Lethality and EROD-inducing potency of chlorinated chrysene in chick embryos.

Gustafsson, E.[A], Brunström, B.[A], Nilsson, U.[B]
[A] Department of Zoophysiology, Norbyvägen 18 A, 752 36 Uppsala, Sweden
[B] Division of Analytical Chemistry, National Institute of Occupational Health, 171 84 Solna, Sweden

Introduction

Chlorinated polycylic aromatic hydrocarbons (Cl-PAHs) are compounds consisting of three or more fused aromatic rings, having one or more chlorine atoms attached to the aromatic ring system. The chlorine can be introduced into the molecule either by substitution or addition. The chloro-added PAHs are highly unstable and reactive and have not yet been detected in environmental samples. Chloro-substituted PAHs are more stable than the parent compounds and have, for instance, been detected in automobile exhaust[1], urban air[1-3], and emissions from municipal waste incinerators[4].
The toxicity of Cl-PAHs has not been extensively studied. However, both chloro-added and chloro-substituted PAHs have been shown to be mutagenic[5-8]. 6-Chlorochrysene and 7-chlorobenz(*a*)anthracene were shown to bind to the Ah-receptor[9] and 6-chlorochrysene induced aryl hydrocarbon hydroxylase (AHH) in rat hepatoma cells *in vitro*[10].
In the present study, the toxic potency of a mixture of chloro-derivatives of chrysene was compared with that of the parent compound. Lethality and induction of 7-ethoxyresorufin *O*-deethylase (EROD) were studied in 72-hour (air-sac injection) and 2-week (yolk-sac injection) toxicity tests in chick embryos.

Methods

Chlorination of chrysene: Chrysene was non-specifically chlorinated by adding gaseous chloride to a solution of chrysene in carbon tetrachloride. The reaction was carried out at room temperature for 20 hours, giving a mixture of non-, mono-, di-, and trichlorinated chrysene in relative amounts of 24, 32, 42 and 2%, respectively (analysis by gas chromatography and mass spectrometry).

Egg injection: For injection into the yolks of fertilized eggs, the compounds were dissolved in an emulsion of peanut oil and lecithin in water. An amount of 100 µl of emulsion was injected into eggs preincubated for 4 days. After fourteen days, mortality rates were determined and livers were removed for determination of EROD activities. For air-sac injections, the compounds were dissolved in peanut oil. An amount of 50 µl was injected into eggs which had been preincubated for 7 days. Seventy-two hours later, mortality rates were determined and livers were removed for determination of EROD activities.

EROD assay: EROD activities of the 18-day-old embryos were determined in liver microsomes, whereas whole homogenates were used for determination of EROD activities in livers from the 10-day-old embryos. EROD was measured using the method described by Pohl and Fouts[11].

Results

In the 2-week test, the mixture of chlorinated chrysene proved to be more embryolethal than chrysene (Table 1). A dose of 2 mg/kg egg of chrysene did not cause an increase in mortality, whereas the same dose of chlorinated chrysene caused 80% mortality. Four embryos were alive by day 18 after treatment with 2 mg/kg of chlorinated chrysene. These embryos exhibited anomalies including pericardial edema (4/4), degenerative hepatic lesions (4/4), shortened beak (3/4), microphthalmia (1/4) and subcutaneous edema (1/4). Also treatment with 0.6 and 0.2 mg/kg of chlorinated chrysene caused pericardial edema (6/15 and 3/13, respectively) and hepatic lesions (13/15 and 11/13, respectively). In embryos treated with 2 mg/kg of chrysene, some cases of pericardial edemas (2/17) and hepatic lesions (5/17) were noted. No anomalies were found in the control embryos. EROD was induced about ten-fold by treatment with 0.6 mg chlorinated chrysene per kg, whereas 2 mg/kg of chrysene did not enhance EROD in comparison with the control value (Table 1).

Table 1. Mortality rates and hepatic EROD activities in chick embryos treated with chrysene and chlorinated chrysene. The substances were injected into the yolks of eggs preincubated for 4 days and mortality rates and enzyme activities were determined on day 18. EROD activities are presented as mean ± S.D. for 8 livers.

Substance	Dose (mg/kg egg)	Embryonic mortality Ratio (%)	EROD activity (pmol/mg liver per min)
Control	-	3/20 (15)	81 ± 38
Chrysene	2.0	3/20 (15)	85 ± 37
Clorinated chrysene	0.2	7/20 (35)	695 ± 176[a]
	0.6	5/20 (25)	886 ± 226[a]
	2.0	16/20 (80)[b]	

[a] Significantly different from the control value (Student's *t* test, $P<0.0001$).
[b] Significantly different from the control value (X^2-test, one-tailed, $P<0.0001$).

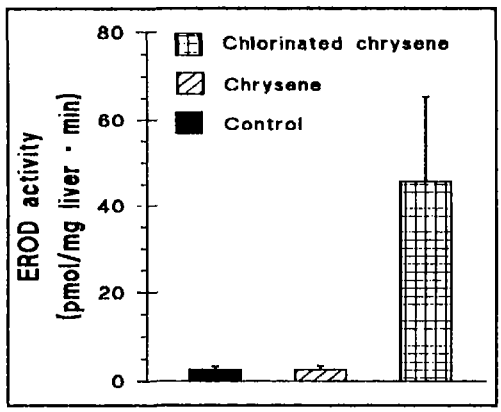

Fig. 1
Hepatic EROD activities of chick embryos treated with chrysene and chlorinated chrysene. A dose of 0.1 mg/kg egg was injected into the air sacs of eggs preincubated for 7 days and EROD was determined 72 hours later. EROD activities are presented as mean ± S.D. for 5-6 livers.

In the 72-h test, the LD_{50} of the chlorinated chrysene was determined to be about 0.13 mg/kg egg. A dose of 0.1 mg/kg induced EROD about 17 times, whereas the same dose of chrysene did not enhance EROD activity (Fig 1).

Discussion

The chlorinated chrysene was considerably more potent than the parent compound in terms of embryolethality in the 2-week test and EROD-induction in the 2-week and 72-hour tests, whereas the embryolethality of the chlorinated mixture in the 72-hour test was similar to that previously found for chrysene[12]. This discrepancy might be due to a higher rate of biotransformation of chrysene than of the chlorinated mixture. Similar differences in effect in these test systems have previously been found between PAHs and more persistent compounds such as polychlorinated biphenyls (PCBs)[13].
The LD_{50} for chlorinated chrysene in the 72-hour test was approximately 15 times lower than that previously found for 2,3,3',4,4'-pentachlorobiphenyl (IUPAC #105)[14] and similar to that for the coplanar PCB 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC #169)[15]. In the 2-week test, the mixture of chlorinated chrysene was about as lethal as PCB #105[14] but less potent than PCB #169[15]. The anomalies caused by chlorinated chrysene were similar to those previously found after treatment of chick embryos with coplanar PCBs[15,16]. Chlorinated chrysene also proved to be as potent an inducer of EROD as some of the most potent PCBs. It thus seems that the mechanism of toxicity of chlorinated chrysene in chick embryos is similar to that of the coplanar PCBs and other Ah receptor ligands.

Conclusions

In this study we showed that non-specific chlorination of chrysene resulted in products having toxic effects similar to those of previously studied Ah receptor ligands. In the

test systems used, the toxic potency of the chlorinated mixture was similar to that of highly toxic PCBs. The fact that numerous different chlorinated PAHs can be formed in industrial processes and at combustion in combination with the results presented in this paper supports the need to further study the toxicity of these compounds and their levels in the environment.

Acknowledgements
This study was supported financially by the Swedish Environmental Protection Agency.

References

1 Haglund P, Alsberg T, Bergman Å, Jansson B. Analysis of halogenated polycyclic aromatic hydrocarbons in urban air, snow and in automobile exhaust. *Chemosphere* 1987;16:2441-50.
2 Östman C, Nilsson U. Coupled LC-GC-MC for on-line clean-up, separation and identification of chlorinated polycyclic aromatic hydrocarbons at pg levels in urban air. *HRC* 1992;15:745-50.
3 Nilsson U, Östman C. Chlorinated polycyclic aromatic hydrocarbons: Method of analysis and their occurrence in urban air. *Environ Sci Technol* 1993; in press.
4 Oehme M, Manø S, Mikalsen A. Formation and presence of polyhalogenated and polycyclic compounds in the emissions of small and large scale municipal waste incinerators. *Chemosphere* 1987;16:143-53.
5 Löfroth G, Nilsson L, Agurell E, Sugiyama T. Salmonella/microsome mutagenicity of monochloro derivatives of some di-, tri- and tetracyclic aromatic hydrocarbons. *Mut Res* 1985;155:91-4.
6 Rannug U, Nilsson U, Colmsjö A. The mutagenicity and chemical structure of certain chloroderivatives of polynuclear aromatic hydrocarbons. In: Ramel C, Lambert B, Magnusson L, eds. *Genetic effects and applied mutagenesis.* New York: Alan R. Liss Inc., 1986:157-64.
7 Bhatia AL, Tausch H, Stehlik G. Mutagenicity of chlorinated polycyclic aromatic compounds. *Ecotoxicol Environ Saf* 1987;14:48-55.
8 Colmsjö A, Nilsson U, Rannug A, Rannug U. The synthesis and mutagenicity of chloroderivatives of pyrene. In: Cooke M, Dennis AJ, eds. *Polynuclear aromatic hydrocarbons: A decade of progress.* Columbus, Ohio: Battelle Press, 1988:147-53.
9 Toftgård R, Franzén B, Gustafsson JÅ, Löfroth G. Characterization of TCDD-receptor ligands present in extracts of urban particulate matter. *Environ Int* 1985;11:369-74.
10 Franzén B, Haaparanta T, Gustafsson JÅ, Toftgård R. TCDD-receptor ligands present in extracts of urban air particulate matter induce aryl hydrocarbon hydroxylase activity and cytochrome P-450c gene expression in rat hepatoma cells. *Carcinogenesis* 1988;9:111-5.
11 Pohl RJ, Fouts JR. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal Biochem* 1980;107:150-5
12 Brunström B, Broman D, Näf C. Toxicity and EROD-inducing potency of 24 polycyclic aromatic hydrocarbons (PAHs) in chick embryos. *Arch Toxicol* 1991;65:485-9.
13 Brunström B. Embryolethality and induction of 7-ethoxyresorufin *O*-deethylase in chick embryos by polychlorinated biphenyls and polycyclic aromatic hydrocarbons having Ah receptor affinity. *Chem-Biol Interactions* 1992;81:69-77.
14 Brunström B. Mono-*ortho*-chlorinated chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin *O*-deethylase (EROD) activity in chick embryos. *Arch Toxicol* 1990;64:188-92.
15 Brunström B, Andersson L. Toxicity and 7-ethoxyresorufin *O*-deethylase-inducing potency of coplanar polychlorinated biphenlys (PCBs) in chick embryos. *Arch Toxicol* 1988;62:263-6.
16 Brunström B, Darnerud PO. Toxicity and distribution in chick embryos of 3,3',4,4'-tetrachlorobiphenyl injected into the eggs. *Toxicology* 1983;27:103-10.