Review

Risk of human health by particulate matter as a source of air pollution —Comparison with tobacco smoking—

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ABSTRACT — Increased air pollution, containing carcinogenic particulate matter smaller than 2.5 μm ($\text{PM}_{2.5}$), has gained particular attention in recent years as a causative factor in the increased incidence of respiratory diseases, including lung cancer. Extensive carcinogenicity studies conducted recently under Good Laboratory Practice conditions by National Toxicology Program in the USA, Ramazzini Foundation in Italy or Contract Research Organizations on numerous chemical compounds have demonstrated the importance of considering dose levels, times and duration of exposure in the safety evaluation of carcinogenic as well as classical toxic agents. Data on exposure levels to chemical carcinogens that produce tumor development have contributed to the evaluation of human carcinogens from extrapolation of animal data. A popular held misconception is that the risk from smoking is the result of inhaling assorted particulate matter and by products from burning tobacco rather than the very low ng levels of carcinogens present in smoke. Consider the fact that a piece of toasted bread contains ng levels of the carcinogen urethane (ethyl carbamate). Yet, no one has considered toast to be a human carcinogen. Future human carcinogenic risk assessment should emphasize consideration of inhalation exposure to higher levels of benzo (a) pyrene and other possible carcinogens and particulate matter present in polluted air derived from automobile exhaust, pitch and coal tar on paved roads and asbestos, in addition to other environmental contaminant exposure via the food and drinking water.

Key words: Air pollution, Particulate matter, Tobacco smoking, Exposure dose level, Carcinogenesis, Lung cancer

INTRODUCTION

As R. A. Weinberg described in his book; “Racing to the Beginning of the Road (1996)”, the incidence of lung cancer increased dramatically over the past two centuries. It became most prevalent as occupational cancers in late 18th century Europe during the industrial revolution from the use of coal for heating (Clayson, 1962; Hueper and Conway, 1964; Arcos *et al.*, 1968). Similar occupational cancers of the skin and urinary bladder were also evident in industrial workers and miners. Unlike these relatively new forms of cancer, resulting from environmental contamination, there are no indications that the worldwide incidence of other forms of cancer have increased. In addition, there is no clear epidemiological evidence

that lung cancer was the result of smoking tobacco in the American Indian or native New Guinea populations.

In addition to Weinberg, M. Enomoto, observed lung cancer was a rare finding at St. Luke’s International Hospital, Tokyo and the University of Tokyo Hospital, Tokyo, where he worked as a human pathologist from 1954 to 1961 (Matsui, 1960; Kitagawa, 1965). During the early 1970’s, dramatic increase in lung cancers were evident in the urban areas of Japan similar to that seen in the United States during the late 1940’s. The incidence of lung cancer in Japanese males is the most prevalent form of cancer in Japan since 1995, exceeding that of stomach cancer (Fig. 1). A similar trend was evident fifty years earlier in the United States from 1940 to 1950 (Fig. 2).

Sir Percivall Pott observed that many patients that had

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Fig. 1. Age-adjusted cancer death rate among males for selected cancer types, Japan, 1960-1999. Compiled from the data of “Kokumin-eisei no Doukou (Current Japanese Health)”, (2001), Health Welfare Statistics, **48**, 416.

cancer of the scrotum were “chimney sweeps” in late eighteenth century. Malignant skin tumors (epitheliomas) were experimentally produced by painting coal tar onto the ears of rabbits by Yamagiwa and Ichikawa in 1918. Interestingly enough, the coal tar used was imported from France and not the tar from burning tobacco leaves (Yamagiwa and Ichikawa, 1918). Since that time carcinogenicity studies using animal models coupled with human epidemiological evidence, comparison of threshold dos-

es and historical data have been used to identify unknown carcinogens (Enomoto, 2000).

The little known fact that cigarette smoke contains carcinogenic aromatic hydrocarbons or nitrosamines; 60 to 200 ng/one cigarette (IARC, 1990) or benzo (a) pyrene; 12.9 to 19.9 ng/cigarette (Nozaki and Kawai, 1978), respectively, comparable to the content of the carcinogen urethane in a piece of whole wheat toast, 312 ng (Zimmerli and Schlatter, 1991) has not been taken into consideration. Such levels are so low they are considered to possess negligible carcinogenic potential and not a risk to humans (Enomoto, 2000; Williams *et al.*, 2004). The classical coal stove produces 1.7 to 3.3 g (per BTU: British thermal units; 0.2520 Kcal.) of benzo (a) pyrene, more than million times of that in tobacco smoke. It should be remembered that coal is the natural decomposition product of plants over millions of years under high pressure and temperature. Indoor air pollution from burning coal was reported to be closely related to the high incidences of lung cancer in Xuan Wei, China, particularly in women who did the cooking (Mumford *et al.*, 1987). Japanese were also exposed to pollution of burning “Rentan”, made from powdered coal for cooking, for approximately twenty years including years of World War II. In addition, an increased incidence of lung cancer, with deposits of particulate matter, was evident in dogs living in large cities from 1962 to 1966 (Takemoto *et al.*, 1968). Churg *et al.* (2003) suggested that chronic exposure to high levels of ambient particulate matter (mean levels of $PM_{2.5}$; $22 \mu g/m^3$) is associated with small airway remodeling of

Fig. 2. Age-adjusted cancer death rate among males for selected cancer types, USA, 1930-1977. Elias, P.S. (1990): Food safety issues; The EEC approach. In: Abstract. on international conference on issues in food safety and technology, pp.13-14, Augt. 29, 1990 Mich. St. Univ., East Lansing, Mich.

human lungs.

Modern lifestyles in industrial cities, including automobile use and exposure to exhausts from coal and petroleum greatly contribute to the accumulation of particulate matter in the atmosphere (Handa *et al.*, 1983; Matsushita, 1989; Valberg and Crouch, 1999; Lena *et al.*, 2002; Churg *et al.*, 2003). Atmospheric reactions of sunlight with hydrocarbons and automobile exhausts and other particulate matter produces an oxidizing, photochemical type of air pollution. Level of atmosphere contamination can reach levels that are hazardous to man and the environment. Aldehydes are the major products in the photo-oxidation of hydrocarbons and in the reactions of hydrocarbons with ozone, oxygen atoms as free radicals. Formaldehyde and acrolein are present in urban atmospheres. Approximately, 50% of the total aldehyde is present as formaldehyde (Menzel and Amdur, 1996). In addition, road pavement contains asphalt (Nozaki, 1988) and produces particulate matter containing residual oil fly ash and many kinds of metals (Hunt *et al.*, 2003). Carcinogens were previously reported to be present in the air of major cities and industrial sites at a level of μg or greater/ m^3 (Table 1). The cause and effect of increased incidences of respiratory cancers resulting from air pollution is clearly evident.

One consideration why smokers have a higher incidence of respiratory disease than non-smokers may be that smokers orally inhale the burnt tobacco smoke directly into the lungs bypassing the filtering effects of the nose

and nasal cavities on airborne pollutants. However, much of $\text{PM}_{2.5}$ in polluted air undergoes peroxidation due to burning tobacco while smoking (Fig. 3). Can moderate smoking be enjoyed safely in a clean environment.

The purpose of this review is to emphasize the importance of exposure levels of toxic and carcinogenic substances present as particulate matter in air pollution. Emphasis must be placed on effects of chemicals on environmental contamination in our modern society and their effects on the development of human disease, especially respiratory diseases (Lewtas *et al.*, 1983; Cupitt and Fitz-Simmons, 1988; Mastrangela, 1996; Woodruff *et al.*, 1998; Moyer *et al.*, 2002; Suwa, *et al.*, 2002; Liu *et al.*, 2003).

Significance of exposure level of carcinogens

A. Animal data

Analyses of the carcinogenic potency database accumulated by NTP in the USA (Huff *et al.*, 1988; Ashby and Tennant, 1991; Huff, 1999) and Ramazzini Foundation in Italy (Soffritti *et al.*, 2002), and of carcinogenicity studies on industrial chemicals including pharmaceuticals and pesticides conducted at worldwide GLP facilities (Enomoto *et al.*, 1996) have supported refined risk assessments based on the evaluation of state-of-the art scientific evidence coupled with sound epidemiology studies and not speculation. Recent studies on mechanisms of carcinogenesis by both genotoxic and non-genotoxic carcin-

Table 1. Air pollution: Measurement data of particulate matter ($\text{PM}_{2.5}$) and benzo (a) pyrene

Contents	Air Pollution
Particulate matter:	Average ambient concentration: 4.9-19.2 $\mu\text{g}/\text{m}^3$, USA ¹⁾
$\text{PM}_{2.5}$	Airborne PM conc.: 19.0-29.9 $\mu\text{g}/\text{m}^3$, Urban areas ²⁾ 120-960 $\mu\text{g}/\text{m}^3$: Air planes, taverns, workrooms ³⁾ Particulate emissions: Diesel vehicle 0.23 g/km, Gasoline vehicle 0.021 g/km ⁴⁾
Benzo (a) pyrene	1.7-3.3 g/BTU*: Coal stove ⁵⁾ 9.6 $\pm$ 2.6 $\mu\text{g}/\text{m}^3$ in traffic tunnels ⁶⁾ Tar stuck on gasoline engine mufflers: 139 $\mu\text{g}/\text{g}$ ⁷⁾ 2.6-7.4 ng/ m^3 ; Room for smokers 1.1-4.4 ng/ m^3 ; Non-smoking room. ⁵⁾ 20-40 ng: Main stream smoke/cigarette ⁸⁾ <*BTU: British thermal units; 0.2520 Kcal.>

Ref.: 1) Schwatz *et al.*, 2002; 2) Lena *et al.*, 2002; 3) Cupitt and Fitz-Simmons, 1988; 4) Strandell *et al.*, 1994; 5) Matsushita, 1989; 6) De Fré *et al.*, 1994; 7) Handa *et al.*, 1983; 8) Nozaki, 1966; Nozaki and Kawai, 1978

Fig. 3. Air pollution and tobacco smoking.

Ref. (Sakabe *et al.*, 1965; Nozaki, 1966; Nozaki and Kawai, 1978; Matsushita, 1989; Zimmerli and Schlatter, 1991)

ogens conducted in parallel with long-term studies have also supported the more relevant evaluation of carcinogenesis data obtained after two years of feeding, inhalation, or other routes of exposure (Tennant, 1993; Gold *et al.*, 1998; Kundsén, 1999; Ward, 2007).

Improved animal study facilities and design, including sufficient number of treatment groups, consisting of three or more study dose levels with sufficient numbers of animals per group in conjunction with gross and histological evaluations of all tissues, lesions and masses led to more meaningful data for realistic interpretations (Maronpot, 1985). Analysis of the results of recently completed carcinogenicity studies revealed for the first time the existence of dose-levels which had no apparent, treatment-related effects on carcinogenic processes and the concept of threshold doses (Enomoto *et al.*, 1990; Enomoto, 2000). Toxicological pathologists have evaluated numerous histological specimens from all organs of animals (Roe, 1972). In addition, they have become aware of "whole body carcinogenesis" as dose-dependent and time-related responses, in view of recent elucidation of the effects of defense mechanisms, including DNA replication and repair, oxidative stress, hormonal influences and cytokines on homeostasis (O'Connor *et al.*, 2000; Enomoto, 2000; Cook and Calabrese, 2006). A number of

more sophisticated mechanistic studies including formation of DNA adducts (Williams *et al.*, 2000), GST-P positive foci and 8-OHdG formation (Fukushima *et al.*, 2002) are suggestive of no risk of cancer to humans at low levels of environmental exposure to DNA-reactive carcinogens. The finding of no-effect levels provides a basis for understanding why very low level environmental exposures of humans, even to DNA-reactive carcinogens, may not be cancer risks (Williams, 2004).

The number of dose groups and dose levels are critical to determine safe dose levels. However, the maximum dose levels as stipulated in regulatory guidelines may result in manifestation of specific biological effects due to unrealistic overdose, saturating adaptation or hormesis exceeding body-burden effects (Calabrese and Baldwin, 2003). Consequently, many of the chemicals evaluated resulted in positive toxic or carcinogenic responses, probably due to oxidative effects including those caused by lack of drug metabolizing enzymes. Using the maximum tolerated dose (MTD) as the highest dose in carcinogenicity studies provoked debate in the classical safety evaluation of chemicals (Haseman and Seilkop, 1992; Hengstler *et al.*, 2003). Most studies that showed positive carcinogenic responses at the MTD, not statistically or biologically significant, were non-genotoxic car-

cinogens, and therefore are not relevant as a real human carcinogenic threat (Lewis *et al.*, 1998; Huff, 1999; Ward, 2007). Recent data, including hormonal, nutritional and age-associated metabolic changes that can influence the occurrence of naturally-occurring tumors have contributed to evaluation of long-term bioassay data, which show an increase or decrease in the incidence of these tumors in the rat or mouse (Enomoto *et al.*, 1990). Large granular lymphocytic (LGL) leukemia or chronic nephropathy-related renal cancer seen predominantly at high dose-levels near the MTD in Fischer 344 rats seems to be due to promotion and not direct effects.

Similar to the results of extensive studies on more than a hundred chemicals conducted by NTP (Huff *et al.*, 1988), approximately 40% of non-genotoxic chemicals evaluated in both rats and mice at the Biosafety Research Center (An-Pyo), Japan, showed positive neoplastic responses in one or more of the sex-species experiments, from 1983 to 1999 (Enomoto *et al.*, 1996). Whereas three genotoxic chemicals were positive in all four groups, non-genotoxic compounds exhibited species or sex-related responses to tumor development. In addition, nearly all chemicals examined were negative at low and mid-dose levels in all studies of both genotoxic and non-genotoxic chemicals.

Table 2 shows the ratio of the highest or middle dose capable of inducing tumor development to the lowest or middle dose that did not induce tumors. Since the highest dose level was selected based on the MTD, 10% reduction in body weight, the ratio was analyzed against the mid-dose, when mid-dose was capable of inducing tumor development. Generally, this ratio was less than a hundred fold, and generally less than ten with pharmaceuticals. Only two pharmaceuticals tested were positive in one sex/species at the low dose level. The reason is unclear. Table 3 lists chemicals with positive neoplastic responses selected from the NTP database, from 1983 to 1999 (Fung *et al.*, 1995; Huff, 1999). For carcinogenicity studies using only two dose levels, both dose groups showed the same positive or negative neoplastic responses occasionally. Similar results were also seen in studies employing three dose levels, when the ratio between low and high dose levels was less than ten (four or five). The Biosafety Research Center used a ratio of ten as a generally employed safety factor for carcinogenicity studies on pharmaceuticals. All databases on exposure levels obtained from extensive carcinogenicity studies appear to suggest that carcinogenic doses generally lie in the range between maximum and the minimum levels of doses required to induce tumors (Enomoto *et al.*, 1996).

Results of the studies listed above revealed that the

ratio of the highest dose capable of inducing tumor development to the lowest dose that did not induce tumor development was generally less than a hundred-fold. Only a few strong carcinogens such as aflatoxin B₁ and some nitroso-compounds exhibited a wider range, between 100 to 1,000 fold (Enomoto, 2000).

B. Human exposure and cancer

Many occupational carcinogens were well known in the past, and were due to the lack of safety procedures for workers (Hueper and Conway, 1964). Some pharmaceutical products, especially anti-neoplastic and immuno-suppressive agents, can cause cancer, when used at high therapeutic dose levels (Dedrick and Morrison, 1992; Fraser, 1996).

Since most chemical carcinogens exhibit their potential to induce tumors at dose levels in the order of one microgram (μg) per kilogram of body weight per day or greater, cancer development would not be expected at exposure levels less than 100 parts per billion (ppb), corresponding to 2.5 $\mu\text{g}/\text{kg}$ body weight per day in humans (Enomoto, 2000). Williams *et al.* have suggested that food-borne carcinogens would not be carcinogenic at a level of less than 1.5 mg/person/day. Even the strongest carcinogen, AFB₁ may be not oncogenic at a level of less than 30 ppb, approximately 45 $\mu\text{g}/\text{person}/\text{day}$ by this assumption (Williams *et al.*, 2004).

In parallel with the extensive research on PM health effects advocated by US Environmental Protection Agency (Lippmann *et al.*, 2003), a number of epidemiologic and biomedical studies have shown that hazardous air pollutants may pose a potential public health threat for the past 50 years. Recent worldwide efforts in this field have resulted in the accumulation of data on air pollutants including carbon, and PM₁₀ or PM_{2.5} containing polycyclic aromatic hydrocarbons derived from diesel and gasoline exhausts (Cupittet *et al.*, 1988; Strandell *et al.*, 1994; Mastrangelo *et al.*, 1996; Woodruff *et al.*, 1998; Lena *et al.*, 2002).

Human risk assessment of complex mixtures of polycyclic organic matter (POM) employs an evaluation of comparative potency for estimating human cancer risks when no data exists for the POM being assessed, but does for similar POM mixtures. Such cancer risk estimations are expressed as 1 μg POM/ m^3 of inhaled air (Lewtas, 1993). Diesel emissions and cigarette smoke were the POM emission sources evaluated for comparison to those from coke ovens or roofing coal tar using the comparative potency method. As illustrated in Fig. 6 of their manuscript, risks of cigarette smoke appear to be less than 1/100 compared to POM estimates of other three

Table 2. Dose levels and ratios of carcinogens showing neoplastic responses from the data of Biosafety Research Center, Shizuoka, Japan <1983-1999>*

Chemicals	Sex	NCD in rat	ECD in rat	Ratio	NCD in mouse	ECD in mouse	Ratio
Genotoxic carcinogens							
(anti-neoplastics)	male	10-30 ppm	100 ppm	3.3	25 ppm	75-225 ppm	3
	female	10 ppm	30-100 ppm	3	25 ppm	75-225 ppm	3
(anti-neoplastics)	male	20-60 ppm	200 ppm	3.3	10-30 ppm	90 ppm	3
	female	20-60 ppm	200 ppm	3.3	10-30 ppm	90 ppm	3
(industrial chemicals)	male	100 ppm	1000-10000 ppm	10	100 ppm	1000-10000 ppm	10
	female	100 ppm	1000-10000 ppm	10	100 ppm	1000-10000 ppm	10
Non-genotoxic carcinogens							
(α -blocker)	male	100-300 ppm	1000 ppm	3.3	100-300 ppm	1000 ppm	3.3
	female	100-300 ppm	1000 ppm	3.3	100-300-1000 ppm	-	-
(anti-ulcer drug)	male	150 ppm	450-1500 ppm	3	100-300-1000 ppm	-	-
	female	-	75-225-750 ppm	-	100-300-1000 ppm	-	-
(anti-bacterials)	male	150 ppm	500-1500 ppm	3.3	100-300-900 ppm	-	-
	female	150-500-1500 ppm	-	-	100-300 ppm	900 ppm	3
(Ca-antagonist)	male	1000-3000-10000 ppm	-	-	32-100 ppm	320 ppm	3.2
	female	1000-3000-10000 ppm	-	-	32 ppm	100-320 ppm	3.1
(central nervous stimulator)	male	8-25-75 mg/kg	-	-	10 mg/kg	30-90 mg/kg	3
	female	8-25-75 mg/kg	-	-	10-30 mg/kg	90 mg/kg	3
(central nervous stimulator)	male	-	120-400-1200 ppm	-	100-300-1000 ppm	-	-
	female	120-400-1200 ppm	-	-	100-300-1000 ppm	-	-
(PAF-antagonist)	male	300-1000-3000 ppm	-	-	150-500-1500 ppm	-	-
	female	300-1000-3000 ppm	-	-	150-500 ppm	1500 ppm	3

Table 2. (Continued)

Chemicals	Sex	NCD in rat	ECD in rat	Ratio	NCD in mouse	ECD in mouse	Ratio
(herbicide)	male	20-100 ppm	6000-12000 ppm	60	10-50-3500-7000 ppm	-	-
	female	20-100 ppm	6000-12000 ppm	60	10-50-3500 ppm	7000 ppm	2
(herbicide)	male	50-200-800-1600 ppm	-	-	100-600 ppm	1200 ppm	2
	female	50-200-800-1600 ppm	-	-	100 ppm	600-1200 ppm	6
(anti-bacterials)	male	50-150-2000-4000 ppm	-	-	70-350 ppm	3500-7000 ppm	10
	female	50-150 ppm	2000-4000 ppm	13.3	70-350 ppm	3500-7000 ppm	10
(plant growth regulator)	male	400-2000 ppm	10000-20000 ppm	5	400-2000-20000-40000 ppm	-	-
	female	400-2000-10000-20000 ppm	-	-	400-2000-20000-40000 ppm	-	-

* All studies employed three or more than three dose levels. All chemicals were administered to both rats and mice. (Ref.: Enomoto *et al.*, 1990, 1996, 2000)

NCD: non-carcinogenicity dose level, ECD: evident carcinogenicity dose level.

Ratio shows the lowest ECD/the highest NCD

Bold numbers represent dose levels showing development: of malignant tumor. -: no data available

Table 3. Dose levels and ratios of carcinogens showing neoplastic responses from the NTP Database <1983-1999> (Ref.: Huff, 1999)

Chemicals (NTP TR No.)	Sex	NCD in rat	ECD in rat	Ratio	NCD in mouse	ECD in mouse	Ratio
Genotoxic carcinogens							
1,3-Butadiene (434) (synthetic rubber or resins)	male	-	-	-	6.25-20 ppm*	62.5-200 ppm*	3.1
	female	-	-	-	6.25-20 ppm*	62.5-200-625 ppm*	3.1
C.I. Disperse blue 1 (299) (hair dyes)	male	1250 ppm*	2500-5000 ppm*	2	-	600-1200-2500 ppm*	-
	female	1250 ppm*	2500-5000 ppm*	2	600-1200-2500 ppm*	-	-
1,2-Epoxybutane (329) (stabilizer in solvents)	male	200 ppm	400 ppm	2	50-100 ppm	-	-
	female	200-400 ppm	-	-	50-100 ppm	-	-
p-Nitroaniline (418) (chemical intermediate)	male	-	-	-	3 mg/kg*	30-100 mg/kg*	10
	female	-	-	-	3-30-100 mg/kg*	-	-
Quercetin (409) (flavonoids)	male	1000 ppm*	10000-40000 ppm*	10	-	-	-
	female	1000-10000-40000 ppm*	-	-	-	-	-

Table 3. (Continued)

Chemicals (NTP TR No.)	Sex	NCD in rat	ECD in rat	Ratio	NCD in mouse	ECD in mouse	Ratio
Non-genotoxic carcinogens							
Phenytol (404) (psycosomatic anticonvulsant)	male	240-800 ppm*	2400 ppm*	3	30-100-300 ppm*	-	-
	female	240-800-2400 ppm*		-	60-200-600 ppm*	-	-
Roxarsone (345) (growth promot., anticocc.)	male	50 ppm	100 ppm	2	100-200 ppm	-	-
	female	50-100 ppm	-	-	100-200 ppm	-	-
Salicylazosulfapyridine (457) (drug for ulcerat. colitis)	male	84 mg/kg*	168-337 mg/kg*	2	-	67.5-135-270 mg/kg*	-
	female	84 mg/kg*	168-337 mg/kg*	2	-	67.5-135-270 mg/kg*	-
Oxazepam (443) (sedative-hypnotic agent)	male	-	2500-5000 ppm	-	125 ppm*	2500-5000 ppm*	20
	female	-	2500-5000 ppm	-	125 ppm*	2500-5000 ppm*	20
Mitrex (313) (insecticide)	male	0.1-1-10 ppm*	25-50-100 ppm*	2.5	-	-	-
	female	0.1-1-10 ppm*	25-50-100 ppm*	2.5	-	-	-
Pentachlorophenol (349) (biocide)	male	-	-	-	100 ppm*	200-600 ppm*	2
	female	-	-	-	100 ppm*	200-600 ppm*	2
Butyl benzyl phthalate (458) (plasticizer)	male	3000-6000 ppm*	12000 ppm*	2	-	-	-
	female	6000-12000 ppm*	24000 ppm*	2	-	-	-
C.I. Pigment red 3 (407) (coloring paints, inks)	male	6000-12500 ppm*	25000 ppm*	4	12500-25000 ppm*	50000 ppm*	2
	female	6000-12500 ppm*	25000 ppm*	2	12500-25000-50000 ppm*	-	-
Rhodamine 6G (364) (dye)	male	120 mg/kg	250 mg/kg	2	1000-2000 ppm	-	-
	female	120 mg/kg	250 mg/kg	2	500-1000 ppm	-	-
2,6-Xylidine (278) (chemical intermediate)	male	300 ppm*	1000-3000 ppm*	3.3	-	-	-
	female	300 ppm*	1000-3000 ppm*	3.3	-	-	-
3,4-Dihydrocoumarin (423) (flavoring agents)	male	150 mg/kg*	300-600 mg/kg*	2	200-400-800 mg/kg*	-	-
	female	150-300-600 mg/kg*	-	-	-	200-400-800 mg/kg*	-

NCD: non-carcinogenicity dose level, ECD: evident carcinogenicity dose level.
Ratio: The lowest ECD/the highest NCD
Bold numbers represent dose levels showing development: of malignant tumor. -: no data available
: the dose in studies which employed 3 to 4 dose levels. No: only 2 dose levels employed.

emission sources (Cupitt *et al.*, 1994).

Concern also focused on the contribution of emissions of carcinogenic polycyclic aromatic hydrocarbons (PAH) from cooking sources to fatal atmospheric PAH concentrations on health effects (Mumford *et al.*, 1987; Li *et al.*, 2003). Research on biological mechanisms for health effect of particulates have revealed a variety of influences on respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), as well as lung cancer, cardiovascular disease, pregnancy and retarded child growth (Lippmann *et al.*, 2003; Moyer *et al.*, 2002; Suwa *et al.*, 2002; Liu *et al.*, 2003).

Epidemiologic data must be analyzed carefully using appropriate statistical and commonsense approaches. However, the validity of such data must be taken in account. Additional work remains to be done to fully understand the implications of available epidemiologic evidence for a cancer-dose response in humans. For more precise estimates of the risks of low-level exposure, a better understanding of the biological mechanisms and more comprehensive knowledge of the dose-response relationships for various types of biological effects are needed (Stavric, 1994; Gold *et al.*, 1998). In the interim, to take into account the reduced risk of chemicals at low doses and low dose rates, a linear-quadratic function is more appropriate than a simple linear function, for estimating the overall risks of late somatic and genetic effects, under conditions of whole-body, low-level exposure.

The high incidence and often unpredictable prevalence of naturally occurring tumors in aged rodents makes low-dose extrapolations difficult in classical life-time bioassays using animal models (Enomoto *et al.*, 1990). Therefore, the importance of a sufficiently large historical control data base can not be overemphasized for meaningful evaluation of results (Kirsch-Volders *et al.*, 2000).

Our confidence in predicting the likelihood of true risks will inevitably be poor for uncommon hazards due to false/negative results which must be rarer than the risk itself. Since most controversial environmental hazards are infrequent, this poses a dilemma for risk management.

State-of-art research is the first priority, "better science for better decisions". Therefore, risk assessment should encompass the evaluation of all available evidence. The argument for or against a threshold hypothesis must be based on theoretical as well as empirical evidence using commonsense for risk assessments. Conversely, regulatory responsibility may focus on chemicals showing the lower ratio between possible low level carcinogenicity within the control range, and the actual human exposure level, rather than assumptions at highly exaggerated estimates (Stöhrer, 1993).

The primary aim of medical and toxicological research is to define real risks to human health, rather than emphasizing the avoidance of tobacco smoking, pesticides or food additives, as often reported sensationally in the media. Cases of human liver cancer caused by man-made chemicals are extremely rare such as thorotrast, oral contraceptives and vinyl chloride. Carbon tetrachloride is a hepatocarcinogen, which induce hepatic tumors in high incidence in experimental animals even by inhalation. Like the American Conference of Governmental Industrial Hygienists (ACGIH) in the USA (1984), Japan Society for Occupational Health (1991) established the safe dose of carbon tetrachloride to be below 5 ppm, which is one fifth of the dose causing hepatocellular adenoma in B6C3F₁ mouse. However, there are no reported human cases of hepatic carcinomas following exposure of carbon tetrachloride to date. It must be emphasized that no clear evidence exists for pesticide induced human hepatic cancers worldwide for the past fifty years excluding accidental overexposure or suicide.

Clinical experts on liver diseases in Japan consider the hepatitis virus to be the major cause for liver cancer in humans. The remainder, less than 5 to 10% of all liver cancers, arise from the consumption of alcoholic beverages. Since alcoholic beverages contain relatively high amount of urethane, from 100 ppb to 20 ppm in brandy (Zimmerli and Schlatter, 1991), there may be a relationship to induction of cancers in the human liver and upper alimental tracts, such as the esophagus and oral cavity. Evaluation of human liver biopsy specimens were conducted for over 30 years with a special attention given to the possibly of exposure to numerous agents, such as hormones. Results have revealed practically no evidence that pesticides-or food-additives induced hepatic cancers (Sugimoto *et al.*, 1982; Enomoto, 1985; Enomoto, 2000).

Evaluation of inhalation studies Comments on tobacco-smoke carcinogenesis

Exposure to toxicants via inhalation is a consequence in all phases of human activity. The actual doses delivered to human or animal lungs are determined from the ambient concentration of the chemical compounds in the air and technically complicated experimental design of inhalation studies. Extensive progress in rodent bioassays has been accomplished in the past half century at several facilities including Oak Ridge National Laboratory (Oak Ridge, Tenn., USA) CIIT and NIEHS (Research Triangle Park, NC., USA), NCI, NIH (Bethesda, Md., USA) Fraunhofer ITEM (Hannover, Germany) and the Biosafety Research Center, JISHA (Hadano, Japan).

At present, toxicologists are devoting at great deal attention to the carcinogenic role of particulate air pollution, particularly inhalation studies on diesel exhausts or other particulate matter (Albert *et al.*, 1986; Kuwabara *et al.*, 1986; Mohr and Dungworth, 1988; HERP, 1987; Nozaki, 1988; Woodruff *et al.*, 1998). Significant and promising fruits of recent exploratory studies on a variety of particulate matter hazards, including health effects of the smallest and potentially most toxic ambient particles and ultrafine particles are worth mentioning (Brown *et al.*, 2001; Ghio *et al.*, 2004; Ogami *et al.*, 2007; Roller, 2007).

Among these studies the mechanistic evaluation of PM-induced oxidative stress performed by Xia *et al.* (2004) has encouraged research on ultrafine particulate toxicity. Another interesting finding was that Kanisawa (1982) demonstrated the possibility of lung cancer induction by oral treatment with nitrofurantoin in mice. As for human lungs, characterization of particulate matter in smog from autopsy lung samples by Hunt *et al.* (2003) provided evidence for the toxicological effects of severe PM exposure to smog with evidence of ultrafine carbonaceous and metallic PM.

Since discussion of data obtained by inhalation in animals is outside the scope of this review, the attention will be focused on a critical review of data that influence respiratory carcinogenesis of tobacco smoking.

The portal of entry of inspired air is the nasopharyngeal structure and includes the nasal turbinates, epiglottis, glottis, pharynx and larynx which remove larger air particles through impaction in the nasal turbinates plus filtration by nasal hairs. These structures condition the incoming air and remove the highest concentrations of chemicals before they can enter the lungs. As would be expected from the fact that the nasopharyngeal cavity can remove 50% or more of inhaled toxicants, individuals who mouth breathe when excited or tobacco smokers are less protected without the filtering action of the nasopharynx.

It is evident that exposure levels to carcinogenic agents including aromatic hydrocarbons in particulate matter exceed their very low natural occurrence in the smoke of dried tobacco leaves and lack clear evidence of carcinogenicity in animal studies (Coggins, 1998). A similar lack of evidence in animal studies is suggested in the IARC monograph (IARC, 1990).

Extremely high exposure to cigarette smoke at 250 mg total particulate matter/m³ (Hutt *et al.*, 2005; Hahn *et al.*, 2007) or over 1mg levels per kg body weight in animals which contained benzo (a) pyrene or carcinogenic nitroso-compounds and caused respiratory and other

tumors in number of past studies (Hoffmann and Hecht, 1985; IARC, 1990; Walaszek *et al.*, 2007), led us to wonder about this relevance to human carcinogenicity. Calculation of the amount of cigarette exposure to animals conducted in whole body exposure chambers, at a level of 250 mg particulate matter/m³ for 6 hr/day, required a total of, at minimum, 2,500 cigarettes/day. The amount of tobacco smoke was determined based on a rate of 8 puffs per cigarette per minute with an approximate content of 2.5 mg particulate matter (a cigarette containing 20 mg tar) in a volume of 35 ml of air per puff (Nozaki and Kawai, 1978). Therefore, it would be expected that the mean body weight of such exposed mice would be more than 10% lower than control animals under such heavily contaminated atmospheric conditions (Hutt *et al.*, 2005), in addition to the increased CO level in the inhaled air.

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Table 4 shows a list of several main chemical carcinogens revealing relatively high exposure to humans throughout normal life. They were compiled from a comprehensive risk assessment and management program for prevention of chemical hazards. Gold *et al.* (1998) have presented, human exposure/rodent potency (HERP) index for ranking possible carcinogenic hazards from exposure data, daily human exposure and carcinogenic doses from long term animal studies. Since the first five chemicals are known to be the major components of air pollution, further detailed examination of their exposure levels as well as the analyses of the particulate matter should be conducted routinely, in industrialized areas. As for urethane and aflatoxin B₁; two major food-born carcinogens, investigation of the dose-response relationship between intake of both chemicals and disorders of the alimentary tract can be of considerable public-health concern, in addition to air pollution in preventive medicine.

The strongest evidence to date that long-term exposure to airborne PM_{2.5} and products of sulfur oxide air pollution, particularly by fossil fuel combustion may be an important environmental risk factor for cardiopulmonary and lung cancer mortality. Particulate matter from the atmosphere contains nitrogen oxides from car exhaust, rubber particles from tires, debris from brake systems, phosphates from residential and agricultural fertilizers, and dozens of other pollutants including bacterial components (endotoxins). Pollutants may be also released from paving materials. Asphalt is known to contain pitch or oil ash. The top coat is likely to be quite toxic.

Table 4. Chemical carcinogens having a relatively high exposure to humans (1)

Contents	Tumor Dose Levels or Safety Level	Animal Data on the Range of Doses Exhibiting Carcinogenic Activity
Benzo (a) pyrene ¹⁾	Usual dose for tumor development	250 ppm (M: 375 mg/kg/day, H: 62.5 mg/kg/day): Skin painting
	The highest level	100 ppm (M: 15 mg/kg/day, H: 2.5 mg/kg/day) Feeding
	The lowest level	(M) 10 mg/kg/day (H: 1.2 mg/kg/day): Painting (R) 10 mg/m ³ , 1 hr/day: Inhalation (M) 25 mg/kg/day: Feeding
	Safety Level	4.5 ppm (M: 6.75 mg/kg, H: 1.13 mg/kg): Painting
NDEA and NDMA ²⁾	Usual dose for tumor development	14 ppm (R: 700 µg/kg/day, H: 117 µg/kg/day): Feeding or Foods
	The highest level	300 ppm (R: 15 mg/kg/day): Feeding
	The lowest level	0.5 ppm (R): Feeding
	Safety Level	10 ppb (H: 250 ng/kg/day): Foods
1,3-Butadiene ³⁾	Usual dose for tumor development	20 ppm (M: 3 mg/kg/day, H: 0.5 mg/kg/day): Inhalation
	The highest level	625 ppm (M: 94 mg/kg/day): Inhalation
	The lowest level	6.25 ppm (M): Inhalation
	Safety Level	
Dioxins ⁴⁾	Usual dose for tumor development	(Animals) 1-137 µg/kg/day: Feeding
	The highest level	
	The lowest level	100 ng/kg/day (R), 70 ng/kg/day (M): Feeding
	Safety Level	10-12500 ng/kg/day (Animals): Feeding
Carbon tetrachloride ⁵⁾	Usual dose for tumor development	
	The highest level	125 ppm (M, R) Inhalation
	The lowest level	25 ppm (M: 30 mg/kg/day) Inhalation, 125 ppm (R) Inhalation
	Safety Level	5 ppm (H: 1 mg/m ³ /day): Inhalation
Urethan: Ethyl carbamate ⁶⁾	Usual dose for tumor development	
	The highest level	600 ppm (M: 90 mg/kg/day): Feeding
	The lowest level	60 ppm (M: 9 mg/kg/day, H: 1.5 mg/kg/day): Feeding
	Safety Level	H: 180 µg/kg/day: Foods

Table 4. (Continued)

Contents	Tumor Dose Levels or Safety Level	Animal Data on the Range of Doses Exhibiting Carcinogenic Activity
Aflatoxin B ₁ ⁷⁾	Usual dose for tumor development	1 ppm (R: 50 µg/kg/day, H: 25 µg/kg/day) Feeding or Foods
	The highest level	
	The lowest level	20 ppb (R: 1 µg/kg/day, H: 0.5 µg/kg/day): Feeding or Foods
	Safety Level	5 ppb (H: 125 ng/kg/day): Foods

M: mouse, R: rat, H: human (estimated dose)
Ref.: 1) Clayson, 1962; Lijinsky and Schubik, 1964; Culp *et al.*, 1998; Enomoto, 2000; Shimizu *et al.*, 2000; 2) Enomoto, 2000; Fukushima *et al.*, 2002; Stöhner, 1993; Williams *et al.*, 2000; 3) Melnick *et al.*, 1990; Fajen *et al.*, 1990; Enomoto, 2000; 4) Huff *et al.*, 1994; De Vito *et al.*, 1995; 5) JBRC Technical Report, 2006; Nagano *et al.*, 2007; 6) Inai *et al.*, 1991; Enomoto, 2000; 7) Enomoto, 2000; Devereux *et al.*, 2001

Table 4. Chemical carcinogens having relatively high exposure to humans (2)

Contents	Exposure Dose Levels & Ratios	Exposure Levels to Humans and Estimated Ratios (carcinogenic potency/exposure level)
Benzo (a) pyrene ¹⁾	Usual exposure levels	15.1 ng/m ³ : Tokyo-202 ng/m ³ : Sapporo (Air)
	The highest level	9.6 ± 2.6 µg/m ³ in traffic tunnels ¹ 141.7-3.3 g/BTU: coal stove (Air) 139 µg/g on gasoline engine muffler (Skin)
	The lowest level	2.6-7.4 ng/m ³ ; Room for smokers, 1.1-4.4 ng/m ³ : Non-smoking room (Air).
	Ratio: T.D.L. (U)**/H.E.L.***	200 (2000/10: Tunnel) (Air) 0.45 (62.5/140) (Skin)
	Ratio: T.D.L. (L)****/H.E.L.	
NDEA and NDMA ²⁾	Usual exposure levels	50 ppb (H: 1.25 µg/kg): Beer
	The highest level	50 ppb (H: 1.25 µg/kg): beer, 2.88 µg Baltimore (Air)
	The lowest level	3 ng/m ³ : Tokyo (Air)
	Ratio: T.D.L. (U)**/H.E.L.***	94 (117/1.25)
	Ratio: T.D.L. (L)****/H.E.L.	4.8 (6/1.25)
1,3-Butadiene ³⁾	Usual exposure levels	
	The highest level	375 ppm (H: 9.4 mg/kg) (Air, Factory)
	The lowest level	
	Ratio: T.D.L. (U)**/H.E.L.***	0.05 (500/9400: HEL)
	Ratio: T.D.L. (L)****/H.E.L.	0.02 (160/9400: HEL)

Table 4. (Continued)

Contents	Exposure Dose Levels & Ratios	Exposure Levels to Humans and Estimated Ratios (carcinogenic potency/exposure level)
Dioxins ⁴⁾	Usual exposure levels The highest level The lowest level Ratio: T.D.L. (U)**/U.E.L.* Ratio: T.D.L. (L)****/U.E.L.	13 ngTCDDequivalents/kg 0.52-3.53 pg/kg/day? (Foods, Japan) 0.3-1.65 pg/m ³ (Air, Large cities, Japan) 5.28 pg (Air) 0.1 pg (Europe) Air 154 (20000/13) 8.4 (109/13)
Carbon tetrachloride ⁵⁾	Usual exposure levels The highest level The lowest level Ratio: T.D.L. (U)**/H.E.L.*** Ratio: T.D.L. (L)****/U.E.L.*	770 ng/m³ (Air), Non-polluted areas 6500 (5000/0.77)
Urethan: Ethyl carbamate ⁶⁾	Usual exposure levels The highest level The lowest level Ratio: T.D.L. (U)**/H.E.L.*** Ratio: T.D.L. (L)****/H.E.L.	100 µg/kg, Brandy: 50 ml 312 ng/piece of Whole wheat toast 125 (12500/100) 30 (3000/100)
Aflatoxin B ₁ ⁷⁾	Usual exposure levels The highest level The lowest level Ratio: T.D.L. (U)**/H.E.L.*** Ratio: T.D.L. (L)****/H.E.L.	40 ppb (H: 1 µg/kg/day: Foods, Kenya) 360 ppb (H: 9 µg/kg/day: Foods, Kenya) 3 ng/kg (H: Foods, USA) 0.1 (1/9) 0.06 (0.5/9)

Ref.: 1) Sakabe *et al.*, 1965; Handa *et al.*, 1983; Matsushita, 1989; De Fré *et al.*, 1994; Culp *et al.*, 1994; Spiegelhalter *et al.*, 1979; Williams *et al.*, 2000; Enomoto, 2000; Lena *et al.*, 2002; 3) Melnick *et al.*, 1990; Enomoto, 2000; 4) Huff *et al.*, 1994; Enomoto, 2000; 5) Matsushita, 1989; JSOH, 1991; Woodruff *et al.*, 1998; 6) Waddell, 1993; Enomoto, 2000; 7) Enomoto, 2000; Azzi Baumgartner *et al.*, 2005;

*1 U.E.L.: Usual exposure level of chemicals *2 T.D.L. (U): Tumor producing dose level (usual) in animals

*3 H.E.L.: Highest exposure level of chemicals *4 T.D.L. (L): Tumor producing dose level (lowest) in animals

Unfortunately, there is very little epidemiological evidence of causal agents in polluted air and their influence on respiratory disease in Japan. Rather, attention should focus on reducing automobile emissions and industrial contamination (Lippmann *et al.*, 2003). Automobile exhausts (Falk, 1970; Matsushita, 1985; Albert and Chen, 1986; HERP Program, 1987; Strandell *et al.*, 1994; Caprino and Tonga, 1998) contribute more than a thousand times the concentration of environmental carcinogens than does tobacco smoke (Sakabe *et al.*, 1965; Matsushita, 1989; IARC, 1990; Witschi *et al.*, 1997).

Secondly, the conventional two-year rodent bioassay should focus on genotoxic carcinogens predicted by Ames testing. The qualitative and quantitative analyses of the rodent bioassay, especially with more meaningful data on low-dose effects (Haseman, 2003) will give a clue to their human carcinogenic potential. Non-genotoxic carcinogens will be evaluated by short to subchronic studies in combination with molecular pathology (Boorman *et al.*, 2002; Preston, 2002). The wealth of the database generated from rodent bioassay in the past quarter of a century and the new genetically altered models for carcinogen screening will certainly contribute to more meaningful hazard identification (Maronpot, 1998; Porta *et al.*, 2003; Ohnishi *et al.*, 2007). Future challenges will involve toxicological approaches to integrate recent advances to elucidate the causation and ultimate prevention of human cancer, together with epidemiology studies as suggested by Carbone *et al.* (2004).

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