

Review

Contribution of toxicologic pathologists for the safety of human health in biomedical research—past, present, and future of the JSTP

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Abstract: The research field of “Toxicologic Pathology” evaluates potentially toxic chemical exposures and chemically mediated illnesses in humans and experimental animals. Comparative studies of chemical exposures between model organisms and humans are essential for the risk assessment of chemicals and human health. Here we review the development and activities of the Japanese Society of Toxicologic Pathology (JSTP) during its 37-year history. Toxicological pathology studies provide many interesting and valuable findings. Rodent cancer bioassay data demonstrate the importance of dose levels, times, and duration of exposures to chemicals that possibly cause human cancers. Studies of toxic injuries in the nasal cavity demonstrate that specific chemical compounds affect different target cells and tissues. These observations are relevant for current air pollution studies in the preventive medicine field. Future toxicological pathology studies will be enhanced by applying molecular pathology with advanced observation techniques. In addition to the nasal cavity, another sense organ such as the tongue should be a potential next program of our mission for risk assessment of inhaled and ingested chemicals. As a message to the younger members of the JSTP, interdisciplinary and global cooperation should be emphasized. Elucidating the mechanisms of toxicologic pathology with a combination of advanced expertise in genetics and molecular biology offers promise for future advances by JSTP members. (DOI: 10.1293/tox.2021-0028; J Toxicol Pathol 2021; 34: 275–282)

Key words: toxicologic pathology, experimental medicine, rodent cancer bioassay, air pollution, lung cancer

Toxicologic Pathology and Experimental Medicine

As you may know, Katsusaburo Yamagiwa and Koichi Ichikawa’s first publication “Experimental study on the pathogenesis of epithelial tumors” in 1915 was a milestone in the history of research on chemical carcinogenesis¹. After their discovery, many studies have been conducted to understand the mechanism of action of chemical carcinogens. The field of “Toxicologic Pathology” evaluates potentially toxic chemical exposures and chemically mediated illnesses in humans and animals. Comparative studies of chemical exposures between model organisms and humans are essential for the risk assessment of chemicals and human health.

In 1985, the American Society of Toxicologic Pathol-

ogy (STP) invited Japanese Society of Toxicological Pathology (JSTP) representatives to their Symposium entitled “Estimating Human Risk from Animal Tumor Data”. Makoto Enomoto described the current level of diagnostic expertise among toxicologic pathologists in Japan. He also presented a comparison of histological data from human stomach and liver cancers with the results from experimental animals with these cancers. By 1970 stomach and liver cancers were most prevalent in Japan. N-Methyl-N’-nitro-N-nitrosoguanidine (MNNG) and N-Ethyl-N’-nitro-N-nitrosoguanidine (ENNG) were used to induce stomach carcinomas in rats and dogs, respectively. Carbon tetrachloride and luteoskyrin are known to induce cirrhosis combined with hepatocellular carcinoma. Luteoskyrin is an anthraquinone derivative that was isolated from mycotoxin which causes the so-called “yellowed rice”. Histological specimens of stomach cancer induced by derivatives of nitrosoguanidine in rats or dogs were compared with those of human endoscopic biopsy specimens (Fig. 1A). Histopathological changes in rodents associated with progression from acute to chronic hepatic damage including cirrhosis and liver cancer induced by carbon tetrachloride and luteoskyrin were compared with biopsy or autopsy specimens from human liver cancer patients (Fig. 1B and 1C)².


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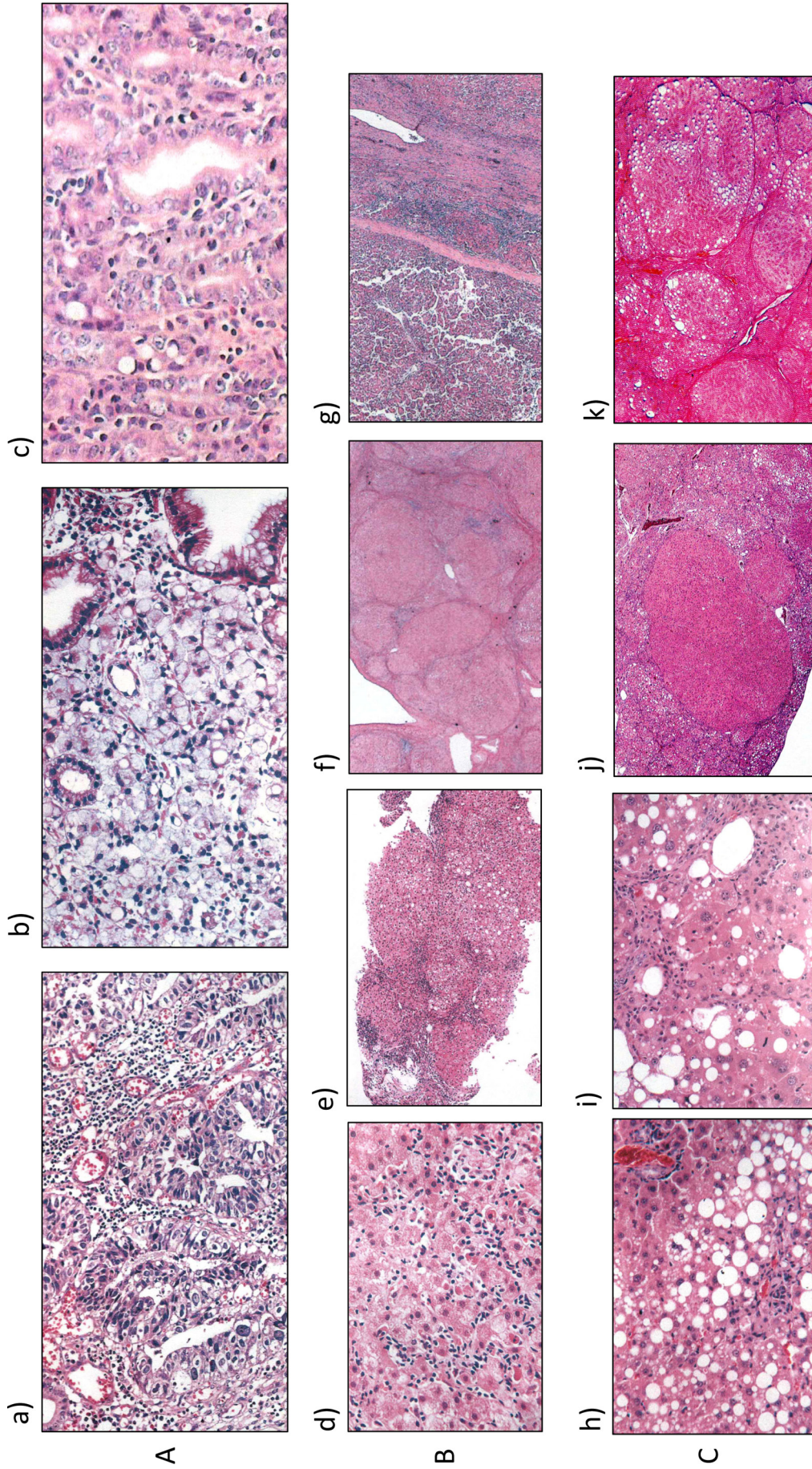


Fig. 1. Development of disease models. A) Stomach cancer in human, dog and rat. H.E. stain. a) biopsy specimen, stomach adenocarcinoma, man, 65 years old, b) gastric mucosal adenocarcinoma, beagle dog, treated with MNNG drinking water, c) early-stage mucosal adenocarcinoma, glandular stomach, rat, treated with MNNG drinking water. B) Human liver cancer, c-type virus hepatitis patient. H.E. stain. d) biopsy specimen, acute hepatitis, man 30 years old, e) biopsy specimen, chronic active hepatitis, 32 years old, f) necropsy liver tissue, liver cirrhosis, 52 years old, g) liver cancer with cirrhosis, 52 years old, C) Liver disease in F344 female rat from inhalation of carbon tetrachloride study. H.E. stain. h) acute liver damage, 4 weeks, i) subacute liver damage, 13 weeks, j) hepatic nodule (chronic damage), 52 weeks, k) liver cirrhosis with carcinoma, 78 weeks.

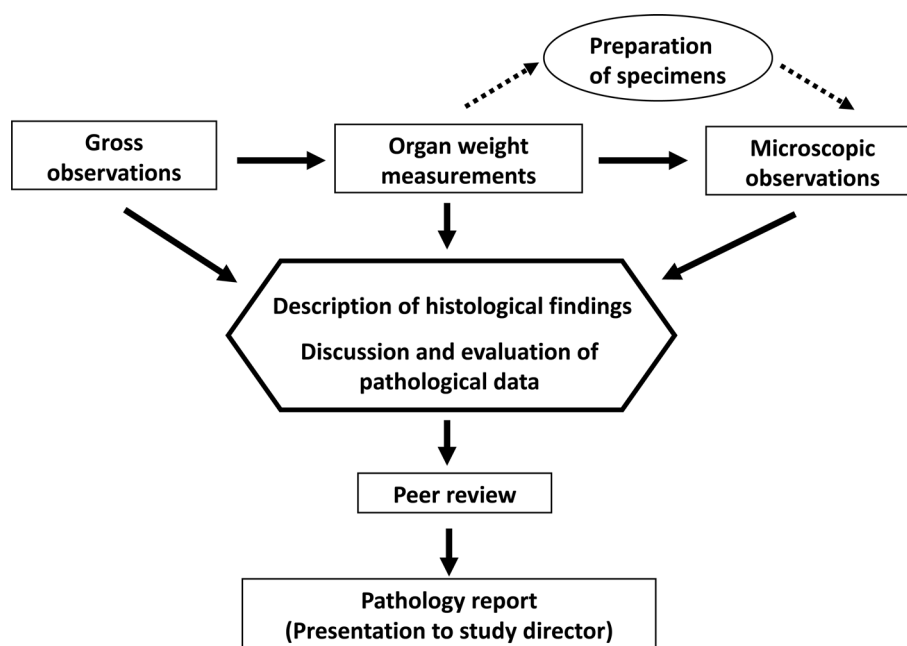


Fig. 2. Outline of the standard tasks of the toxicologic pathologist.

Progress and Contributions of JSTP during 37 years of History

The history of the JSTP was surveyed comprehensively a decade ago by the late Yoichi Konishi, one of our founding members³. This review described the development of JSTP independence from the Japanese Society of Toxicology, interactive communication with the Japanese Association for Laboratory Animal Science (JALAS), and cooperative activities with the STP as well as the European Society of Toxicologic Pathology (ESTP). He also stated that education of the toxicologic pathologists was the most important goal. The late Nobuyuki Ito and Yoichi Konishi organized and started the annual JSTP meeting in Nara, inviting internationally famous pathologists from around the world including the USA, Germany, Japan, and faculty members of the International Life Sciences Institute (ILSI). Tremendous efforts and a variety of the other smaller seminars organized by experimental pathologists in Japan contributed to the mentoring of young toxicologic pathologists who graduated from a variety of schools such as veterinary, human medical pathology, pharmacology, or basic biology. Thus, safety assessments of chemicals including medical drugs, pesticides, and food additives were completed by many institutes/laboratories in compliance with GLP requirements of Japan, Europe, and the USA over the last half-century. The basic roles of the toxicologic pathologist and key points for their routine safety assessments are shown in Fig. 2 and Table 1.

Table 1. Key Observations for the Safety Assessments

Comparison: untreated (control) vs. treated groups
Relationship: treated volumes of chemicals
Sex-related lesions
Evaluation: target organs, tissues, cells or organelles
Discussion: exposure-related changes
Complex findings
Evaluation: individual-specific lesions and naturally-occurring lesions
Age-related lesions
Relationship: gross and histological findings; cause of death
Whole body analysis

Development and Advancement of Toxicologic Pathology

A. Toxicology contributions

Among the interesting results obtained from the experimental assessment of the toxicologic effects of drugs and pesticides, three important examples are illustrated below. 1) Physiological and pharmacological effects of estrogen. A variety of effects of estrogen in addition to its function as a female sex hormone have been described (Fig. 3). It is notable that estrogen itself can be a carcinogen causing mammary carcinoma in humans, similar to the early induction of pituitary tumors observed in mice. Since estrogen is made in the body, the possible differences between its "in situ" and artificial exogenous effects need to be elucidated. 2) Disturbance of the hormone balances by psycho-neurology drugs. Psycho-neurological drugs resulted in disturbed hormonal imbalances in experimental animals as side effects; these included a variety of toxicological injuries and tumori-

genic endpoints (Table 2). 3) Side effects of the quinoline drugs. Figure 4 summarizes the toxicological side effects

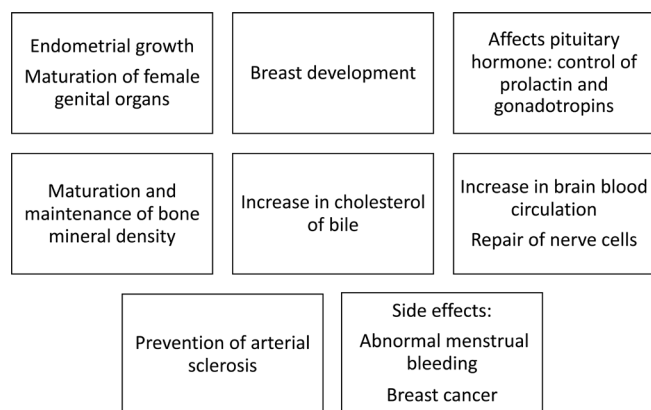


Fig. 3. Physiological and pharmacological effects of estrogen.

of quinoline compounds. The difference in the appearance of toxicological disorders among animal species is also notable. Pathological effects of these medical quinoline drugs could be the key factors for the discovery of the new generation of improved quinoline drugs.

B. Rodent cancer bioassays

As with Ito's medium-term assays for chemical carcinogenicity studies⁴, two-year cancer bioassay programs were carried out extensively in Japan, the USA, and Europe. In order to improve the design of rodent cancer bioassays, joint meetings were held in Tokyo and Hakone, Japan between 1975 and 1980. Members of the National Cancer Institute, USA, and experimental Japanese scientists participated in these meetings. They agreed on guidelines for: 1) well-controlled animal testing facilities, 2) proper animal handling and husbandry by appropriately trained technical personnel, and 3) the standardization of preclinical safety evaluation

Table 2. Disturbance of Hormone Balances by Psycho-neurology Drugs

Drugs	Pharmacological mechanisms	Hormonal changes	Toxicological effects
Anti-psychotic	Dopamine receptor inhibition	Prolactin↑ FSH and LH↓ ACTH↑ Estrogen↓	Mammary gland hyperplasia, Uterine atrophy Ovarian atrophy (Menstrual disturbance) Harder's gland: porphyrin deposit, adenoma Cleft palate Osteoporosis, Arteriosclerosis
Anti-depression	Neurotransmission stimulation	Prolactin↑ Estrogen↓	Mammary gland hyperplasia Osteoporosis Arteriosclerosis
Tranquilizer	GABA receptor stimulation	TSH↑ LH↓	Thyroid disorder Spermatogenesis dysfunction, Uterine atrophy
Anti-Parkinson's	Dopamine receptor agonist	Prolactin↓	Uterine cancer
Sympathomimetics		GH↓	

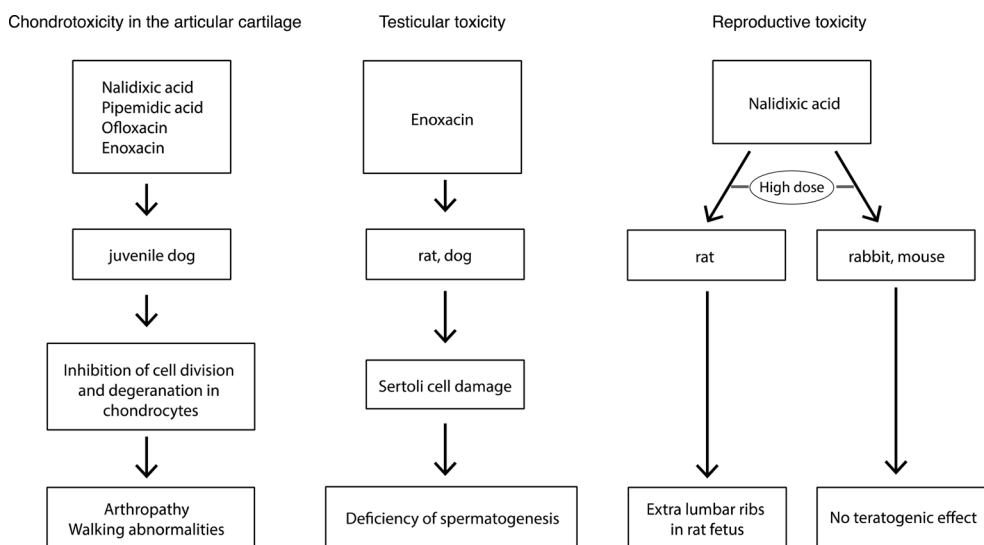


Fig. 4. Toxicological side effects of the quinoline drugs.

testing guidelines. Finally, the Good Laboratory Practice (GLP) regulations became law in 1979. As a result, data obtained from studies conducted since 1980 show higher quality and more reproducibility between different testing facilities, providing better accuracy in predicting and evaluating the potential toxicity and carcinogenicity of chemicals for humans (Table 3). Also, the incorporation of mechanistic and toxicokinetic data of chemicals obtained from rodent bioassays greatly deepened the scientific insight on tumor development⁵⁻⁷. Table 4 showed that the guidance for obtaining the reliability of toxicity and carcinogenicity studies. Groups of 50 males and females of selected strains of rats (F344/SD/Wistar) or mice (B6C3F₁/ICR) are assigned to each of the control or treatment groups. Following the 13-week repeated dose toxicity studies, top doses are chosen to expose animals to a minimally toxic change. And lower doses are selected within the linear range of kinetics. Animals are given three or four dose levels of test substances by gavage, feeding or drinking water consumption, dermal painting, or inhalation exposure. Dosing to animals starts at age 5–6 weeks and lasts for 2 years. Then surviving animals

receive a complete histopathologic examination. All studies include clinical biochemical measurements, as well as assessments of gross behavioral changes, body and organ weights, food and water consumption. Because of the assistance of the animal care specialists trained by the JALAS and technical specialists for clinical examination, the data of carcinogenicity studies are reliable. Further, the historical control data of gross macroscopic lesions and histological lesions are useful for evaluating the toxicological and/or carcinogenic potential of chemical substances. These data also may contribute to the elucidation of age-associated spontaneous morphologic changes in a commonly used strain of rat and mouse in the long term animal studies⁸⁻¹⁰ (Table 5).

The most significant fact in a large-scale bioassay is recognizing the importance of dose levels, times, and duration of exposure in the safety evaluation of carcinogenic as well as classical toxic agents. A great deal of attention will have to be paid to the concept of threshold doses and exposure levels that may be required to achieve preneoplastic conditions or the induction of carcinogenic processes. Thus, bioassay studies successfully identify tumor-causing agents in rodents by providing information on dose-responses and characterizing other chemical-related toxicities. A no-effect level can exist for tumor development and the exposure-response can be supralinear in range. We suggest that linear extrapolation from high toxic exposures to postulated low exposure effects of DNA-reactive carcinogens can yield overestimates. The finding of no-effect levels provides a basis for understanding why very low-level environmental exposures to humans of even DNA-reactive carcinogens may convey insignificant cancer risks^{7, 11-13}.

Table 3. Human Carcinogens (WHO and IARC)

Chemicals	Target organs	Induced species
4-Aminobiphenyl	Bladder	Mouse, Rat, dog
Phenacetin	Renal pelvis	Rat
Asbestos	Lung, Pleural cavity	Mouse, Rat, Hamster
Arsenic, arsenic compounds	Lung	Mouse, Hamster
Azathioprine	Lymph node	Mouse
Benzene	Hematopoietic system	Mouse, Rat
Benzidine	Bladder	Dog
Bis (chloromethyl) ether	Lung	Mouse, Rat
Chlorambucil	Hematopoietic system	Mouse, Rat
Hexavalent chromium	Lung	Rat
Cyclophosphamide	Bladder	Rat
Melphalan	Hematopoietic system	Mouse
Diethylstilbestrol	Vagina, Uterus	Mouse, Hamster
Mustard gas	Lung	Mouse
Busulfan, Myleran	Hematopoietic system	Mouse
2-Naphthylamine	Bladder	Dog, Hamster, Monkey
Soot carbon, Coal, Coal tar	Skin, Trachea	Mouse
Polyvinyl chloride	Liver (Blood vessel)	Mouse, Rat, Hamster

Table 4. Guidance for Long-term Carcinogenicity Studies

1. Groups of 50 male and female rodents for better statistical power
2. High quality and reproducible testing facilities
3. Perform dose finding study
4. Select high doses with minimally toxic effects
5. Specific strains of rats (F344/SD/Wistar) or mice (B6C3F₁/ICR)
6. Test substances given at three or four dose levels
7. Improve clinical biochemical measurements: a rich source of pathology information
8. Supplementation with medium-term test
9. Consideration of naturally occurring lesions in historical control animals

Table 5. Comparison of the Species for Carcinogenicity Studies

Considerations	Rat	Mouse
Two-year survival	Slightly reduced	Superior: B6C3F ₁
Handling	Slightly superior	More challenging (small size)
Naturally occurred Lesions	Common: Endocrine, Kidney, Joint lesions	Fewer spontaneous lesions (except for the proliferative lesions in liver)
Target organs for tumor induction	Liver, Urinary system, Forestomach, Thyroid, Hematopoietic system	Liver, Lung, Forestomach, skin, Hematopoietic system

C. Air pollution and inhalation study

Inhalation studies over the past half century by toxicologic pathologists have provided ample evidence for human health risks associated with air pollution particulate matter (PM). Both Ulrich Mohr and the late Donald L. Dungworth are owed a great deal for their contributions in the field of inhalation toxicology. Their efforts led to large respiratory pathology research programs throughout the world. In Japan, extensive studies on toxic and carcinogenic effects of nano-level size particulates¹⁴⁻¹⁸ and nanotubes^{19, 20}, along with earlier rodent bioassays that exposed the whole body of animals to a variety of industrial chemicals, have demonstrated the effects of airborne chemical hazards. Air pollution containing carcinogenic PM derived from automobile exhaust, paved roads containing toxic pitch, coal tar, and asbestos are sources of injury to the respiratory organs including the nasal cavity. Toxic injuries of the nasal cavity

caused by chemical compounds were studied extensively by toxicologic pathologists, demonstrating differences of target cells and tissue sites depending on chemicals (Fig. 5)²¹. It should be also noted that there are significant increases of macrophages in rodents following inhalation of pharmaceutical materials²². The importance of exposure levels to toxic and carcinogenic substances present as PM air pollution needs to be emphasized with respect to human health risks. Pathologists have reported that chronic exposure to high levels of ambient PM (daily average of PM_{2.5}; 22 µg/m³) is associated with small airway remodeling of human lungs^{23, 24}. The public health burden associated with the air pollution-related carcinogens has been forecast based on exposure levels at which there is a measurable cancer risk. Table 6 shows that carcinogenic particulates were present in the air of major cities and industrial sites at greater than µg/m³ levels. Furthermore, Fig. 6 demonstrates the differ-

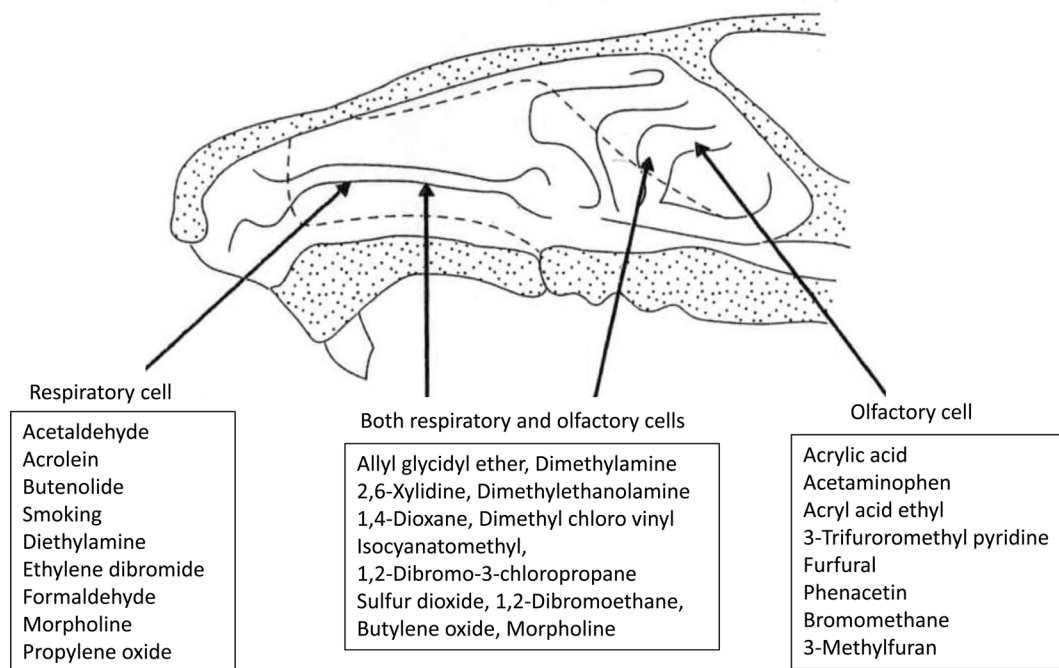


Fig. 5. Effects of chemicals in the nasal cavity – difference between sites and target cells.

Table 6. Air Pollution: Measurement Data of Particulate Matter - PM_{2.5} and Benzo(a)pyrene

Contents	Air Pollution
Particulate matter (PM _{2.5})	Average ambient concentration 4.9–19.2 µg/m ³ , USA Airborne PM concentration 19.0–29.9 µg/m ³ , NY, USA Airplanes, taverns, workrooms 120–960 µg/m ³ Particulate emissions: Diesel vehicle 230 mg/km Gasoline vehicle 21 mg/km
Benzo(a)pyrene	Coal stove 1.7–3.3 g/BTU: 0.252 kcal Tar suck on engine mufflers 139 mg/kg Traffic tunnels 9.6 ± 2.6 µg/m ³ Smoker's room 2.6–7.4 ng/m ³ Non-smoker's room 1.1–4.4 ng/m ³ Mainstream smoke 20–40 ng/cigarette

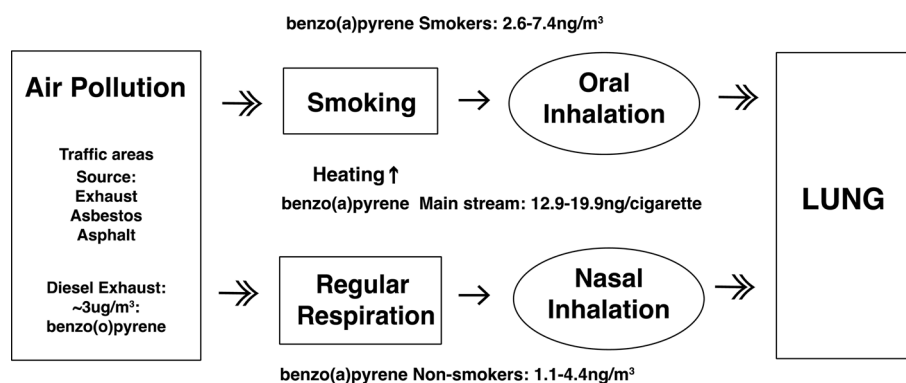


Fig. 6. Air pollution and oral (tobacco smoking) versus nasal inhalation.

ent effects between oral and nasal breathing of the inhaled polluted air.

Future Development of the JSTP and Message

The practice of identifying cancer hazards through rodent cancer bioassays is being replaced or supplemented by evaluating molecular biomarkers to characterize hazards^{25–27}. Future experimental animal studies are expected to contribute to the safety assessment of chemical agents by applying advanced immunohistological assays for early detection and diagnoses of cancer cells²⁸. Since a study on toxicologic pathology of the nasal cavity emphasized the important role of nasal breath for filtering airborne pollutants, further investigation on sense organs such as the nose for smell and the tongue for taste should be carried out for safety assessments of both humans and animals.

Interdisciplinary research cooperation with areas of genetics, embryology, pharmacological toxicology, and clinical pathology should be necessary to improve and deepen the research success of our mission²⁹. Global corporation should also be a key factor in the further development of our society, as the 37th JSTP annual meeting president Hijiri Iwata stressed at the conference. Many of the biggest mistakes in history have been based on consensus thinking. Consensus should never be regarded as a substitute for scientifically based facts³⁰.

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