[101–103] induced carcinogenic responses in both sexes of rats (kidney, male and female; and testes) and of mice (lung in males and liver in females) in NTP inhalation studies. Tumors of the head (mainly nasal cavity) and total malignant tumors were increased in rats in the RF gavage studies. Both studies showed carcinogenic activity; the NTP inhalation studies were perhaps more convincing in that cancers appeared in four target organs, and ethylbenzene was carcinogenic in both sexes of rats and mice. This may have been due to the different routes of exposure, strain of rats, or a combination of the two. Nonetheless, taken together these findings show convincing evidence of carcinogenicity for ethylbenzene.

Methylene Chloride (Dichloromethane)

Methylene chloride (dichloromethane)[104–106] exhibited multisite carcinogenesis in both the RF and the NTP studies. Given by gavage (RF), dichloromethane induced lung tumors in male mice, and a marginal increase in total malignant tumors occurred in female rats. By inhalation exposure, dichloromethane induced tumors of the mammary gland in both laboratories, albeit marginally at RF. A nonsignificant increase in total malignant tumors in rats was reported (RF). In the NTP inhalation studies, lung and liver tumors were induced in both sexes of mice. In the NTP studies mammary gland tumors, not a usual occurrence in males, were increased in both male and female rats.

Propylene

Propylene[107–111] exposures by inhalation did not cause any increases in tumors at either laboratory. In the NTP studies, rats and mice were exposed to 0, 5000, and 10,000 ppm, while in the RF studies, rats (104 weeks) and mice (78 weeks) were exposed to 0, 200, 1000, and 5000 ppm. Conversely, the oxide of propylene via inhalation caused a small number of papillary adenomas of the nasal turbinates in male and female rats, and hemangiomas or hemangiosarcomas of the nasal turbinates in male and female mice (NTP).

Styrene

Styrene[112–117] given by inhalation (0, 25, 50, 100, 200, 500 ppm for 52 weeks), by gavage (0, 50, 250 mg/kg for 52 weeks), and by injection (1 sc or 4 times ip at 50 mg) caused only tumors of the mammary gland in rats and total benign/malignant tumors (RF) by inhalation. In addition, by gavage (rats: 0, 500, 1000, 2000 mg/kg; mice: 0, 150, 300 mg/kg), styrene was associated with a marginal increase in tumors of the lung in male mice (NTP).

Styrene Oxide

Styrene oxide[112–117] was studied using the gavage route of administration: RF used 0, 50, 250 mg/kg 4 or 5 times/week for 52 weeks (animals then lived out their life without exposure) and NTP used 0, 275, 550 mg/kg for rats and 0, 375, 750 mg/kg for mice 3 times per week for 104 weeks. The main pathologic findings from both laboratories were high incidences of squamous cell carcinomas or papillomas of the

forestomach in both sexes of both rats and mice. Additionally, there was a statistically significant increase in the incidence of hepatocellular neoplasms in male mice receiving 375 mg styrene oxide/kg (NTP). These virtually single target site carcinogens like styrene oxide often cause cancer in the first organ exposed (so-called "application site" carcinogenesis): gavage and forestomach tumors; inhalation and nose or lung tumors; and dermal and skin tumors.

Toluene

Toluene[118–122] did not cause any tumors using the inhalation route with exposures as high as 1200 ppm (NTP). By the gavage route, tumors of the mammary glands, head, total malignant tumors, and leukemias were increased in rats (RF). Tumors of the mammary glands were somewhat increased in the low-dose group (500 mg/kg: 27% versus 14%) but not in the high-dose group (800 mg/kg). The total number of malignant tumors was doubled in the exposed animals compared to controls. Combined tumors of the head were elevated only in the top-dose male rats. Only in the low-dose group of female rats was the incidence of leukemias/lymphomas convincing (2% vs. 17.5%). Thus even though there were four positive responses, the findings overall, while being evidence of carcinogenic activity, were considered less than overwhelming.

Trichloroethylene

Trichloroethylene[123–126] has been evaluated in several laboratories, and in all there is evidence of carcinogenic activity. The two major routes of exposure, oral and inhalation, gave convincing carcinogenesis. Inhalation (RF): Sprague-Dawley rats showed increases in Leydig cell tumors, non-dose-related leukemias, and some rare renal tumors. Swiss mice exhibited lung and liver tumors. B6C3F1 mice had increases in lung, liver (?), and total malignant tumors. Oral gavage (NTP): tumors of the liver in B6C3F1 mice; a few rare tumors of the renal tubular cells in ACI, August, Marshall, Osborne-Mendel, and Fischer rats; interstitial cell tumors of the testis in Marshall rats. Thus, trichloroethylene in these and other studies clearly shows carcinogenic activity in the liver, lung, kidney, lymphoma/leukemia, and testis. Importantly there is evidence in human studies as well: liver and biliary tract, non-Hodgkin's lymphoma, and kidney (NTP report on carcinogens, 2000). These trichloroethylene target tissues/organs in both rodents and humans are particularly consistent.

Trichlorofluoromethane

Trichlorofluoromethane[127,128] exposures by inhalation and by gavage were uniformly negative. Inhalation exposures were 0, 1000, and 5000 ppm to Sprague-Dawley rats and Swiss mice (RF). Oral exposures to Fischer rats were 0, 500, and 1000 mg/kg and to B6C3F1 mice were 0, 2000, and 4000 mg/kg (NTP). The rat gavage experiments were considered inadequate due to high and early chemical-associated mortality.

Vinylidene Chloride

Vinylidene chloride[129–134] was studied using two routes of exposure. The RF inhalation experiments showed increases in leukemias and total malignant tumors in

Sprague-Dawley rats whose exposure began *in utero*. There was a marginal increase in mammary gland tumors in female rats. The NTP gavage experiments (0, 1, 5 mg/kg rats; 0, 2, 10 mg/kg mice), while uniformly negative, were considered less than adequate because the use of a maximum tolerated dose had not been clearly demonstrated. There was a slight increase in the low-dose female mice for lymphoma or leukemia (7/48, 15/49, 7/50).

Xylenes, Mixed

Xylenes, mixed[135–138] were evaluated for carcinogenicity in both laboratories by the gavage route. The NTP studies showed no evidence of carcinogenic activity of mixed xylenes at exposures of 0, 250, or 500 mg/kg for Fischer rats and to B6C3F1 mice at 0, 500, or 1000 mg/kg. The commercial mixture contained 60% *m*-xylene, 14% *p*-, 9% *o*-, and 17% ethylbenzene. The RF bioassay used xylenes composed of 50% *m*-, 27% *o*-, 22% *p*-, and 0.3% toluene at exposures of 0, 500, and 800 mg/kg. Sprague-Dawley rats (RF) showed increases in total malignant tumors, mammary gland tumors, lymphomas/leukemias, and tumors of the head; all were non-dose related.

DISCUSSION

Both the Ramazzini Foundation and the National Toxicology Program are pioneers in the study, design, conduct, evaluation, and interpretation of long-term chemical carcinogenesis bioassays, which serve to identify chemicals that cause cancer in experimental animals and that are most likely to cause cancer in exposed humans.

This paper compares the carcinogenesis results for 14 chemicals studied, evaluated, and reported by both RF and NTP laboratories (TABLE 8)**.** Results between the RF and the NTP for the 14 chemicals studied by both laboratories are remarkably consistent regarding whether a chemical showed a positive or negative carcinogenic effect: that is, 11 of 14 chemicals are concordant. The RF and NTP studies had at least one target organ in common for eight chemicals; for six chemicals, there was no common target organ.

Only three chemicals gave inconsistent results: xylene, vinylidene, and toluene. RF had positive carcinogenicity findings, and the NTP did not. Each of these is discussed in an attempt to ascertain the differences in carcinogenic responses found by the two laboratories.

In the RF studies of xylenes, neither total malignant tumors nor those of the oral cavity were significantly increased until after 112 weeks. Increases in hemolymphorecticular leukemias were not seen until week 144, long after a two-year study would have been terminated. Thus, it appears that the difference in detecting carcinogenicity of xylenes is simply the duration of the experiments. The RF typically exposes animals for 52–104 weeks and then allows the animals to live out the remainder of their natural lives. The NTP bioassay terminates at 104 weeks. The NTP study design was an attempt to mimic an occupational lifetime for workers—that is, from young adulthood through retirement age, the human age that is comparable to 2 years of age for rats and mice. Because of great increases in life span over the past 50 years, it may be beneficial to extend studies in rodents beyond 2 years, particularly because most human tumors occur later in life.

TABLE 8. Summary bioassay findings from RF and NTP

1. Chemicals studied by RF	200
2. Chemicals studied by NTP	500
3. Bioassay designs basically similar, except	
a. RF exposures of 52–104 weeks	
b. NTP exposures 104 weeks	
c. RF duration: natural life span	
d. NTP 104 weeks (2 years)	
e. RF rat species: Sprague-Dawley	
f. NTP rat species: Fischer 344	
4. Same chemicals studied by RF and NTP	21
5. Bioassays evaluated and published	14
6. Routes of exposure	
a. Same for a chemical	8
b. Different for a chemical	6
c. Most used route: gavage	16
d. Next most used route: inhalation	13
7. Carcinogenicity (+,+ or –,–) concordance	11/14
8. Organ/tissue site commonality	8/14
9. Most common tumor sites (per chemical)	
a. Total malignant tumors	10
b. Mammary glands	8
c. Lung or liver	6
d. Forestomach or leukemia	5

Results in the vinylidene chloride bioassays did not agree between the two laboratories. Experiment duration was shorter in the NTP studies, and exposure concentrations were lower. Perhaps more importantly, the RF studies began with *in utero* exposure from 12 days of gestation and continued for two years after birth. The animals then lived out their life span. Tumors were increased in the offspring but not in the breeders. This underscores the importance of evaluating carcinogenicity of chemicals in offspring of women exposed to potentially carcinogenic chemicals before or during a pregnancy.

In the case of vinylidene chloride, one of the tumor categories that showed increases in the RF study was the category of "total malignant tumors," a carcinogenesis category not used by the NTP. The low-dose vinylidene chloride doubling of total malignant tumors (22 versus 9) in female mice (NTP) is similar to the doubling reported by RF (34.1 versus 17.9). The counting of total malignancies also explains the discordance between the RF and NTP for toluene-induced tumors. Thus, it may be prudent to include the total number of tumors caused by a given agent in evaluating its carcinogenicity and potential for causing tumors in humans.

ACKNOWLEDGMENTS

I thank Morando Soffritti and Fiorella Belpoggi for inviting me to this meeting to honor Cesare Maltoni and David P. Rall, and for their long collaboration and friendship. In addition, I thank Myron Mehlman for his efforts in organizing this meeting and clearing the way for me to attend. For their useful and valuable remarks on my slides and on this paper, I thank John Bucher and Ronald Melnick.

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Benzene

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Chlorine

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Ethylbenzene

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Methylene Chloride (Dichloromethane)

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Toluene

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