Dioxin-receptor Ligands in Urban Air and Vehicle Exhaust

Grant G. F. Mason

Department of Medical Nutrition, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden

The ability of extracts of urban air and vehicle exhaust particulates to bind to the dioxin receptor has been determined. It was shown that such extracts do contain significant amounts of dioxin-receptor binding activity. The level of dioxin-receptor binding found in ambient air reflects its pollution level as determined by mutagenic activity. Furthermore, it was shown that the extracts of both urban air and vehicle exhaust particulates could provoke the induction of cytochrome P450A1 in cultured rat hepatoma cells. Chemical fractionation of the extracts revealed that the majority of the dioxin-receptor binding activity from urban air and gasoline vehicle samples fractionated with the polycyclic aromatic compounds. However, unknown polycyclic aromatic compounds were responsible for the majority of the binding activity measured. In the case of diesel vehicle exhausts, the majority of the dioxin-receptor binding activity was found to be associated with nitro-polycyclic aromatic compounds. Studies with a variety of diesel fuels showed that the amount of dioxin-receptor ligands present in exhaust emissions are fuel-dependent and that substantial amounts of dioxin-receptor ligands are present in the semivolatile phase of exhaust emissions. — Environ Health Perspect 102(Suppl 4):111–116 (1994).

Key words: diesel, dioxins, polycyclic aromatic compounds, risk assessment, vehicle exhaust

Introduction

Bound to particles found in urban air is a highly complex mixture of organic compounds, many of which are produced during the incomplete combustion of organic matter such as from the burning of fossil fuels and from motor vehicle exhausts. The adverse health effects of these compounds is known only partly.

In man and other mammals, metabolism of these compounds in the tissues can have one of two effects. On one hand, metabolism may lead to products that are relatively nontoxic and are cleared from the body rapidly. On the other hand, even though it may represent a minor metabolic pathway for the compounds in question, many of these compounds are metabolized to a more chemically reactive species capable of reacting with cellular macromolecules such as DNA (e.g., the metabolism of benzo[a]pyrene to benzo[a]pyrene-7,8-diol-9,10-epoxide) (1). Such metabolic activation is a key step in the sequence of events leading to genotoxicity and carcinogenicity (2). With regard to polycyclic aromatic compounds (PACs), two cytochrome P450 isoforms, P450IA1 and P450IA2 (3), preferentially catalyze this activation, and their activity generally is known as aryl hydrocarbon hydroxylase (AHH) (4).

One of the most commonly studied effects of the PACs is their ability to induce AHH (4,5). Thus, these compounds are capable of inducing their own metabolism as well as that of related compounds. Although this induction can lead to increased toxicity such as the increased formation of benzo[a]pyrene adducts, the effects of AHH induction on PAC toxicity are complex, depending upon the PAC in question and its route of administration, bioavailability and target organ. Thus, induction of AHH per se, may play a role in the mechanism of toxicity and carcinogenicity of the nonhalogenated PACs that are metabolized extensively by this monooxygenase system in chemically reactive species capable of binding to cellular macromolecules.

Studies have shown that AHH induction is due to an increased rate of transcription of the cytochrome P450 gene(s) in question (6–8). It is believed that the PACs elicit these effects through initial binding to the receptor protein that specifically binds the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the dioxin receptor (otherwise termed the Ah receptor) (6,7,9–12). The binding of PAC or TCDD to the dioxin receptor results in a ligand-dependent transformation of the receptor to an activated receptor-ligand complex that translocates to the cell nucleus. In that position, it subsequently interacts with specific nuclear components to affect transcriptional regulation of target genes (6,7,9,11,12), including cytochrome P450IA1. Induction of AHH correlates well with the inducer affinity for the dioxin receptor, both in vivo and in vitro (9,13–15).

The highly toxic halogenated PACs such as chlorinated dibenzo-p-dioxins and dibenzo-p-dioxin analogs only are metabolized slowly to relatively nontoxic metabolites, which are rapidly cleared from the body (9). Many of the parent compounds, however, share a common characteristic spectrum of toxic effects including thymic involution, teratogenesis (e.g., cleft-palate formation), tumor promotion, and the stimulation of epidermal cell proliferation and differentiation, leading to chloracne in humans (9). A number of nonhalogenated PACs also have been shown to produce some of these toxic effects in model systems (16–19). Importantly, it is apparent that these toxic effects of the halogenated and nonhalogenated PACs are dioxin-receptor mediated. Thus, it is evident that the determination of a compound's dioxin-receptor binding affinity and its ability to induce activation of the receptor to its DNA-binding state, allowing it to regulate target genes, would provide a measure of its potential for eliciting the toxic effects associated with this large group of compounds.

Methods

During the Swedish Urban Air Project, we determined the ability of various sample extracts to bind to the dioxin receptor. The dioxin-receptor binding affinities of samples were measured using an hydroxylapatite assay developed in our laboratory (20) and...
are described in full elsewhere (21). Relative binding affinities are expressed as IC$_{50}$, the amount of sample required to reduce the specific binding of a standard amount of [3H]TCDD (1 pmole/ml) to its receptor by 50%. The ability of particle extracts to induce AHH in the cell line H4IIE $in vivo$ was determined as described by Franzen et al. (22). Sample extracts were prepared and their PAC content was analyzed as noted in later references. Particulate material was collected over 24-hr periods from inner-city, suburban, and rural air using high-volume filter samplers situated at roof top level (21). Particulate emissions from gasoline- and diesel-fueled vehicles were collected (23).

**Results**

**Presence of Dioxin-receptor Ligands in Urban Air**

Extracts of ambient air particulate samples were shown to contain components which bound to the dioxin receptor (21) (Figure 1). The much lower IC$_{50}$ (0.1 m$^3$/ml) of inner-city air indicates that it elicits significantly higher dioxin-receptor binding competition per m$^3$ air than rural air (IC$_{50}$ 7.8 m$^3$/ml). The IC$_{50}$ of a sample taken at a suburban site was approximately twice that of the inner-city sample (0.19 m$^3$/ml), reflecting a decreased presence of dioxin-receptor binding activity. There appeared to be agreement between the dioxin-receptor binding competition determined for each site and the pollution level as determined by measuring direct mutagenic effects of the same samples by Ames test (21).

The amounts of 26 known PACs present in some urban air particulate extracts were determined. Assuming that all analyzed PACs present at concentrations $\geq$ 0.1 ng/m$^3$ had the same receptor binding affinity as benzo[a]pyrene, between 1 and 30% of the observed binding could be accounted for (21). This is a conservative estimate based on the fact that only two of the 16 PACs present for which receptor binding affinity data is available have binding affinities greater than benzo[a]pyrene. Any significant contribution to the observed binding activity due to TCDD or tetrachlorodibenzo-p-dioxin was ruled out because these compounds are below the detection limit of 2 pg/m$^3$.

**Presence of Dioxin-receptor Ligands in Vehicle Exhaust Emissions**

A substantial amount of the particulate matter in city air comes from vehicle exhaust emissions. Therefore, extracts were made of vehicle exhaust particulate matter and assayed for the presence of dioxin-receptor ligands. As illustrated in Figure 2, more dioxin-receptor binding activity was emitted per driving distance from the diesel-fueled car (IC$_{50}$ 0.002 m/ml) than from the gasoline-driven car (IC$_{50}$ 0.030 m/ml) (23). However, a number of factors play significant roles in the magnitude of dioxin-receptor binding competition displayed by vehicle exhaust including vehicle type and fuel composition. This presumably results from the effect these factors have on the PAC content of the emissions (24). Indeed, IC$_{50}$ values as low as 0.005 m driving distance/ml (Figure 2) have been obtained from emission particulate extracts from gasoline driven vehicles (R. Tofgård, personal communication).

**Dioxin-receptor-mediated Biological Activity of Urban Air Particulate Extracts**

Having demonstrated the presence of dioxin-receptor ligands in association with urban air particulate matter, it was important to know whether these ligands could elicit biological effects. Therefore, the ability of particle extracts to induce the enzyme system AHH in the rat hepatoma cell line H4IIE was investigated. H4IIE cells were treated with extracts of particulate matter from urban air, vehicle exhaust, and pure compounds known to be present in such extracts (22). The results obtained are given in Table 1.

**Table 1.** Induction of aryl hydrocarbon hydroxylase (AHH) activity compared with competition for dioxin receptor binding by pure substances, extracts of vehicle exhaust particulate matter, and urban air particulate matter (22).

| Compound/sample     | AHH induction (EC$_{50}$) | Dioxin receptor (IC$_{50}$) |
|---------------------|---------------------------|-----------------------------|
| 5,6-Benzoflavone    | 45 nM                     | 26 nM                       |
| Dibenzo[a,h]pyranthracene | 62 nM                   | 6 nM                        |
| 6-Chloroanthracene  | 96 nM                     | 14 nM                       |
| 1-/3-nitrobenzo[a]pyrene (1:2) | 220 nM                | 2 nM                        |
| Benzo[a]pyrene      | 680 nM                    | 42 nM                       |
| Ben[a]pyrene        | 720 nM                    | 80 nM                       |
| Gasoline car exhaust| 0.01 m/ml                 | 0.03 m/ml                   |
| Ambient air 1       | 25 μg/ml                  | 6 μg/ml                     |
| Ambient air 2       | 17 μg/ml                  | 4 μg/ml                     |

$^a$Data taken from Franzen et al. (22). $^b$The EC$_{50}$ is the concentration of compound or sample giving 50% of the maximum AHH activity. $^c$The IC$_{50}$ is the concentration of competitor that competes for 50% of the specific binding of [3H] TCDD. $^d$Data taken from Greibrok et al. (32). $^e$Concentration of tested particulate extract corresponding to meter driving distance per milliliter cytosol. $^f$Microgram of extracted particulate matter per milliliter cytosol. $^g$Represents a second 24-hr sample taken at a similar site to sample 1 (22).
exception of the nitro-PAC, there was a high overall rank correlation between induction of AHH by the pure PACs and particle extracts and their receptor binding affinities ($r = 0.85$). These and further data from the use of specific inhibitors of cytochrome P450s, analysis of mRNA levels, and immunoblotting (22) indicate that substances present in extracts of urban air particulates can interact with the dioxin receptor in cells and cause an accumulation of cytochrome P450IA1 mRNA leading to an increase in enzyme activity.

Thus it has been shown that compounds associated with urban air or vehicle exhaust particulate matter not only demonstrate dioxin-receptor binding but also can elicit a subsequent biological response in intact cells.

**Fractionation of Particulate Extracts**

It had been estimated that only 1 to 30% of the dioxin-receptor binding competition activity of an urban air particulate extract theoretically could be attributed to known PAC determined in the sample (21). In order to understand which compounds present in particulate extracts contribute to the measured receptor binding activity, particulate extracts from urban air as well as diesel and gasoline exhaust emissions were fractionated on the basis of increasing polarity into five fractions, I to V (23,25,26). The dioxin-receptor binding activity of the fractions (Figure 3) was determined in addition to their chemical composition. The five fractions collected were fraction I, light aliphatic hydrocarbons; fraction II, heavy aliphatic hydrocarbons and PAC; fraction III, nitro-PAC; fraction IV, dinitro-PAC and quinones; and fraction V, polar material.

Dioxin-receptor binding activity was elicited by all the fractions of the urban air particulate extract. The highest dioxin-receptor binding activity was associated with fraction II, which contains the majority of PACs, members of which are presumed to be responsible for the observed receptor binding. Considerable activity was also observed in fraction IV, which contains more polar dinitro- and oxygen-containing PACs. However, the chemical identity of the compounds that contribute to the activity of this fraction is not known.

Somewhat similar results were seen for the gasoline emission extract. The highest dioxin-receptor binding activity was observed again in fraction II, with some activity also in the later fractions. If it is assumed that the PACs determined in fraction II with potential affinity for the dioxin receptor have the same binding affinity as benzo[a]pyrene, most of the observed receptor binding competition in this fraction can be accounted for. In contrast, the considerable activity present in fraction III could not be explained on the basis of those receptor ligands such as fluorone derivatives determined to be present. Possible candidates are halogenated derivatives of oxygenated PAC (23).

In the case of the diesel emission extract, more than 85% of the measured activity was present in fraction III, which contains the majority of nitro-PACs. Fraction II elicited only approximately 10% of the determined dioxin-receptor binding competition, the remaining minor activity was in fraction IV.

Recently, fraction II has been fractionated further on the basis of molecular size. This took place in initial experiments designed to determine more precisely which compounds in these extracts are responsible for the observed dioxin-receptor binding competition (26). Fraction II was divided into seven subfractions (Table 2). Although more than 90% of the PACs detected in the subfractions was found in fractions II-1 to II-4, the greatest dioxin-receptor binding competition was seen in fractions II-5 and II-6. Although these two fractions contain PACs that are a size expected of good dioxin-receptor ligands (27), it is not known whether the PACs

**Table 2.** Dioxin receptor affinity test IC$_{50}$ (mg/ml) of the subfractions.

| Subfraction | IC$_{50}$ (mg/ml) |
|-------------|------------------|
| Fraction II-1 | >66              |
| Fraction II-2 | 27               |
| Fraction II-3 | 6                |
| Fraction II-4 | 1.8              |
| Fraction II-5 | 0.8              |
| Fraction II-6 | 1.1              |
| Fraction II-7 | 6.2              |

*IC$_{50}$ values are expressed as mg/ml of original particulate material.*
present in these fractions account for the observed competition.

The Impact of Fuel on Diesel Exhaust Emissions

A large study to investigate the impact of fuel on the chemical composition and biological effects of diesel exhaust emissions has been carried out (28). As part of this study, the ability of particulate and semivolatile phase exhaust emission extracts competing for dioxin-receptor binding was determined for eight fuels. Each fuel was tested in two vehicle types, a bus and a truck (28). Diesel fuel D6 represented commercially available diesel at fuel stations in Sweden.

Figure 4 shows the IC$_{50}$ values for competition obtained from particulate extracts from burning different fuels in the bus. The IC$_{50}$ values varied over an approximate 8-fold range. Because of large variation about the mean, the IC$_{50}$ calculated for fuel D7 was not significantly different from that of any other fuel. Insufficient sample prohibited further estimations, and D7 has been excluded from the following comparisons. Fuel D1 displayed the lowest competition in the binding assay with the highest (p<0.01) IC$_{50}$ of 0.24 m driving distance/ml. Fuel D6 produced the emission sample with the highest binding competition with the lowest IC$_{50}$ of 0.04 m/ml. This IC$_{50}$ was significantly lower than those for fuels D1, D2, D5 (p<0.01), and D8 (p<0.05), but it was not significantly different from those of the remaining fuels.

The IC$_{50}$ values for dioxin-receptor binding competition of the particulate phase exhaust emissions from the truck are shown in Figure 5. It was observed that fuel D6 once again produced the emission with the highest receptor binding affinity with an IC$_{50}$ of 0.04 m driving distance/ml. In contrast to that seen with the bus, there were no significant differences between any of the fuels with regard to the receptor binding competition IC$_{50}$ of their emissions from the truck. However, only a 2-fold variation in IC$_{50}$ values was observed in the samples from the truck. The IC$_{50}$ values for dioxin-receptor binding competition of particulate associated emission products in this study compare favorably with those determined in previous studies of both diesel- and gasoline-fueled exhaust emissions.

From the data derived from the bus experiment where there was a greater spread in the IC$_{50}$, it was observed that there was a gross correlation between the affinity of an exhaust sample for the dioxin receptor and the PAC content of the sample for the original fuel sample. Fuels with high PAC content gave rise to particulate emissions with high PAC contents and high dioxin-receptor binding activities and vice versa; fuels with low PAC content gave rise to emissions with lower PAC contents and lower dioxin-receptor binding activities. This observation is not surprising because it is generally believed that compounds of the PAC-type are responsible for the binding activities. All fuel and emission data were subjected to multivariate analysis in this study (28). This analysis revealed that a major factor correlating with the biological effect of emissions (dioxin-receptor binding, Ames test) was the PAC content of the fuel. However, it is not certain which compounds are responsible for the dioxin-receptor binding activity elicited by vehicle exhaust particulate extracts. It is probably not because of dibenzo-p-dioxins or related compounds because the levels of these compounds are too low to produce the observed receptor binding activities (29).

The IC$_{50}$ values for the dioxin-receptor binding competition of the semivolatile phase exhaust emissions from the bus are shown in Figure 6. Dioxin receptor binding activity was observed in all the samples except that from fuel D7 (IC$_{50}$>0.5 m/ml). Fuel D6 gave rise to the semivolatile phase emission with the highest affinity for the dioxin receptor with the lowest IC$_{50}$ of 0.02 m/ml. With the exception of the sample from fuel D1, the dioxin-receptor binding activities of the semivolatile phase emissions were similar or lower than those of the corresponding particulate-phase emissions.

This study demonstrates the presence of significant amounts of compounds with affinity for the dioxin receptor in the semivolatile
phase of diesel exhaust emissions. This was a surprising result because it was expected that compounds emitted in this phase would be of lower molecular weight and size than good ligands to the dioxin receptor. The majority of any ligand of the correct size was expected to appear in the particulate phase. It has been shown that, characteristically, good ligands for the dioxin receptor are planar molecules that, when their atomic van der Waals radii are included, fit into a rectangle of $6.8 \times 13.7$ Å (27). Such compounds might be expected to have a low volatility and then would appear to a much greater degree in the particulate phase. Indeed, the majority of larger PACs were found in the particulate phase, whereas the smaller 2- and 3-ringed PACs were mainly in the semivolatile phase. It has been suggested, based on mathematical modeling of particle/vapor partitioning, that possibly 20 to 60% of TCDD in ambient air would exist in the vapor phase (30). Indeed, it has been reported that tetra- and pentachlorinated dioxins and dibenzofurans pass through glass fiber filters during urban air sampling (31). It will be of interest to identify those compounds present in the semivolatile phase with affinity for the dioxin receptor.

**Discussion**

Extracts of particulate material collected from urban air have been shown to contain compounds capable of binding to the dioxin receptor. Furthermore, these extracts have been shown to be capable of eliciting a receptor-mediated biological effect in cultured cells, namely, the induction of AHH activity. Thus, it is demonstrated that compounds present in these extracts have the potential to elicit the dioxin-receptor mediated effects discussed above.

It is not fully known which compounds in these extracts are responsible for the observed dioxin-receptor binding. Initial studies have shown that for extracts of ambient air particulates only 1 to 30% of the measured dioxin-receptor binding activity could be accounted for and that perhaps nitro-compounds do not contribute in any major way. A number of urban air particulate samples were collected in the presence of increased concentrations of reactive gases to investigate the effect of chemical transformation on dioxin-receptor binding and mutagenicity (21). Studies had shown that certain nitro-PACs have high affinities for the dioxin receptor (32). However, the presence of increased concentrations of nitrogen dioxide, nitrous acid, nitric acid, or ozone had no effect on the dioxin-receptor binding activities determined. Thus, although nitro-PACs are present in these extracts, they do not appear to be the major dioxin-receptor binding species in extracts of urban air particulate samples.

Although most of the receptor binding associated with the major PAC-containing fraction in the case of a fractionated particulate extract from gasoline emissions could be accounted for, a substantial amount of receptor binding activity present in the other fractions could not be accounted for. In contrast to what is seen for ambient air particulates, it would appear that for diesel particulate emission extracts the large majority of dioxin-receptor binding activity resides with nitro-derivatives of the PAC. A comparison of gasoline and diesel exhaust emissions with respect to nitro-PAC shows that the latter contains 100- to 1000-fold more of these compounds (33,34). Moreover, a number of mono-nitro-PACs have been shown to bind with high affinities to the dioxin receptor (29), and nitro-PACs have been shown to cause induction of AHH activity (35). This may account for the different profiles of receptor binding across the fractions for gasoline and diesel exhaust emissions. Which of these compounds elicits the dioxin-receptor affinity associated with the environmental samples is, however, primarily unknown. Thus, more detailed fractionation studies are required to elucidate the most potent dioxin-receptor ligands in these extracts. Then we would know on which compounds to focus future toxicity and risk assessment studies.

Finding substantial dioxin-receptor binding activity in the semivolatile phase of diesel-fueled vehicle emissions is important. By concentrating our efforts on particulate-associated PAC and their derivatives, we may be underestimating the burden of these compounds in our environment grossly. It will be of interest to determine the identity of the dioxin-receptor ligands in the semivolatile phase.

As the dioxin-receptor binding assay gives results that can be related to a given amount of TCDD, it is possible to express the results obtained with complex samples as TCDD equivalents. This method of expressing results has been validated for use with complex mixtures of halogenated-PACs (36). However, as the nonhalogenated PACs are metabolized and cleared from the body much more rapidly, using TCDD equivalents probably has little relevance to estimating the potential toxicity of mixtures of these compounds. The use of a model nonhalogenated PAC as a standard against which to compare potencies would be of greater relevance in this case. More information concerning the dioxin-receptor binding affinities and the toxicities of PACs occurring in association with urban particulate matter is required before a suitable prototype compound can be chosen.

In conclusion, the dioxin-receptor binding assay is a useful tool for screening samples for potentially toxic compounds acting through the dioxin receptor. It also compliments other short-term toxicity tests that detect potential toxins with differing mechanisms of action.

### REFERENCES

1. Gelboin HV. Benzo[a]pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. Physiol Rev 60:1107-1166 (1980).
2. Weinstein IB, Jeffrey AM, Leffler S, Pulkarabek P, Yamaski H, Grunberger D. Interactions between polycyclic aromatic hydrocarbons and cellular macromolecules. In: Polycyclic Aromatic Hydrocarbons and Cancer, Vol 2. Molecular and Cell Biology (Gelboin HV, Ts'o POP, eds). New York: Academic Press, 1978:4-36.
3. Nebert DW, Adensnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R, Waterman MR. The P450 gene superfamily: recommended nomenclature. DNA 6:11-11 (1987).
4. Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polyaromatic hydrocarbons. Cancer Res 42:4875-4917 (1982).
5. Nebert DW, Eisen HJ, Negishi M, Lang MA, Hjelmeland LM, Okey AB. Genetic mechanisms controlling the induction of poly-substrate monoxygenase (P450) activities. Annu Rev Pharmacol Toxicol 21:431-462 (1981).
6. Israel DI, Whitlock JP Jr. Regulation of cytochrome P450 gene transcription by 2,3,7,8-tetrachlorodibenzo-p-dioxin in wild type and variant mouse hepatoma cells. J Biol Chem 259:5400-5402 (1984).
7. Gonzales FJ, Tukey RH, Nebert DW. Structural gene products of the Ah locus. Transcriptional regulation of cytochrome P450 and
P450 mRNA levels by 3-methylcholanthrene. Mol Pharmacol 26:117–121 (1984).
8. Tukey RH, Nebert DW, Negishi M. Structural gene product of the (Ah) complex. Evidence for transcriptional control of cytochrome P450 induction by use of a cloned DNA sequence. J Biol Chem 256:6969–6974 (1981).
9. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 22:517–554 (1982).
10. Poland A, Glover E, Kende AS. Stereosepecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is the receptor for the induction of aryl hydrocarbon hydroxylase. J Biol Chem 251:4936–4946 (1976).
11. Tukey RH, Hannah RR, Negishi M, Nebert DW, Eisen HJ. The Ah locus: correlation of intranuclear appearance of inducer-receptor complex with induction of cytochrome P450 mRNA. Cell 31:275–284 (1982).
12. Whitlock JP Jr. The regulation of cytochrome P450 gene expression. Annu Rev Pharmacol Toxicol 26:333–369 (1986).
13. Mason G, Sawyer T, Keys B, Bandiera S, Romkes M, Piskorska-Pliszczynska J, Zmudzka B, Safe S. Polychlorinated dibenzo-furans (PCDFs): correlation between in vivo and in vitro structure-activity relationships. Toxicology 37:1–12 (1985).
14. Mason G, Farrell K, Keys B, Piskorska-Pliszczynska J, Safe L, Safe S. Polychlorinated dibeno-p-dioxins: quantitative in vitro and in vivo structure-activity relationships. Toxicology 41:21–31 (1986).
15. Safe S. Comparative toxicology and mechanism of action of polychlorinated dibeno-p-dioxins and dibenzofurans. Annu Rev Pharmacol Toxicol 26:371–399 (1986).
16. Rhewald JG, Green H. Formation of keratinizing epithelium in a culture by a cell line derived from a teratoma. Cell 6:317–330 (1975).
17. Knutson JC, Poland AP. Keratinization of mouse teratoma cell line XB produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin: an in vitro model of toxicity. Cell 22:27–36 (1980).
18. Osborne R, Greenlee WF, 2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal-cells. Toxicol Appl Pharmacol 77:434–443 (1985).
19. Poland A, Glover E, 2,3,7,8-Tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Mol Pharmacol 17:86–94 (1980).
20. Poellinger L, Lund J, Dahlberg E, Gustafsson J-Å. A hydroxylapatite microassay for receptor binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3-methylcholanthrene in various target tissues. Anal Biochem 144:371–384 (1985).
21. Toftgård R, Löfroth G, Carlstedt-Duke J, Kur L, Gustafsson J-Å. Compounds in urban air compete with 2,3,7,8-tetrachlorodibenzo-p-dioxin for binding to the receptor protein. Chem Biol Interact 46:335–346 (1983).
22. Franžén B, Haaparanta T, Gustafsson J-Å, Toftgård R. TCDD receptor ligands present in extracts of urban air particulate matter induce aryl hydrocarbon hydroxylase activity and cytochrome P450c gene expression in rat hepatoma cells. Carcinogenesis 9:111–115 (1988).
23. Al sb erg T, Stenberg U, Westerholm R, Strandell M, Rannug U, Sundvall A, Romert L, Berson V, Pettersson B, Toftgård R, Franžén B, Jansson M, Gustafsson J-Å, Egebäck K-E, Teije G. Chemical and biological characterization of organic material from gasoline exhaust particles. Environ Sci Technol 19:43–50 (1985).
24. Al sb erg T. ed. Swedish Environmental Protection Agency Report No. 3680. Solna, Sweden: Swedish Environmental Protection Agency, 1989.
25. Löfroth G. ed. Swedish Environmental Protection Agency Report No. 3841. Solna, Sweden: Swedish Environmental Protection Agency, 1990.
26. Mason GGF, Gustafsson J-Å, Li H, Westerholm R. Chemical fractionation of particulate extracts from diesel vehicle exhaust: distribution of ligands for the dioxin receptor. Environ Sci Technol (in press).
27. Gillner M, Bergman J, Cambillau C, Fernström B, Gustafsson J-Å. Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. Mol Pharmacol 28:357–363 (1985).
28. Westerholm R, Egebäck K-E, eds. Swedish Environmental Protection Agency Report No. 3968. Solna, Sweden: Swedish Environmental Protection Agency, 1991.
29. Toftgård R, Franžén B, Gustafsson J-Å, Löfroth G. Characterization of TCDD receptor ligands present in extracts of urban particulate matter. Environ Int 11:369–374 (1985).
30. Bidelman TF. Atmospheric processes. Environ Sci Technol 22:361–387 (1988).
31. Eitzer BD, Hites RA. Concentrations of dioxins and dibenzofurans in the atmosphere. Int J Environ Anal Chem 27:215–230 (1986).
32. Greibrokk T, Löfroth G, Nilsson L, Toftgård R, Carlstedt-Duke J, Gustafsson J-Å. Nitroarenes: mutagenicity in the Ames Salmonella/microsome assay and affinity to the TCDD-receptor protein. In: The Toxicity of Nitroaromatic Compounds (Rickert DE, ed). Washington:Hemisphere Publishing, 1985:167–183.
33. Westerholm RN, Alméen J, Li H, Rannug UJ, Egebäck K-E, Grägg K. Chemical and biological characterization of particulate-phase-associated, semivolatile-phase-associated, and gas-phase-associated compounds in diluted heavy-duty diesel exhaust: a comparison of three different semivolatile-phase samplers. Environ Sci Technol 25:332–339 (1991).
34. Westerholm R, Alméen J, Li H, Rannug U, Rosén Å. Exhaust emissions from gasoline fuelled light duty vehicles operated in different driving conditions: a chemical and biological characterization. Atmos Environ (in press).
35. Åström A, Birberg W, Pilotti Å, DePierre JW. Induction of different isozymes of cytochrome P450 and of microsomal epoxide hydroxylase in rat liver by 2-acetylaminofluorene and structurally related compounds. Eur J Biochem 154:125–134 (1986).
36. Safe S, Mason G, Sawyer T, Zacherek W, Harris M, Yao C, Keys B, Farrell K, Holcomb H, Davis D, Safe L, Piskorska-Pliszczynska J, Leece B, Denommme M, Hutzinger O, Thoma H, Chittim B, Madge J. Development and validation of in vitro induction assays for toxic halogenated aromatic mixtures: a review. Toxicol Ind Health 5:757–775 (1989).