Annu. Rev. Pharmacol. Toxicol. 1993. 32:383–407

RESPIRATORY TRACT UPTAKE OF INHALANTS AND METABOLISM OF XENOBIOTICS[1]

A. R. Dahl
Inhalation Toxicology Research Institute, Albuquerque, New Mexico 87185

J. L. Lewis
Benchmark Environmental Corporation, and Inhalation Toxicology Research Institute, Albuquerque, New Mexico 87185

KEY WORDS: cytochrome P450, olfactory, lung

INTRODUCTION

Recent progress in understanding and predicting toxicity in the respiratory tract has been made largely through advances in two areas. New concepts regarding the uptake and clearance of inhalants have led to rethinking of long-held principles. At the same time, advances in understanding the role of respiratory tract metabolism in determining the fate of xenobiotics is improving the clarity with which we explain the causes of respiratory tract toxicity. The purpose of this review is to provide updated commentary on recent developments in the study of the dosimetry of inhaled materials and of the metabolism of xenobiotics in the respiratory tract. Our purpose is to highlight recent important developments in the area rather than to exhaustively review the literature.

Several reviews cover aspects of metabolism in the respiratory tract: xenobiotic metabolism in the entire respiratory tract (1, 2); detailed reviews of xenobiotic metabolism in the nasal cavity (3) and lung (4–6); the relationship of pulmonary metabolism to the toxicity of compounds (7, 8) and future research needs in terms of toxicity in the nasal passages (9). The factors

[1]The US Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

affecting the concept of dose for inhalants have been examined (10); and interspecies comparative dosimetry for inhaled materials was the subject of a symposium proceedings (11). The interested reader is referred to these articles.

UPTAKE AND CLEARANCE

Progress in Gas and Vapor Uptake Modeling

Traditionally, gas and vapor uptake has been described by lung ventilation/perfusion models (e.g. 12). An assumption of such models is that uptake occurs in the alveolar region of the lung. These models predict that the uptake of highly water-soluble vapors (more accurately, those with high water/air partition coefficients) is ventilation-limited (i.e. uptake depends only on respiratory tidal volume and breathing rate), whereas the uptake of poorly water-soluble vapors is perfusion limited (i.e. uptake depends only on blood flow through the alveoli). Many workers have recognized, however, that experimentally determined uptake often varies from that predicted by ventilation/perfusion models (e.g. 13). Because of the inadequacies of simple ventilation/perfusion models, new interest in the factors affecting the uptake of inhalants has been kindled. Sophisticated modeling methods have been used to calculate and to validate the effects of airflow patterns on vapor uptake and site of toxicity (14, 15). The role of metabolism of vapors on uptake in the nasal cavity has been examined (16); an experimentally validated mathematical model describing the nasal uptake of vapors and gases has been developed (17); and a similar model, albeit assuming that uptake into blood occurs only in the gas exchange region, has been developed for vapor uptake in the lung (13). The groups involved in this research continue to produce both data and explanations that expand our understanding of how inhaled gases and vapors interact with the respiratory tract. Among recent advances are the findings that air flow patterns correlate with sites of toxicity for inhaled, highly reactive vapors (14); that metabolism in the nasal cavity contributes substantially to the uptake of organic esters (16); and that substantial desorption of vapor molecules from the nasal and lung airways occurs during exhalation (13, 17).

New Concepts in the Clearance of Lipophilic Compounds

Based on earlier work by Gerde & Scholander (18), Gerde et al (19) published a theoretical model describing the uptake of highly lipophilic compounds (those compounds with oil/water partition coefficients exceeding ~100,000). As a consequence of strong affinities for the lipoidal parts of the mucosal tissue, diffusion of highly lipophilic compounds through the mucosa into the blood stream is slowed; therefore, much longer half-times for clearance are

expected for highly lipophilic compounds compared to less lipophilic ones. In turn, the postulated long residency for highly lipophilic compounds, such as benzo(a)pyrene (BaP), suggests that metabolism will contribute substantially to clearance from the respiratory tract. The theory further predicts that less lipophilic compounds will clear rapidly without substantial metabolism and that even highly lipophilic compounds will pass relatively quickly through the thin air/blood barrier of the gas exchange region.

RESPIRATORY TRACT METABOLISM

Nasal

CYTOCHROMES P450, ASSOCIATED ACTIVITIES, AND POTENTIAL TOXIC EFFECTS

The uniqueness of the nasal tissue cytochrome P450s was recognized initially from studies of its metabolic activities, and examples of unusual activities continue to be reported. For example, rat nasal tissues have very high capacities for the production of hydrogen cyanide from organonitriles (20). Although volatile organonitriles are commonly inhaled and, because of their high water solubility, may undergo extensive uptake and metabolism in the nasal cavity, the toxic effects of the released cyanide may be ameliorated by the presence in the nasal tissues of the enzyme rhodanese, which catalyzes the formation of thiocyanate from cyanide (21).

Methylenedioxyphenyl (MDP) compounds are widely dispersed in nature and are also used in the production of pesticides—especially in household sprays, where they potentiate the pyrethroid toxicants. MDP compounds are metabolized by cytochrome P450-dependent monooxygenases, putatively to carbenes that bind to and inactivate P450 at the heme iron. MDP compounds are much more potent inhibitors of rabbit nasal demethylases than of liver demethylases (22), and inhalation of these compounds may have important effects on nasal metabolic activities.

MDP compounds are not the only inhibitors of P450-mediated nasal metabolism encountered in nature. Hong et al (23) showed that a single oral dose of diallyl sulfide, a component of garlic, substantially decreases the capacity of nasal mucosa to metabolize several nitrosamines.

The unique characteristics of nasal P450s may also make nasal tissue particularly vulnerable to the effects of procarcinogenic nitrosamines because nasal tissue homogenates activate certain nitrosamines to mutagenic products, even when liver and lung tissue homogenates do not (24).

Major progress has been made in the identification and localization of cytochromes P450 in the nasal cavities of test animals and in the determination of cytochrome P450-mediated activities in the human nose. The cDNA-derived amino acid sequence has been determined for a nasal olfactory

tissue-specific isozyme, rabbit P450 NMb (2G1) (see Ref. 25 for P450 nomenclature) (26). 2G1 metabolizes testosterone while a second isozyme found at high levels in rabbit olfactory tissue, NMa (a member of P450 family 2), is apparently largely responsible for metabolism of the potent rat nasal procarcinogen, hexamethylphosphoramide (HMPA), to the carcinogen formaldehyde (27). Cytochrome P450 isozymes 2G1 and a 2A subfamily member were localized in the rat olfactory mucosa using immunohistochemical techniques. These enzymes are highly concentrated in the Bowman's glands (28). A P450 isozyme belonging to the 4A subfamily is possibly responsible for the metabolism of arachidonic acid to 20-hydroxyeicosatetraenoic acid in rabbit olfactory tissue (28a).

P450 isozymes 1A1 (typical substrate, BaP), 2B1 (typical substrates pentoxyresorufin, BaP, aminopyrine), and 4B1 (typical substrate, 2-aminofluorene) have all been reported to occur in nasal tissue (see Ref. 3). Adams et al (29) closely examined the distributions of these isozymes in rat and Syrian hamster nasal tissue. Few differences are found in the distribution of cytochrome P450 isozymes 2B1, 4B1, and 1A1 between the hamster and rat nasal cavities. The nasal transitional epithelia (see Ref. 3 for summary of nasal anatomy) in both species have high concentrations of form 2B1 and lower concentrations of forms 4B1 and 1A1. The relative concentrations of these enzymes is similar in the respiratory epithelium, olfactory sustentacular cells, acinar cells of the Bowman's glands, and in the septal organ. The vomeronasal gland in both species has low P450 levels. NADPH-cytochrome P450 reductase levels tend to parallel those of isozyme 2B1 (29).

Differences between activities in monkey nasal tissue from those in human nasal tissue may indicate that the monkey is not necessarily the best model for humans with regard to nasal metabolism (30). P450-dependent metabolic activities reported in human nasal respiratory tissue include dealkylases for HMPA, aminopyrine, ethoxycoumarin, ethoxyresorufin, diethylnitrosamine, and dimethylnitrosamine (3, 31). Among other differences, the nasal respiratory mucosa of cynomolgus monkeys has ethoxycoumarin dealkylase activity 100-fold higher than that in humans (30). Other P450 activities reported in the monkey nasal tissue include aminopyrine, ethoxyresorufin and erythromycin dealkylases, and aniline hydroxylase.

The nasal cavity is increasingly reported as a site for metabolite-induced lesions. Orally administered phenacetin causes nasal tumors—as well as kidney and urinary bladder tumors—in rats. Both squamous cell carcinomas and adenocarcinomas occur in the nasal cavities of rats administered phenacetin chronically. Degenerative changes in the olfactory epithelium and necrosis in Bowman's glands occur following acute phenacetin administration (32). Acetaminophen, like structurally similar phenacetin, is toxic to nasal cells as well as to bronchiolar Clara cells after oral administration (33).

Within the nasal cavity, the olfactory mucosa is particularly vulnerable to metabolite-induced toxic effects (3). For example, iminodipropionitrile (IDPN) metabolites cause olfactory toxicity in the rat after administration by noninhalation routes (34), and olfactory tissue lesions in rats and mice result from inhaled ferrocene vapor (35, 36). The toxicity of both compounds is probably related to the high metabolic capacity of the olfactory tissue. When administered by inhalation, the chemical intermediate, 3-trifluoromethylpyridine (3-TMP), is a potent olfactory tissue toxicant in rats, but much less so in monkeys. The role of P450-mediated metabolism in the toxicity of 3-TMP is shown by the inhibition of binding of the compound after treatment with metyrapone, a P450 inhibitor (37).

CARBOXYLESTERASES Volatile carboxylic esters are common in the natural environment and in the occupational setting, and their hydrolysis catalyzed by esterases may play a role in determining their biological fate and potential toxicity. As mentioned previously, Morris (16) showed that nasal hydrolysis of ethyl acetate contributes to the uptake of ethyl acetate in rats. Nasal esterase-mediated hydrolysis also activates some esters to toxic metabolites. For example, a mixture of dibasic esters used as industrial solvents is hydrolyzed by rat nasal tissue to produce toxicants (38, 39).

Human nasal respiratory mucosa, like that of other species, contains high levels of esterases as measured using naphthyl butyrate as the substrate (40). The enzyme responsible is localized to the apical portions of the epithelial cells and to some glands in the lamina propria (41). The pathological state of the nasal mucosa may be an important modifier of esterase activity, and hence, of toxicity of inhaled esters. Thus, human nasal tissues with evidence of hyperplasia or squamous metaplasia have greatly decreased immunohistochemical staining for carboxylesterase (41).

OTHER ENZYMES AND COFACTORS Decreased tissue levels of the antioxidant, glutathione (GSH), may increase the susceptibility of the affected tissues to the toxic effects of chemicals normally detoxicated by reaction with GSH. Among heart, kidney, liver, lung, nasal mucosa, and testes, the nasal tissue shows the sharpest drop in GSH content during inhalation exposure to low levels of 1,3-dichloropropene (DCP)(42). After the GSH is depleted, further exposure to DCP or other xenobiotics such as aflatoxin (42a) or the herbicide dichlobenil (42b)—administered by inhalation or by other routes—may result in unexpected nasal toxicity.

Other factors, such as age, may affect tissue GSH levels. For example, GSH levels decrease with age in several brain regions, including the olfactory bulb. However, GSH levels in the rat olfactory epithelium apparently do not vary with age (43).

The cyanide-metabolizing enzyme, rhodanese, shows very high levels of activity in the nasal tissues of rats (21). High rhodanese activity has been reported in human nasal respiratory tissue as well, and the activity may be modulated by smoking (44). The subcellular and glandular distribution of rhodanese differs in bovine and rat nasal tissues (45), emphasizing the importance of characterizing species-specific enzyme activity and localization before making interspecies extrapolations with respect to toxicity.

Nasopharyngeal and Tracheal

Although the nasopharynx and trachea are at times the target tissues for protoxicants, only a few recent studies have examined metabolic activities in these tissues. Parenteral 3-methylcholanthrene (3-MC) administration induces P4501A1 in the rat nasopharynx as well as in the lung, whereas P4501A2 is not induced in the respiratory tract at doses effective in the liver. The ratio of aryl hydrocarbon hydroxylase (AHH) activity to 1A1 for rat lung is tenfold that for the nasopharynx, in which there is little AHH activity even after induction (46).

Regenerating hamster tracheal epithelium contains cytochrome P450 form 2 and NADPH-cytochrome P450 reductase. The secretory cells of the trachea, in particular the apical portions, have the highest concentrations of these enzymes (47). Adult hamsters may be particularly sensitive to toxic effects in the trachea. Thus, after parenteral administration of naphthalene, the tracheae and lobar bronchi of hamsters, but not rats, show cytotoxic changes (47a).

Bronchiolar and Alveolar and Whole Lung

CYTOCHROMES P450 AND ASSOCIATED ACTIVITIES AND TOXICITIES The lungs from a number of species, including humans, have striking similarities in the types of P450 isozymes constitutively present or inducible, but there are also some possibly important differences (Table 1). A notable interspecies difference is found in the levels of constitutive enzymes present, as indicated in the following comments on lung P450s in six species.

Mouse Murine lung, like that of other species, contains very low constitutive levels of P4501A isozymes; they are, however, readily inducible. Murine pulmonary P4501A1 is nearly maximally induced 48 hr after parenteral treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and remains at that level for 4 wk (63). A monoclonal antibody to a 3-MC-induced P450 indicates the induced isozyme is present in the alveolar walls and in the blood vessel endothelium of parenterally 3-MC-administered mice but not in that of control mice (64). Similar studies in the lungs of "responsive" and "non-responsive" mice localized 3-MC and phenobarbital-inducible cytochromes

P450 using antibodies against the appropriate isozymes. Forms 1A1 and1A2 are present in 3-MC-induced lungs, but are not constitutive. Forms 2B1 and 2B2 are constitutively present, but are not inducible. 1A1 is induced in Type II cells of the septa, but not in endothelial cells; whereas 1A2 is induced in both cell types. P4502B1 or 2B2 or both are present in Type II alveolar cells and bronchiolar epithelial cells, including Clara cells (48). Clara cells isolated from mouse bronchiolar epithelium lack cytochrome P4501A1, but do contain cytochrome P450 "mN", which can metabolize the pneumotoxicant, naphthalene. In addition, three other major components of the P450 monooxygenase system present in isolated murine Clara cells are P450 isozymes 2B1/2 and 4B1, and NADPH cytochrome P450 reductase (49).

A number of murine pneumotoxicants require metabolic activation. Naphthalene has been well studied with regard to its capacity to necrose Clara cells in the mouse lung (65). A P450 isozyme termed P450m50b may be the major one involved in the pulmonary metabolism of naphthalene (50). In addition to naphthalene, both acetaminophen and phenacetin require metabolic activation—possibly by P4502E1 in the case of acetaminophen—to exert toxic effects on mouse bronchiolar Clara cells (33).

Rat In untreated rat lung, P4502B1 is the major isozyme, but isozyme 1A1 has also been detected (66). P4501A1 is induced in the rat lung by 3-MC administration, whereas, unlike the case for the mouse, P4501A2 is not. The ratio of AHH activity to 1A1 is larger in rat liver than in lung, indicating that isozymes besides 1A1 may be important in providing lung AHH activity (46). Quite possibly, 2B1 contributes substantially to this activity.

Baron & Voigt (51) and Voigt et al (67) used immunohistochemical and histochemical techniques to study the localization, distribution, and induction of xenobiotic metabolizing enzymes and AHH activities in rat lung. P450 isozymes 1A1 and steroid-inducible 3A2 (responsible for steroid 6β-hydroxylation, among other activities) are about equally distributed among bronchial epithelial cells, Clara cells, and Type II pneumocytes. Isozyme 2B1 and NADPH P450 reductase are at significantly higher concentrations in the Clara cells than in the other cell types examined. BaP hydroxylase is uniformly distributed among the three cell types from untreated rats and is uniformly increased among the cell types after treatment with 3-MC or Arochlor 1254. All three cell types also show dramatically increased levels of isozyme 1A1 in rats pretreated with Arochlor 1254. 3-MC pretreatment dramatically increases 1A1 levels in Clara cells and Type II pneumocytes, but not in bronchial epithelial cells (51). These findings corroborate those reported by Keith et al (66) who reported P4502B1 to be localized in rat Type II cells and Clara cells. These workers also reported that P4501A1 is highly inducible by 3-MC administration in the rat lung, especially in endothelial cells, Clara

Table 1 Some P450 isozymes reported in lungs of mice, rats, rabbits, Syrian hamsters and humans*

	Isozyme	Comments	References
Mouse	1A1	Induced in type II cells	48
	1A2	Induced in type II cells and endothelial cells	48
	2B1	Constitutive in type II cells and Clara cells	48
	2B2	Constitutive in type II cells and Clara cells	48
	4B1	Rabbit form activates ipomeanol and 2-aminofluorene	49
	"mN"	In Clara cells, metabolizes naphthalene	49
	"m50b"	In Clara cells, major naphthalene metabolizing enzyme	50
Rat	1A1	Induced in bronchial epithelium, Clara cells and type II cells	51
	2A3	Absent in rat liver	68a
	2B1	Constitutive at highest levels in Clara cells	51
	2E1	Induced by hyperoxygen	52
	3A1/2	Induced in bronchial epithelium, Clara cells and type II cells	51
	4B1	Absent in rat liver	62
	"FI"	Constitutive. Induced by O_3	53
	"FII"	Cross reacts with rat anti 2B1. Constitutive. Induced by O_3	53
Rabbit	1A1	Highly inducible. Occurs in endothelial cells without reductase	54
	2B1	With 4B1, accounts for 80% of uninduced P450 in lung	55
	2E1, 2E2	2E1 5% that of liver; 2E2 2.5% that of liver	55a
	4A4	P450 prostaglandin ω-hydroxylase. Occurs only in pregnant rabbits or after induction by progesterone	56
	4B1	Activates 2-aminofluorene and ipomeanol	57
Hamster	"MC"	Possibly 1A1. Highly inducible	58
	2B	Along with reductase, absent from mesothelium in adults	59
	4B	Present in mesothelium in adult	59
Human	1A1	Inducibility related to smoking and lung cancer	60
	2F1	Ethoxycoumarin, pentoxyresorufin and 3-methylindole are substrates	61, 61a
	4B1	Unlike rabbit form, does not activate 2-aminofluorene	62

*NOTE ADDED IN PROOF
Kato et al report racial differences in human lung 2E1 polymorphisms (138).

cells, and Type II cells. P4501A2, however, is not found in rat lung microsomes (66).

MDP compounds, normally thought of as P450 inhibitors, can also induce P450s, and they differentially induce P4501A1 in rat lung Clara cells. P4502B1 decreases as a result of treatment, but the level of mRNA encoding 2B1 is not decreased (68). The authors conclude that these compounds regulate P450s by both transcriptional and post transcriptional mechanisms. A 3-methylcholanthrene inducible P450 unique to the rat lung, 2A3, shares 85% amino acid similarity with human 2A3 and 94% similarity to mouse testosterone 15α-hydroxylase (68a). In contrast to the rat 2A3, the human and mouse forms do appear in liver.

Ethoxyresorufin deethylase activity, but not *p*-nitrophenol hydroxylase, is reported to increase in rat lung microsomes after oral ethanol administration (69). Because *p*-nitrophenol is an excellent substrate for ethanol-inducible P4502E1, whereas ethoxyresorufin probably is not, these results offer intriguing possibilities for unusual mechanisms for induction in the lung. For some assays, however, rat alveolar Type II cell P450 activities exhibit complex kinetics, especially at low enzyme concentrations (70). Thus, the findings regarding ethanol induction may have been influenced by the enzyme concentration range examined.

Ethanol-inducible cytochrome P4502E1 is induced fourfold in rat lung and liver by oxygen (95% O_2 for 60 hr), indicating that this isozyme may moderate oxygen-mediated toxicity (52). Possibly related results indicate that inducers of cytochrome P450 protect against oxygen toxicity. β-Naphthaflavone or 3-MC induction provides better protection than does phenobarbital induction (71).

Ozone induction of P450 in rat lung is ascribed to induction of constitutive forms and not of new forms. Two constitutive P450s (FI and FII) are induced by 2 wk of ozone exposure. FII cross-reacts with antibody to rat liver form 2B1 (53). A role for P450 induction in ozone toxicity, however, has not been established, and induction may simply result from changes in relative ratios of cell types.

Rabbit Reminiscent of the rat, P450s in the rabbit are localized primarily in Clara cells and Type 11 cells, in addition to alveolar macrophages. The total rabbit lung P450 concentration is considerably greater than that of the rat. In untreated rabbit lungs, cytochrome P450 forms 2B1 and 4B1 account for 80% of the total pulmonary P450. Form 1A1 contributes only a few percent of the total P450 in untreated rabbit lung but is induced 26-fold by 3-MC or TCDD administration. Form 1A2 has not been reported in either untreated or 3-MC-induced rabbit lungs (55). Both P4501A1 and its encoding mRNA are strongly induced by TCDD in lung Clara cells and endothelial cells; neither

P450 reductase, a key component of the mixed function oxidase system, nor its encoding mRNA is detected in endothelial cells. The explanation for this finding is the subject of continuing research (54).

A P450 isozyme apparently unique to pregnant rabbit lung or lungs from progesterone-induced rabbits (56) is described in the section of this review subtitled, *Influence of Hormones and Inflammation on Respiratory Tract Metabolism*.

Syrian hamster Suggestive of virtually all species examined to date, AHH, an activity linked to the activation of some important procarcinogens, is highly inducible in the hamster lung. P450 MC has been isolated from 3-MC-induced Syrian hamster lung. The lung form appears to differ from liver forms. It is related to rat 1A1 and has a high (11.4 mol/min/mol P450) catalytic activity for BaP metabolism (58).

P4502E1 is another isozyme that, like 1A1, is linked to the activation of some protoxicants, including nitrosodimethylamine. Orally administered acetone, an inducer of ethanol-inducible cytochrome P4502E1, induces aniline hydroxylase, a 2El activity, in the lungs of hamsters, but does not affect nitrosodimethylamine demethylase or the overall levels of cytochromes P450 or b_5. Acetone administration markediy decreases hamster lung microsomal BaP hydroxylase, a P4501A1 activity, and 7-ethoxycoumarin O-deethylase activities (72). These findings contrast with those reported in the rat (69) in which ethoxyresorufin metabolism, a P4501A1 activity, increases after ethanol administration, whereas *p*-nitrophenol hydroxylase, a 2E1 activity, decreases.

Other isozymes studied in hamster lung include forms found in the lungs of most other species. P450 isozymes of the 2B and 4B subfamilies and cytochrome P450 reductase follow different developmental pathways in the lungs of hamsters (59). P4502B and reductase first appear in the trachea and bronchi on gestational day 14 and in all other conducting airways and in the lung vasculature on day 15. Isozyme 4B follows 2B and reductase by about 24 hr. Type II cells and the mesothelium contain all three enzymes on neonatal day 1. At 3.5 wk, the mesothelium no longer contains either reductase or 2B. Other sites retain all three enzymes in the adult.

Human Wheeler & Guenthner (73) have reviewed cytochrome P450-dependent metabolism in human lung. These authors indicate that total human lung P450 is 2 pmoles/mg of microsomal protein, only 5% of that in rat lung and less than 1% of that found in untreated rabbit lung. Shimada et al (74) solubilized and purified microsomal P450 isozymes from nine human lung specimens. They reported ~10 pmole of P450/mg microsomal protein. The reason for the fivefold higher P450 concentration compared to that reported

by Wheeler & Guenthner is not clear. Two potentially new P450 isozymes were found. In addition, isozyme 1A1 capable of metabolizing BaP was present but isozyme 1A2 was absent in all samples.

Human lung AHH activity is only a few percent that of human liver and about 20% that of uninduced rat lung. Human lung homogenates are reported not to activate mutagens requiring AHH activity, although bronchial explants have been reported to have AHH activity. Although isozyme P4501A1, often associated with AHH activities, has been identified in human lung by immunochemical methods, AHH in human lung may be catalyzed to a significant degree not only by 1A1 (or 1A2) isozymes but also by other P450 isozymes (73), a conclusion similar to that drawn for rat lung by Wilson et al (46).

Using a technique involving discontinuous sucrose gradient centrifugation to remove particulate contaminants interfering with the measurement of the low levels of lung P450, Wheeler & Guenthner (75) compared the cytochrome P450 content of human lung to that of baboons and found them to be nearly equivalent to each other but <1% that in rabbits. The human lung P450 measurements correlated well with metabolic activities in the microsomal fraction (75).

cDNAs for human lung 4B1 (62) and 2F1 (61), the latter possibly unique to the human lung, have been isolated and expressed in human cell lines. Expressed human 4B1 lacks 2-aminofluorene and lauric acid hydroxylase activity. Aminofluorene is a procarcinogen and a good substrate for rabbit 4B1. Thus, this finding may affect the interpretation of carcinogenicity data obtained using test animals. Expressed 2F1 has ethoxycoumarin, pentoxyresorufin O-demethylase (61), and 3-methylindole oxidase (61a) activities, but no ethoxyresorufin O-demethylase activity (61). Thus, the exclusivity of phenobarbital-inducible P4502B1/2 for the metabolism of pentoxyresorufin (76) appears to be broken by 2F1. As a consequence, metabolism of this substrate in human lung cannot be taken as an indication of the relative abundance of 2B1/2.

Twelve human P450s and two P450s from other species were expressed in human hepatoma cells (57). These Hep G2 cells were examined for their ability to activate the pneumotoxicant (77), ipomeanol. Forms 1A2, 3A3, and 3A4 (see Ref. 78 for a review of human P450s) catalyze ipomeanol oxidation to metabolites that bind DNA. Except for 1A2 in the mouse, these enzymes are not known to be expressed in either human or rodent lung. Cytochromes P4502F1 and P4504B1, isozymes occurring in human lung and discussed previously, have only modest ability to metabolize ipomeanol. The most active enzyme for ipomeanol metabolism is 4B1 from rabbit, which, as mentioned, also can metabolize 2-aminofluorene, another substrate that the human form does not metabolize (57).

Fast-emerging and important research may soon pinpoint environmental and genetic influences on susceptibility to lung cancer. Cigarette smoke has long been known to induce lung AHH activity in animals (79). Cigarette smoke also has pronounced effects on pulmonary xenobiotic metabolism and lipid peroxidation in human lungs. Although enzyme preparations from the bronchial tree and the peripheral lung have low capacities to activate promutagens in the Ames test, and they even decrease the activity of several direct mutagens, there is, nonetheless, support for the hypothesis that P4501A1 levels in tobacco smokers are associated with lung cancer risk (80). The presence of peripheral lung adenocarcinoma and lung P4501A1 levels correlate, but there is no strong correlation between bronchial cancer and lung P4501A1 levels. Macrophages in all lung cancer patients are consistently negative for antigen to a 1A1 monoclonal antibody. Type II cells from bronchial cancer and peripheral lung cancer patients tend to have more 1A1 activity than do Type I cells. Among patients with peripheral lung cancers, but not those with bronchial cancer, the bronchiolar epithelium tends to have more P4501A1 in the cuboidal cells than in ciliated cells (60).

Among patients with peripheral lung cancers, the vascular endothelium in the bronchi tends to have measurable 1A1 (60). The finding of a high degree of inducibility for P4501A1 in rabbit pulmonary endothelium and the absence of endothelial P450-reductase, suggesting that 1A1 in this case may serve as a binding protein and not as an enzyme (54), may be important in indicating the role of 1A1 in human carcinogenesis if the reductase is also absent in human lung endothelium.

P4501A1 is not the only P450 isozyme that correlates with susceptibility to lung cancer. P4502D6—which metabolizes the anti-hypertensive, debrisoquine, and which is apparently absent or defective in ~10% of Caucasians—metabolizes the tobacco procarcinogen, NNK (81). Because poor metabolizers of debrisoquine are at low risk for lung cancer (82), the inference that 2D6 participates in lung carcinogenicity would be obvious, *except* that it has not been reported to occur in lung tissue.

Major differences between humans and rodents in the ratios of potentially activating enzyme activities to detoxicating activities may contribute to the apparent resistance of humans to the toxic effects of some inhalants (83, 84). Human Type II cell BaP 4,5-oxide hydrolase activity, a detoxication pathway for this BaP metabolite, is reported at levels of 1.08 nmol/min/mg protein—compared to only 0.152 and nmol/min/mg protein for rabbit and rat Type II cells, respectively. In contrast,7-ethoxycoumarin 0-deethylation, a P450-dependent activity that may indicate a capacity to activate BaP, is not detected in either human Type II cells or human macrophages, but occurs at levels of ~100 pmol/min/mg protein in both rabbit and rat Type II cells (85). P450-mediated metabolism, however, may not be the only, or even the major,

source of activated metabolites in the human lung. Devereaux et al (85) conclude that, in human lung, non-P450-mediated oxidations, such as cooxidation by prostaglandin synthtase, may be important in xenobiotic metabolism. In this regard, the bioactivation of cyclophosphamides by prostaglandin-H synthtase has been suggested as an explanation for the susceptibility of the lung to the toxic effects of this drug (86). The role of prostaglandin-H synthtase in xenobiotic metabolism may be extensive (87, 88).

CARBOXYLESTERASES Carboxylesterases play an important role in the detoxication of organophosphorus esters, a class of compounds that includes many chemical weapons as well as pesticides that may pose an inhalation hazard (89). In part for this reason, interest continues in lung carboxylesterases. Inhalation of soman, an organophosphorus ester used as a nerve gas, at 7.3 or 32 ppb for 40 hr is reported to inhibit rat blood plasma carboxylesterase to a greater extent than lung carboxylesterase. A closer examination of the accessibility of lung esterases to inhaled soman may help to explain this finding (90).

Rat alveolar macrophage carboxylesterase plays a role in detoxicating malathion and phenthoate. Interestingly, lung injury produced by 0,0,S-trimethyl phosphorothioate, paraquat, or bromobenzene results in an increase in macrophage esterase activity (91). Human alveolar macrophage serine esterase has been characterized and found to be related to a liver microsomal enzyme (92).

The enzymology of lung esterases, like that of esterases in general, is not nearly as well understood as is that of the P450s. Six carboxylesterases have been isolated from rat liver microsomal fractions, and several have been reported in the rat lung (93). Carboxylesterases in guinea pig lung have been identified through differential inhibition by organophosphorus compounds (94).

OTHER ENZYMES Flavin-containing monooxygenases (FMO) in rabbit lung differ from those expressed in liver (95), although expression of FMO enzymes in COS-1 cells indicates that both rabbit lung and liver have more than one form (96).

At least two alpha-class glutathione transferases (GSH-T) are regulated by different genes in the rabbit lung. Both are apparently closely related to the human Ha subunit. Tissue- and species-dependent expression of the cytosolic GSH-T implies unique functions for each isozyme and unique contributions to the differential susceptibilities of tissues and animals to toxicants (97).*

*NOTE ADDED IN PROOF

Singhal et al report three α-class, two μ-class, and one π-class GSH-Ts in human lung (139).

UPTAKE AND METABOLISM: CONSIDERATIONS FOR DRUG DELIVERY AND INFLUENCES OF HOST FACTORS AND EXPOSURE ROUTE

Intranasal and Intrabronchial Drug Delivery

Interest in the nasal route for systemic administration of drugs has been rising rapidly (98, 99). Reviewing the mechanisms involved in nasal absorption, Fisher (98) concludes that, although both aqueous and lipoidal routes exist, research investigating the factors affecting nasal uptake is fragmentary, and systematic research results are lacking. The presence of substantial enzyme activities for xenobiotic metabolism (3) as well as peptidases (100) in the nasal cavity should certainly be considered in planning a systematic study.

The isolated perfused rabbit lung has been used as a model for both intravascular and intrabronchial administration of bronchodilator drugs. Diester prodrugs are 30–50% metabolized on the first pass through the lungs following endotracheal administration (101). Taking advantage of such first-pass lung metabolism in drug design for diseases such as asthma, for which treatment by inhalation is common (102), may provide a means for ameliorating undesirable systemic effects.

The efficacy of inhaled pharmaceuticals and the potency of inhaled toxicants will be influenced by the basic principles affecting their clearance. Inhaled compounds seem to fall into three categories for clearance from the lung: highly lipophilic, less lipophilic, and water soluble. Highly lipophilic compounds, such as BaP, are probably retained in the respiratory tract tissue and are metabolized substantially prior to elimination (18, 103, 104). Less lipophilic compounds, such as resorcinol derivatives of salmetrol (105) and antipyrine (106), apparently pass quickly into and through lipid membranes and clear rapidly into the blood. As lipophilicity further decreases toward water-soluble compounds, such as mannitol, uptake appears to depend on diffusion through aqueous membrane pores and lung retention increases substantially above that of lipophilic compounds, although it does not reach the level attained by highly lipophilic compounds (18, 103, 104).

To achieve higher persistence of drugs administered via inhalation, particular attention should be given to lipophilicity/retention relationships in drug design. Thus, for cyclosporine-type drugs and drugs against bronchial asthma—a disease that is thought to result from chronic inflammation (102)—drug design including consideration for novel lipophilicity/retention relationships (18, 19) might lead to increased persistence in the targeted tissues.

Noninhalation Routes of Pulmonary Exposure

Xenobiotics administered by noninhalation routes can interact with respiratory tract enzymes (107) and, under certain circumstances, the lungs may play a significant role in the metabolic elimination of xenobiotic agents in the circulation (108). BaP is metabolized extensively in the isolated perfused rat lung (108), suggesting metabolism in endothelial cells despite the reported lack of P450 reductase in rabbit lung endothelium (54). On the other hand, extrapulmonary metabolism can be involved in the mechanisms of pulmonary toxicity (109) and may play a key role in the activation of some procarcinogens. Thus, Wall et al (110) used orthotopic liver transplantation in rats to show that, once BaP reaches the liver, liver release accounts for virtually all circulating polar metabolites. Of course, ingested or inhaled BaP must first pass through the mucosa of the gastrointestinal tract or respiratory tract before reaching the blood for delivery to the liver. During this passage, considerable metabolism may occur (19).

Influence of Hormones and Inflammation on Respiratory Tract Metabolism

The influence of hormones on respiratory tract disease and on the function of respiratory tract enzymes in the metabolism of hormones is an emerging research area. Testosterone is a substrate for olfactory enzymes in the rabbit and the rat. In the rabbit, metabolism may be catalyzed by an isozyme unique to olfactory tissue, P4502G1. Sex-related steroids are also metabolized in the olfactory epithelium of the sow (see Ref. 3). Testosterone may play a key role in nasal cavity tumors induced by nitrosamines (111), and certain nitrosamine-induced nasal cavity tumors can be prevented in rats by orchiectomy (112). For example, castration of rats decreases the capacity of the nasal mucosal tissue to metabolize N-nitrosobis(2-oxopropyl)amine, whereas castration increases the capacity of the liver from rats to activate the same compound (113).

Rabbit olfactory microsomes metabolize arachidonic acid to 20-hydroxyeicosatetraeonic acid, a mediator of inflammation. The isozyme responsible is probably a member of the P450 4A subfamily (28a). A unique eicosanoid-metabolizing enzyme, termed $P450_{PG-\omega}$, was isolated from the lungs of pregnant rabbits. This isozyme may be responsible for physiologically significant processes other than xenobiotic metabolism (114). Pregnant rabbit lung $P450_{PG-\omega}$ is apparently induced at the transcriptional level, and induction is tightly regulated at the protein and the mRNA levels (115). Selective inhibition of $P450_{PG-\omega}$, has been suggested as a useful tool for studying the role of prostaglandin hydroxylation in the rabbit lung during pregnancy (116).

The relationships among mediators of respiratory tract inflammation, the presence of respiratory tract inflammation, and enzyme activities, continue to yield tantalizing results that may prove significant in the control of lung disease. Thus, xenobiotic metabolism in alveolar Type II cells may be modified by inflammatory mediators, such as platelet-activating factor, produced by alveolar phagocytes (117). In sensitized guinea pigs, P450 content and associated activities in the lung are significantly decreased. Because P450s play an important role in the arachidonate cascade (118), reduced P450 activities may lead to decreased metabolism of compounds in the arachidonate cascade that, in turn, decreases the capacity to terminate the action of cysteinyl leukotrienes (119).

The antioxidant, GSH, also plays a role in the synthesis of leukotrienes and proteins and in the activation of enzymes, in addition to its role in the protection of cells from toxic xenobiotics and oxidants (120). Oxidative stress may substantially influence lung metabolism. As mentioned previously, hyperoxygen exposure induces cytochrome P4502E1 in both lung and liver (52), and ozone induces some constitutive forms in the lung (53).

Exposure of the Brain to Inhalants Via the Olfactory Tissue

The olfactory neurons extend from the airway lumen directly into the olfactory bulb, providing a partition that is literally only one cell in thickness. The adequacy of the nose/brain barrier (121) to prevent entry of inhalants into the brain is coming under increasing scrutiny, particularly in light of the relationship of certain diseases, including Alzheimer's, to olfactory deficits (122).

Metabolism may play a role in providing or preventing access of inhalants to the brain. Nonvolatile metabolites of ^{14}C-labeled toluene and xylene accumulate in the olfactory bulbs during a 1-hr inhalation exposure to these solvents (123). For xylene, biochemical analysis indicates that the olfactory bulbs primarily accumulate methyl hippuric acid, representing glycine-conjugation subsequent to P450-dependent hydroxylation. Radioactivity progressively clears from the olfactory epithelium and deposits in the olfactory bulbs over a time course of 1 to 24 hr, a time course consistent with that expected for fast axonal transport (10cm to 1 m/day depending on species) (124). Nasal xenobiotic metabolizing enzymes may play a role in producing xylene metabolites for transportation to the brain during and following inhalation. However, immunohistochemical localization of cytochrome P450 isozymes in the olfactory bulbs is consistent with the potential metabolism of xylene in this tissue as well (125, 126).

The apical portion of the olfactory epithelium and Bowman's glands and ducts contain high levels of glucose-6-phosphate dehydrogenase (G-6-Pd) (127). In both young and aged rats, the olfactory epithelium and olfactory

bulb have much higher G-6-Pd activities than do nasal respiratory epithelium or the occipital cortex. In the olfactory bulbs, the highest concentrations occur in the nerve and glomerular layers, but enzyme activity decreases with age in olfactory tissues. G-6-Pd plays a key role in the production of cofactors for P450 and other enzymes important in xenobiotic metabolism. Decreased G-6-Pd is suggested to increase exposure of tissues to oxidative stress and to increase vulnerability to cell degeneration, as seen in aged patients, Alzheimer's disease patients, and in certain other patients with neurodegenerative disease (127).

Cancer of the Respiratory Tract

NASAL Among men in southeast China and Hong Kong, one third of all cancers occur in the nasal cavity. The putative procarcinogens are nitrosamines ingested in salted fish (128). In light of the influence of testosterone on the potency of nitrosamines in causing nasal cancer in animals (see *Influence of Hormones and Inflammation on Respiratory Tract Metabolism*), the special susceptibility of men to this disease can hardly be dismissed as coincidental. In rodents, N-nitrosodimethylamine causes nasal tumors after long-term inhalation exposure to 0.04, 0.2, or 1 ppm. Oddly, although 13 of 36 nasal tumors were observed in the low-dose animals, the survival time was 2 mo longer for these than for controls (129).

Environmental factors can influence the course of nasal cancer. Thus, oral alcohol administration favors the development of olfactory neuroepitheliomas that infiltrate the frontal lobe of the brain in mice co-administered N-nitrosodimethylamine or N-nitrosonornicotine (130, 131).

LUNG In recent years, there has been a decrease in the incidence of bronchiogenic cancer and an increase in cancer occurring in the peripheral lung (132). As mentioned before, peripheral adenocarcinomas and P4501A1 in lungs correlate, but there is no strong correlation between bronchial cancer and P4501A1. Among patients with peripheral cancers, the vascular epithelium in the bronchi tends to have measurable 1A1 (60). What all this may mean from either a causal or diagnostic view is not clear at this time. The observation that in the rabbit, P450 reductase—an important component for P450-mediated activities—is absent in the endothelium (54); and the observation that there are at least two forms of P4501A1, only one of which is closely related to lung cancer (133), may be important clues at the molecular level for the elucidation of the role of lung P450 in the cause of lung cancer.

Hepatic P4502D6, the major enzyme involved in the metabolism of debrisoquine, activates NNK and several other nitrosamines. Virtually all lung, urinary bladder, and gastrointestinal cancer patients (but not breast

cancer patients) have high metabolic capacities for debrisoquine metabolism (134,135).

Tobacco-specific nitrosamines, including NNK, are metabolized at different rates by different cells in the respiratory tract (136), and complex dose-response relationships exist between the O^6-methylguanine formed from activated NNK in Clara cells and the induction of pulmonary neoplasia by NNK in the rat (137). Studies such as these may be very important for understanding the causes of lung cancer because the initial activation of procarcinogens is only the first of many enzyme-mediated reactions resulting in mutated cells.

SUMMARY AND CONCLUSIONS

The combined impact of new research regarding the dosimetry of inhalants, discussed in early paragraphs of this review, and the rapidly developing knowledge regarding the location and substrate specificities of the enzymes responsible for xenobiotic metabolism should soon lead to new insights into the causes and prevention of cancer and other diseases of the respiratory tract and may provide insight into the design of drugs used in the treatment of respiratory tract disease. Among the developments to be expected within the next decade are the following:

1. The issue of extrapulmonary versus intrapulmonary activation of lung prodrugs and protoxicants will be resolved by validation of the different dosimetries predicted for highly lipophilic inhalants compared to less lipophilic ones (19).
2. The possibly complex roles of P450 isozymes 1A1 and 2D6 and other forms in the causation of human lung cancer will undoubtedly be better understood in the next few years.
3. Interspecies comparisons of respiratory tract enzyme activities—both activating and detoxicating—will lead to improved use of laboratory animals as models for expected toxicological and pharmacological effects in humans.
4. The potential role of nasal uptake and metabolism in causing brain disease will be established or denied experimentally.
5. The complex relationships between host factors—such as hormone levels and the presence of inflammation—and metabolism-mediated toxicity will become clearer.
6. As new research results continue to illuminate the complexities of the interactions of xenobiotics with respiratory tract tissue, clues as to how best to administer drugs via the respiratory tract and understanding of changes in disease patterns—such as the recent shift in sites for lung cancer—will follow.

ACKNOWLEDGMENT

Studies carried out in the authors' laboratory were supported by the National Institute of Environmental Health Sciences grant no. ES04422 and the Office of Health and Environmental Research of the US Department of Energy under Contract DE-AC04-76EV01013, in facilities fully accredited by the American Association for Laboratory Animal Care.

Literature Cited

1. Benford, D. J., Bridges, J. W. 1986. Xenobiotic metabolism in lung. In *Progress in Drug Metabolism,* ed. J. W. Bridges, L. F. Chasseaud, 9:54–81. New York: Taylor & Francis
2. Gram, T. E., Okine, L. K., Gram, R. A. 1986. The metabolism of xenobiotics by certain extrahepatic organs and its relation to toxicity. *Annu. Rev. Pharmacol. Toxicol.* 26:259–91
3. Dahl, A. R., Hadley, W. M. 1991. Nasal cavity enzymes involved in xenobiotic metabolism: Effects on the toxicity of inhalants. *CRC Crit. Rev. Toxicol.* 21:345–72
4. Gram, T. E. 1985. The pulmonary mixed-function oxidase system. In *Toxicology of Inhaled Materials,* ed. H. P. Witschi, J. D. Blair, pp. 421–70. New York: Springer-Verlag
5. Philpot, R. M., Wolf, C. R. 1981. The properties and distribution of the enzymes of pulmonary cytochrome P450-dependent monooxygenase systems. *Rev. Biochem. Toxicol.* 3:51–76
6. Gram, T. E. 1980. The metabolism of xenobiotics by mammalian lung. In *Extrahepatic Metabolism of Drugs on Other Foreign Compounds,* ed. T. E. Gram, pp. 159–209. Lancaster, UK
7. Cohen, G. M. 1990. Pulmonary metabolism of foreign compounds: Its role in metabolic activation. *Environ. Health Perspect.* 85:31–41
8. Smith, B. R., Brian, W. R. 1991. The role of metabolism in chemical-induced pulmonary toxicity. *Toxicol. Pathol.* 19:470–81
9. Bonnefoi, M., Monticello, T. M., Morgan, K. T. 1991. Toxic and neoplastic responses in the nasal passages: Future research needs. *Exp. Lung Res.* 17:853–68
10. Dahl, A. R. 1990. Contemporary issues in toxicology. Dose concepts for inhaled vapors and gases. *Toxicol. Appl. Pharmacol.* 103:185–97
11. Dahl, A. R., Schlesinger, R. B., Heck, H. D'A., Medinsky, M. A., Lucier, G. W. 1991. Comparative dosimetry of inhaled materials: Differences among animal species and extrapolation to man. *Fundam. Appl. Toxicol.* 16:1–13
12. Fiserova-Bergerova, V., ed. 1983. *Modeling of Inhalation Exposure to Vapors: Uptake, Distribution, and Elimination.* Boca Raton, FL: CRC Press
13. Johanson, G. 1991. Modelling of respiratory exchange of polar solvents. *Ann. Occup. Hyg.* 35:323–39
14. Morgan, K. T., Kimbell, J. S., Monticello, T. M., Patra, A. L., Fleishman, A. 1991. Studies of inspiratory airflow patterns in the nasal passages of the F344 rat and Rhesus monkey using nasal molds: Relevance to formaldehyde toxicity. *Toxicol. Appl. Pharmacol.* 110:223–40
15. Morris, J. B., Blanchard, K. T. 1992. Upper respiratory tract deposition of inspired acetaldehyde. *Toxicol. Appl. Pharmacol.* 114:140–46
16. Morris, J. B. 1990. First-pass metabolism of inspired ethyl acetate in the upper respiratory tracts of the F344 rat and Syrian hamster. *Toxicol. Appl. Pharmacol.* 102:331–45
17. Gerde, P., Dahl, A. R. 1991. A model for the uptake of inhaled vapors in thenose of the dog during cyclic breathing. *Toxicol. Appl. Pharmacol.* 109: 276–88
18. Gerde, P., Scholander, P. 1987. A hypothesis concerning asbestos carcinogenicity: The migration of lipophilic carcinogens in adsorbed lipid bilayers. *Ann. Occup. Hyg.* 31:395–400
19. Gerde, P., Medinsky, M. A., Bond, J. A. 1991. Particle-associated polycyclic aromatic hydrocarbons—A reappraisal of their possible role in pulmonary carcinogenesis. *Toxicol. Appl. Pharmacol.* 108:1–13
20. Dahl, A. R., Waruszewski, B. A. 1989. Metabolism of organonitriles to

cyanide by rat nasal tissue enzymes. *Xenobiotica* 19:1201–5
21. Dahl, A. R. 1989. The cyanide-metabolizing enzyme rhodanese in rat nasal respiratory and olfactory mucosa. *Toxicol. Lett.* 45:199–205
22. Dahl, A. R., Brezinski, D. A. 1985. Inhibition of rabbit nasal and hepatic cytochrome P450-dependent hexamethylphosphoramide (HMPA) N-demethylase by methylenedioxyphenyl compounds. *Biochem. Pharmacol.* 34: 631–36
23. Hong, J.-Y., Smith, T., Lee, M.-J., Li, W., Ma, B.-L., et al. 1991. Metabolism of carcinogenic nitrosamines by rat nasal mucosa and the effect of diallyl sulfide. *Cancer Res.* 51:1509–14
24. Dahl, A. R. 1985. Mutagenicity of some dialkylnitrosamines, cyclic nitrosamines and N,N-diethanolnitrosamine in *Salmonella typhimurium* with rat and rabbit nasal, lung and liver S9 homogenates. *Mutat. Res.* 158:141–47
25. Nebert, D. W., Nelson, D. R. 1991. P450 gene nomenclature based on evolution. *Methods Enzymol.* 206:3–11
26. Ding, X., Porter, T. D., Peng, H.-M., Coon, M. J. 1991. cDNA and derived amino acid sequence of rabbit nasal cytochrome P450NMb (P450IIG1), a unique isozyme possibly involved in olfaction. *Arch. Biochem. Biophys.* 285:120–25
27. Ding, X., Coon, M. J. 1990. Immunochemical characterization of multiple forms of cytochrome P450 in rabbit nasal microsomes and evidence for tissue-specific expression of P450s NMa and NMb. *Mol. Pharmacol.* 37: 489–96
28. Zupko, K., Poria, Y., Lancet, D. 1991. Immunolocalization of cytochromes P450olf1 and P450olf2 in rat olfactory mucosa. *Eur. J. Biochem.* 196:51–58
28a. Laethem, R. M., Laethem, C. L., Ding, X., Koop, D. R. 1992. P450-dependent arachidonic acid metabolism in rabbit olfactory microsomes. *J. Pharmacol. Exp. Ther.* 262:433
29. Adams, D. R., Jones, A. M., Plopper, C. G., Serabjit-Singh, C. J., Philpot, R. M. 1991. Distribution of cytochrome P450 monooxygenase enzymes in the nasal mucosa of hamster and rat. *Am. J. Anat.* 190:291–98
30. Longo, V., Mazzaccaro, A., Ventura, P., Gervasi, P. G. 1992. Drug-metabolizing enzymes in respiratory nasal mucosa and liver cynomolgus monkey. *Xenobiotica* 22(4):427–31
31. Gervasi, P. G., Longo, V., Naldi, F., Panattoni, G., Ursino, F. 1991. Xenobiotic-metabolizing enzymes in human respiratory nasal mucosa. *Biochem. Pharmacol.* 41:177–84
32. Bogdanffy, M. S., Mazaika, T. J., Fasano, W. J. 1989. Early cell proliferative and cytotoxic effects of phenacetin on rat nasal mucosa. *Toxicol. Appl. Pharmacol.* 98:100–12
33. Jeffery, E. H., Haschek, W. M. 1988. Protection by dimethylsulfoxide against acetaminophen-induced hepatic, but not respiratory toxicity in the mouse. *Toxicol. Appl. Pharmacol.* 93:452–61
34. St. Clair, M. B. G., Llorens, J., O'Callaghan, J. P., Peclc, D. B., Morgan, K. T., et al. 1992. Olfactory toxicity of β,β′-iminodipropionitrile (IDPN) in the rat. *J. Neurosci.* Submitted
35. Sun, J. D., Dahl, A. R., Gillett, N. A., Barr, E. B., Crews, M. L., et al. 1991. Two-week, repeated inhalation exposure of F344/N rats and B6C3F$_1$ mice to ferrocene. *Fundam. Appl. Toxicol.* 17:150–58
36. Nikula, K. J., Sun, J. D., Barr, E. B., Bechtold, W. E., Haley, P. J., et al. 1992. Thirteen-week, repeated inhalation exposure of F344/N rats and B6C3F$_1$ mice to ferrocene. Submitted
37. Hext, P. M., Lock, E. A. 1992. The accumulation and metabolism of 3-trifluoromethylpyridine by rat olfactory and hepatic tissues. *Toxicology* 72:61–75
38. Trela, B. A., Bogdanffy, M. S. 1991. Cytotoxicity of dibasic esters (DBE) metabolites in rat nasal explants. *Toxicol. Appl. Pharmacol.* 110:259–67
39. Bogdanffy, M. S., Kee, C. R., Hinchman, C. A., Trela, B. A. 1991. Metabolism of dibasic esters by rat nasal mucosal carboxylesterase. *Drug Metab. Dispos.* 19:124–29
40. Mattes, P. M., Mattes, W. B. 1992. α-naphthyl butyrate carboxylesterase activity in human and rat nasal tissue. *Toxicol. Appl. Pharmacol.* 114:71–76
41. Lewis, J. L., Nikula, K. J., Novak, R., Dahl, A. R. 1992. Comparative localization of carboxylesterase in rat, dog, and human nasal tissue. Submitted
42. Fisher, G. D., Kilgore, W. W. 1988. Tissue levels of glutathione following acute inhalation of 1,3-dichloropropene. *J. Toxicol. Environ. Health* 23:171–82
42a. Larsson, P., Tjälve, H. 1992. Binding of afloxin B$_1$ metabolites in extrahepatic tissues in fetal and infant mice and in adult mice with depleted glutathione levels. *Cancer Res.* 52:1267–77

42b. Brittebo, E. B., Eriksson, C., Brandt, I. 1992. Effects of glutathione-modulating agents on the covalent binding and toxicity of dichlobenil in the mouse olfactory mucosa. *Toxicol. Appl. Pharmacol.* 1144:31–40

43. Kirstein, C. L., Coopersmith, R., Bridges, R. J., Leon, M. 1991. Glutathione levels in olfactory and non-olfactory neural structures of rats. *Brain Res.* 543:341–46

44. Lewis, J. L., Rhoades, C. E., Gervasi, P.-G., Griffith, W. C., Dahl, A. R. 1991. The cyanide-metabolizing enzyme rhodanese in human nasal respiratory mucosa. *Toxicol. Appl. Pharmacol.* 108:114–20

45. Lewis, J. L., Rhoades, C. E., Bice, D. E., Harkema, J. R., Hotchkiss, J. A., et al. 1992. Interspecies comparison of cellular localization of the cyanide metabolizing enzyme rhodanese within olfactory mucosa. *Anat. Rec.* 232:620–27

46. Wilson, J. D., Miller, H., Gelboin, H. V., Friedman, F. K. 1990. Variation in inducibility of cytochrome P450c and aryl hydrocarbon hydroxylase in rat liver, lung, kidney, pancreas and nasopharynx. *Pharmacology* 41:256–62

47. McDowell, E. M., Zhang, X. M., Philpot, R. M., De Santi, A. M., Strum, J. M. 1990. Immunohistochemical demonstration of cytochrome P450 monooxygenase in regenerating tracheal epithelium: A recapitulation of fetal development. *Virchows Arch. B* 59: 243–49

47a. Plopper, C. G., Suverkropp, C., Morin, D., Nishio, S., Buckpitt, A. 1992. Cytochrome P-450 activity in clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J. Pharmacol. Exp. Ther.* 261:353

48. Forkert, P. G., Vessey, M. L., Park, S. S., Gelboin, H. V., Cole, S. P. C. 1989. Cytochromes P450 in murine lung: An immunohistochemical study with monoclonal antibodies. *Drug Metab.* 17:551–55

49. Chichester, C. H., Philpot, R. M., Weir, A. J., Buckpitt, A. R., Plopper, C. G. 1991. Characterization of the cytochrome P450 monooxygenase system in nonciliated bronchiolar epithelial (Clara) cells isolated from mouse lung. *Am. J. Respir. Cell Mol. Biol.* 4:179–86

50. Nagata, K., Martin, B. M., Gillette, J. R., Sasame, H. A. 1990. Isozymes of cytochrome P450 that metabolize naphthalene in liver and lung of untreated mice. *Drug Metab. Dispos.* 18:557–64

51. Baron, J., Voigt, J. M. 1990. Localization, distribution, and induction of xenobiotic-metabolizing enzymes and aryl hydrocarbon hydroxylase activity within lung. *Pharmacol. Ther.* 47:419–45

52. Tindberg, N., Ingelman-Sundberg, M. 1989. Cytochrome P450 and oxygentoxicity. Oxygen-dependent induction of ethanol-inducible cytochrome P450(2E1) in rat liver and lung. *Biochemistry* 28:4499–504

53. Takahashi, Y., Miura, T. 1990. Responses of cytochrome P450 isozymes of rat lung to in vivo exposure to ozone. *Toxicol. Lett.* 54:327–35

54. Overby, L. H., Nishio, S., Weir, A., Carver, G. T., Plopper, C. G., et al. 1992. Distribution of cytochrome P450 1A1 and NADPH-cytochrome P450 reductase in lungs of rabbits treated with 2,3,7,8 - tetrachlorodibenzo - *p* - dioxin: Ultrastructural immunolocalization and in situ hybridization. *Mol. Pharmacol.* 41:1039–46

55. Domin, B. A., Devereux, R. R., Philpot, R. M. 1986. The cytochrome P450 monooxygenase system of the rabbit lung: Enzyme components, activities, and induction in the nonciliated bronchiolar epithelial (Clara) cell, alveolar type II, and alveolar macrophage. *Mol. Pharmacol.* 30:296–303

55a. Porter, T. D., Khani, S. C., Coon, M. J. 1989. Induction and tissue-specific expression of rabbit cytochrome P450IIE1 and IIE2 genes. *Mol. Pharmacol.* 36:61–65

56. Muerhoff, A. S., Williams, D. E., Masters, B. S. S. 1990. Purification and properties of pregnancy-inducible rabbit lung cytochrome P450 prostaglandin ω-hydroxylase. *Methods Enzymol.* 187:253–67

57. Czerwinski, M., McLemore, T. E., Philpot, R. M., Nhamburo, P. T., Korzekwa, K., et al. 1991. Metabolic activation of 4-ipomeanol by complimentary DNA-expressed human cytochromes P450: Evidence for species-specific metabolism. *Cancer Res.* 51: 4636–38

58. Fujii, H., Sagami, I., Ohmachi, T., Kikuchi, H., Watanabe, M. 1991. Tissue difference in expression of cytochrome P450 between liver and lung of Syrian golden hamsters treated with 3-methylcholanthrene. *Adv. Exp. Med. Biol.* 283:413–17

59. Strum, J. M., Ito, T., Philpot, R. M., DeSanti, A. M., McDowell, E. M.

1990. The immunocytochemical detection of cytochrome P450 monooxygenase in the lungs of fetal, neonatal, and adult hamsters. *Am. J. Respir. Cell Mol. Biol.* 2:493–501

60. Anttila, S., Hietanen, E., Vainio, H., Camus, A.-M., Gelboin, H. V., et al. 1991. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P4501A in lung cancer patients. *Int. J. Cancer* 47:681–85
61. Nhamburo, P. T., Kimura, S., McBride, O. W., Kozak, C. A., Gelboin, H. V., Gonzalez, F. J. 1990. The human CYP2F subfamily: Identification of a cDNA coding for a new cytochrome P450 expressed in lung, cDNA-directed expression and chromosome mapping. *Biochemistry* 29:5491–99

61a. Thornton-Manning, J. R., Ruangyuttikarn, W., Gonzalez, F. J., Yost, G. S. 1991. Metabolic activation of the pneumotoxin, 3-methylindole, by vaccinia-expressed cytochrome P450s. *Biochem. Biophys. Res. Commun.* 181: 100

62. Nhamburo, P. T., Gonzalez, F. J., McBride, O. W., Gelboin, H. V., Kimura, S. 1989. Identification of a new P450 expressed in human lung: Complete cDNA sequence, cDNA-directed expression, and chromosome mapping. *Biochemistry* 28:8060–66
63. Beebe, L., Park, S. S., Anderson, L. M. 1990. Differential enzyme inductionof mouse liver and lung following a single low or high dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Biochem. Toxicol.* 5:211–19
64. Anderson, L. M., Ward, J. M., Park, S. S., Jones, A. B., Junker, J. L., et al. 1987. Immunohistochemical determination of inducibility phenotype with a monoclonal antibody to a methylcholanthrene-inducible isozyme of cytochrome P450. *Cancer Res.* 47: 6079–85
65. Kanekal, S., Plopper, C., Morin, D., Buckpitt, A. 1991. Metabolism and cytotoxicity of naphthalene oxide in the isolated perfused mouse lung. *J. Pharmacol. Exp. Ther.* 256:391–401
66. Keith, I. M., Olson, E. B., Wilson, N. M., Jefcoate, C. R. 1987. Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P450. *Cancer Res.* 47:1878–82
67. Voigt, J. M., Kawabata, T. T., Burke, J. P., Martin, M. V., Guengerich, F. P., et al. 1990. In situ localization and distribution of xenobiotic-activating enzymes and aryl hydrocarbon hydroxylase activity in lungs of untreated rats. *Mol. Pharmacol.* 37:182–91
68. Marcus, C. B., Wilson, N. M., Keith, I. M., Jefcoate, C. R., Omiecinski, C. J. 1990. Selective expression of cytochrome P450 isozymes by 4-N-alkyl-methylene dioxybenzenes in rat lung cells. *Arch. Biochem. Biophys.* 277:17–25

68a. Kimura, S., Kozak, C. A., Gonzalez, F. J., 1989. Identification of a novel P450 expressed in rat lung:cDNA cloning and sequence, chromosome mapping and induction by 3-methylcholanthrene. *Biochemistry* 28:3798–803

69. Carlson, G. P. 1991. Influence of ethanol on microsomal p-nitrophenol hydroxylation and ethoxyresorufin deethylation in rat liver and lung. *J. Toxicol. Environ. Health* 32:153–59
70. Rabovsky, J., Judy, D. J., Goddard, M., Pailes, W. H., Castranova, V. 1990. Lung cytochrome P450-dependent benzyloxyphenoxazone debenzylase and ethoxyphenoxazone deethylase activities in total microsomal and isolated alveolar type II cells: Responses to changes in assay conditions with special reference to non-linear dependence at low enzyme concentration. *Int. J. Biochem.* 22:171–77
71. Mansour, H., Brun-Pascaud, M., Marquetty, C., Gougerot-Pocidalo, M.-A., Hakim, J., et al. 1988. Protection of rat from oxygen toxicity by inducers of cytochrome P450 system. *Am. Rev. Respir. Dis.* 137:688–94
72. Ueng, T.-H., Tsai, J.-N., Ju, J.-M., Ueng, Y.-F., Iwasaki, M., et al. 1991. Effects of acetone administration on cytochrome P450-dependent monooxygenases in hamster liver, kidney, and lung. *Arch. Toxicol.* 65:45–51
73. Wheeler, C. W., Guenthner, T. M. 1991. Cytochrome P450-dependent metabolism of xenobiotics in human lung. *J. Biochem. Toxicol.* 6:163–69
74. Shimada, T., Yun, C.-H., Yamazaki, H., Gautier, J. C., Beaune, P. H., Guengerich, F. P. 1992. Characterization of human lung microsomal cytochrome P-4501A1 and its role in the oxidation of chemical carcinogens. *Mol. Pharmacol.* 41:856–64
75. Wheeler, C. W., Guenthner, T. M. 1990. Spectroscopic quantitation of cytochrome P450 in human lung microsomes. *J. Biochem. Toxicol.* 5:269–72

76. Bartlomowicz, B., Friedberg, T., Utesch, D., Molitor, E., Platt, K., Oesch, F. 1989. Regio- and stereoselective regulation of monooxygenase activities by isoenzyme-selective phosphorylation of cytochrome P450. *Biochem. Biophys. Res. Commun.* 160: 46–52
77. Gram, T. E. 1989. Pulmonary toxicity of 4-ipomeanol. *Pharmacol. Ther.* 43:291–97
78. Wrighton, S. A., Miller, J. C. 1992. The human hepatic cytochromes P450 involved in drug metabolism. *Crit. Rev. Toxicol.* 22(1):1–21
79. Welch, R. M., Cavallito, J., Loh, A. 1972. Effect of exposure to cigarette smoke on the metabolism of benzo(a)pyrene and acetophenetidin by lung and intestine of rats. *Toxicol. Appl. Pharmacol.* 23:749–58
80. Bartsch, H., Petruzzelli, S., De Flora, S., Hietanen, E., Camus, A.-M., et al. 1991. Carcinogen metabolism and DNA adducts in human lung tissues as affected by tobacco smoking or metabolic phenotype: a case-control study on lung cancer patients. *Mutat. Res.* 259:103–14
81. Crespi, C. L., Penman, B. W., Gelboin, H. V., Gonzales, F. J. 1991. Atobacco smoke-derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1 -butanone, is activated by multiple human cytochrome P450s including the polymorphic human cytochrome P4502D6. *Carcinogenesis* 12:1197–201
82. Caporaso, N. E., Tucker, M. A., Hoover, R. N., Hayes, R. B., Pickle, L. W., et al. 1990. Lung cancer and the debrisoquine metabolic phenotype. *J. Natl. Cancer Inst.* 82:1262–72
83. Dahl, A. R., Sun, J. D., Birnbaum, L. S., Bond, J. A., Griffith, W. C., et al. 1991. Toxicokinetics of inhaled 1,3-butadiene in monkeys: Comparison totoxico kinetics in rats and mice. *Toxicol. Appl. Pharmacol.* 110:9–19
84. Sabourin, P. J., Burka, L. T., Bechtold, W. E., Dahl, A. R., Hoover, M. D., et al. 1992. Species differences in urinary butadiene metabolites; identification of 1,2-dihydroxy-4-(N-acetylcysteinyl)butane, a novel metabolite of butadiene. *Carcinogenesis.* In press
85. Devereux, T. R., Domin, B. A., Philpot, R. M. 1989. Xenobiotic metabolism by isolated pulmonary cells. *Pharmacol. Ther.* 41:243–56
86. Smith, B. J., Curtis, J. F., Eling, T. E. 1991. Bioactivation of xenobiotics by prostaglandin H synthtase. *Chem. Biol. Interact.* 79:245–64
87. Eling, T. E., Thompson, D. C., Foureman, G. L., Curtis, J. F., Hughes, M. F. 1990. Prostaglandin H synthtase and xenobiotic oxidation. *Annu. Rev. Pharmacol. Toxicol.* 30:1–45
88. Guengerich, F. P. 1990. Enzymatic oxidation of xenobiotic chemicals. *Crit. Rev. Biochem. Mol. Biol.* 25(2): 91–153
89. Fonnum, F., Sterri, S. H., Aas, P., Johnsen, H. 1985. Carboxylesterases,importance for detoxification of organophosphorus anticholinesterases and trichothecenes. *Fundam. Appl. Toxicol.* 5:S29–38
90. Walday, P., Paål, A., Fonnum, F. 1991. Inhibition of serine esterases in different rat tissues following inhalation of soman. *Biochem. Pharmacol.* 41: 151–53
91. Imamura, T., Schiller, N. L., Fukuto, T. R. 1983. Malathion and phenthoate carboxylesterase activities in pulmonary alveolar macrophages as indicators of lung injury. *Toxicol. Appl. Pharmacol.* 70:140–47
92. Munger, J. S., Shi, G.-P., Mark, E. A., Chin, D. T., Gerard, C. 1991. A serine esterase released by human alveolar macrophages is closely related to liver microsomal carboxylesterases. *J. Biol. Chem.* 266:18832–38
93. Gaustad, R., Sletten, K., Lovhaug, D., Fonnum, F. 1991. Purification and characterization of carboxylesterases from rat lung. *Biochem. J.* 274:693–97
94. Gaustad, R., Johnsen, H., Fonnum, F. 1991. Carboxylesterases in guinea pig: A comparison of the different isoenzymes with regard to inhibition of organophosphorus compounds in vivo and in vitro. *Biochem. Pharmacol.* 42:1335–43
95. Lawton, M. P., Gasser, R., Tynes, R. E., Hodgson, E., Philpot, R. M. 1990. The flavin-containing monooxygenase enzymes expressed in rabbit liver and lung are products of related but distinctly different genes. *J. Biol. Chem.* 265:5855–61
96. Lawton, M. P., Kronbach, T., Johnson, E. F., Philpot, R. M. 1991. Properties of expressed and native flavin-containing monooxygenases: Evidence of multiple forms in rabbit liver and lung. *Mol. Pharmacol.* 40:692–98
97. Gardlik, S., Gasser, R., Philpot, R. M., Serabjit-Singh, C. J. 1991. The major alpha-class glutathione S-transferases of rabbit lung and liver. *J. Biol. Chem.* 266:19681–87
98. Fisher, A. N. 1990. Absorption across the nasal mucosa of animal species:

Compounds applied and mechanisms involved, See Ref. 1, pp. 12:88–131
99. Chien, Y. W., Su, K. S. E., Chang, S. F. 1989. In *Nasal Systemic Drug Delivery*. New York: Marcel Dekker
100. Kaliner, M. A. 1991. Human nasal respiratory secretion and host defense. *Am. Rev. Respir. Dis.* 144:S52–56
101. Brazzell, R. K., Kostenbauder, H. B. 1982. Isolated perfused rabbit lung as a model for intravascular and intrabronchial administration of bronchodilator drugs II: Isoproterenol prodrugs. *J. Pharmacol. Sci.* 71:1274–81
102. Giembycz, M. A. 1992. Could isoenzyme-selective phosphodiesterase inhibitors render bronchodilator therapy redundant in the treatment of bronchial asthma? *Biochem. Pharmacol.* 43: 2041–51
103. Gerde, P., Scholander, P. 1989. An experimental study on the penetration of polycyclic aromatic hydrocarbons through a model of the bronchial lining layer. *Environ. Res.* 48:287–95
104. Henderson, R. F., Bechtold, W. E., Medinsky, M. A., Fischer, P. J., Lee, T. T. 1988. The effect of molecular weight/lipophilicity on clearance of organic compounds from lungs. *Toxicol. Appl. Pharmacol.* 95:515–21
105. Jeppsson, A. B., Roos, C., Waldeck, B., Widmark, E. 1989. Pharmacodynamic and pharmacokinetic aspects on the transport of bronchodilator drugs through the tracheal epithelium of the guinea-pig. *Pharmacol. Toxicol.* 64:58–63
106. Schanker, L. S., Mitchell, E. W., Brown, R. A. Jr., 1986. Species comparison of drug absorption from the lung after aerosol inhalation or intratracheal injection. *Drug Metab. Dispos.* 14:79–88
107. Brittebo, E. B., Brandt, I. 1990. Interactions of xenobiotics in the respiratory tract following non-inhalation routes of exposure. *Int. J. Epidemiol.* 19:S24–31
108. Roth, R. A., Vinegar, A. 1990. Action by the lungs on circulating xenobiotic agents, with a case study of physiologically based pharmacokinetic modeling of benzo(a)pyrene disposition. *Pharmacol. Ther.* 48:143–55
109. Yost, G. S., Buckpitt, A. R., Roth, R. A., McLemore, T. L. 1989. Contemporary issues in toxicology: Mechanisms of lung injury by systemically administered chemicals. *Toxicol. Appl. Pharmacol.* 101:179–95
110. Wall, K. L., Gao, W., Koppele, J. M., Kwei, G. Y., Kauffman, F. C., Thurman, R. G. 1991. The liver plays a central role in the mechanism of chemical carcinogenesis due to polycyclic aromatic hydrocarbons. *Carcinogenesis* 12:783–86
111. Pour, P. M., Stepan, K. R. 1988. The role of testosterone in the nasal cavity tumors induced by N-nitrosobis(2-oxopropyl)amine in rats. *Carcinogenesis* 9:1417–20
112. Pour, P. M., Götz, U. 1983. Prevention of N-nitrosobis(2-oxopropyl)amine-induced nasal cavity tumors in rats by orchiectomy. *J. Natl. Cancer Inst.* 70: 353–57
113. Lawson, T. A. 1990. Activation of N-nitrosobis(2-oxopropyl)amine by liver and nasal mucosa tissue from intact and castrated male rats. *Cancer Lett.* 53:39–43
114. Masters, B. S. S., Okita, R. T., Muerhoff, A. S., Leithauser, M. T., Gee, A. 1989. Pulmonary P450-mediated eicosanoid metabolism and regulation in the pregnant rabbit. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 19:335–38
115. Muerhoff, A. S., Williams, D. E., Leithauser, M. T., Jackson, V. E., Waterman, M. R., et al. 1987. Regulation of the induction of a cytochrome P450 prostaglandin ω-hydroxylase by pregnancy in rabbit lung. *Proc. Natl. Acad. Sci. USA* 84:7911–14
116. Muerhoff, A. S., Williams, D. E., Reich, N. O., CaJacob, C. A., Ortiz de Montellano, P. R., et al. 1989. Prostaglandin and fatty acid ω- and (ω-1)-oxidation in rabbit lung. *J. Biol. Chem.* 264:749–56
117. Rabovsky, J., Pailes, W. H., Judy, D. J., Castranova, V. 1991. Cytochrome P450-dependent metabolism in alveolar type II epithelial cells: Modulation by platelet-activating factor. *Biochim. Biophys. Acta* 1095:223–29
118. Capdevila, J. H., Falck, J. R., Estabrook, R. W. 1992. Cytochrome P450 and the arachidonate cascade. *FASEB* 6:731–36
119. Grant, M. H., Rodger, I. W. 1991. The metabolism of leukotrienes, detoxification of reactive oxygen species and the activity of the cytochrome P_{450} mixed function oxidase system in sensitized guinea-pig lungs. *Pulmonary Pharmacol.* 4:165–69
120. Cantin, A. M., Bégin, R. 1991. Glutathione and inflammatory disorders of the lung. *Lung* 169:123–38
121. Lewis, J. L. 1991. Olfactory biotransformation enzymes: Transduction and

toxicology merge. Oral summary presented at Special Symp. Assoc. Chemorecept. Sci. Ann. Meet., Sarasota, FL
122. Roberts, E. 1986. Alzheimer's disease may begin in the nose and may becaused by aluminosilicates. *Neurobiol. Aging* 7:561–67
123. Ghantous, H., Dencker, L., Gabrielsson, J., Danielsson, B. R. G., Bergman, K. 1990. Accumulation and turnover of metabolites of toluene and xylene in nasal mucosa and olfactory bulb in the mouse. *Pharmacol. Toxicol.* 66:87–92
124. Cooper, J. R., Bloom, F. E., Roth, R. H. 1986. *The Biochemical Basis of Neuropharmacology*, pp. 247–49. London: Oxford Univ. Press. 5th ed.
125. Kolher, C., Eriksson, L. G., Hansson, T., Warner, M., Ake-Gustafsson, J. 1988. Immunohistochemical localization of cytochrome p-450 in the rat brain. *Neurosci. Lett.* 84(2):109–14
126. Hansson, T., Tindberg, N., Ingelman-Sundberg, M., Kohler, C. 1990. Regional distribution of ethanol-inducible cytochrome P450 IIE1 in the rat central nervous system. *Neuroscience* 34(2): v 451–63
127. Miller, S., Coopersmith, R., Leon, M. 1991. Biochemical quantitation and histochemical localization of glucose-6-phosphate dehydrogenase activity in the olfactory system of adult and aged rats. *Neurochem. Res.* 16:475–81
128. Tricker, A. R., Preussmann, R. 1991. Carcinogenic N-nitrosamines in the diet: Occurrence, formation, mechanisms and carcinogenic potential. *Mutat. Res.* 259:277–89
129. Klein, R. G., Janowsky, I., Pool-Zobel, B. L., Schmezer, P., Hermann, R., et al. 1991. Effects of long-term inhalation of N-nitrosodimethylamine in rats. *IARC Sci. Publ.* 105:322–28
130. Griciute, L., Castegnaro, M., Béréziat, J.-C. 1981. Influence of ethyl alcohol on carcinogenesis with N-nitrosodimethylamine. *Cancer Lett.* 13:345–52
131. Griciute, L., Castegnaro, M., Béréziat, J. C., Cabral, J. R. P. 1986. Influence of ethyl alcohol on the carcinogenic activity of N-nitrosonornicotine. *Cancer Lett.* 31:267–75
132. Auerbach, O., Garfinkel, L. 1991. The changing pattern of lung carcinoma. *Cancer* 68:1973–77
133. Hayashi, S., Watanabe, J., Nakachi, K., Kawajiri, K. 1991. Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J. Biochem.* 110:407–11
134. Huober, J., Bertram, B., Petru, E., Kaufmann, M., Schmähl, D. 1991. Metabolism of debrisoquine and susceptibility to breast cancer. *Breast Cancer Res. Treat.* 18:43–48
135. Nebert, D. W. 1991. Role of genetics and drug metabolism in human cancer risk. *Mutat. Res.* 247:267–81
136. Belinsky, S. A., White, C. M., Trushin, N., Hecht, S. S. 1989. Cell specificity for the pulmonary metabolism of tobacco-specific nitrosamines in the Fischer rat. *Carcinogenesis* 10:2269–74
137. Belinsky, S. A., Foley, J. F., White, C. M., Anderson, M. W., Maronpot, R. R. 1990. Dose-response relationship between O6-methylguanine formation in Clara cells and induction of pulmonary neoplasia in the rat by 4-(methlnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res. 50:3772–80
138. Kato, S., Shields, P. G., Caporaso, N. E., Hoover, R. N., Trump, B. F., et al. 1992. Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. *Cancer Res.* 52:6712–15
139. Singhal, S. S., Saxena, M., Ahmad, H., Awasthi, S., Hague, A. K., Awasthi, Y. C. 1992. Glutathione *S*-transferases of human lung: characterization and evaluation of the protective role of the α-class isozymes against lipid peroxidation. *Arch. Biochem. Biophys.* 299:232–41