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Biological Function of Xenobiotics through Protein Binding and Transportation in Living Cells

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Abstract: The main objective of this study is to find out how xenobiotics (especially pesticides) bind to special protein and transported through the main living cell to reach its targeting. Because pesticides residues exists in foods, water, plants and underground then transported to human through the above sources, a lot of disease were detected in high intensity in the population in different countries. Therefore, research carried out to find out relationship between pesticides contamination in human breast milk and animal serum and protein profiles. Pollution with xenobiotics induce different form of cytochrome P-450 in human liver and directly accelerate the rate of α-synuclein fibril formation in living cell. Therefore, our investigation concern to find out the relationship between xenobiotics and protein binding and transportation in living cell. Breast milk and rat serum proteins as well as fish were used as examples of pollution materials with xenobiotics to find out the above relation.

Key words: Xenobiotics, protein binding, pesticides, breast milk, rat serum protein pollution

INTRODUCTION

To fulfill and find out the relationship between xenobiotics and proteins involved during transportation in living cell , we start first to define xenobiotics and transport proteins and the possibility xenobiotic-protein Interactions in living cell.

Definition of Xenobiotic

A xenobiotic is a chemical which is found in an organism but which is not normally produced or expected to be present in it. It can also cover substances which are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal diet. Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defences produced by some organisms as protection against predators. However, the term xenobiotic is very often used in the context of pollutants such as dioxins and polychlorinated biphenyls and their effect on the biometabolism, because xenobiotics are understood as substances foreign to an entire biological system, i.e., artificial substances, which did not exist in nature before their synthesis by humans.

Xenobiotics in the Environment

Xenobiotics substances are becoming an increasingly large problem in sewage treatment systems, since they are relatively new substances and are very difficult to categorize. Antibiotics, for example, were derived from plants originally and so mimic naturally occurring substances. This, along with the natural monopoly nature of municipal waste water treatment plants makes it nearly impossible to remove this new pollutant load. Some xenobiotics are resistant to degradation ,for example, they may be synthetic organochlorides such as plastics and pesticides, or naturally occurring organic chemicals such as polyaromatic hydrocarbons (PAHs) and some fractions of crude oil and coal. However, it is believed that microorganisms are capable of degrading all the different complex and resistant xenobiotics found on the earth.

Xenobiotic-Protein Interactions

Both the beneficial and harmful effects of xenobiotics are determined by xenobiotic-protein interactions or how xenobiotic and living cell organisms react to each other. To do its job, a xenobiotic must: 1) penetrate the organism, 2) move or be transported to the site of action and 3) there disrupt or alter the vital function.

Protein Involved During Xenobiotics Transportation

Transport Protein

Membrane transport protein (or simply transporter) is a protein involved in the movement of ions, small molecules, or macromolecules, such as another protein across a biological membrane. Transport proteins are integral membrane proteins; that is they exist within and span the membrane across which they transport substances. The proteins may assist in the movement of substances by facilitated diffusion protein or active transport. The mechanism of action of these proteins is known as carrier-mediated transport. There are two forms of carrier-mediated transport, active transport and facilitated diffusion (Crompton, 1999).

Facilitated diffusion Protein speeds the movement of a chemical through a membrane in the absence of energy input; therefore, the transported chemical can move only down a concentration gradient. This can be accomplished by the formation of a high-specificity pore or channel that spans the membrane. These polar "holes" through the membrane are lined by specific amino acids residues that lower the energy barrier to the movement of polar molecules (Fig. 1).

Now we will deal with some specific proteins involved in binding with xenobiotic especially pesticides and transportation of its for targeting and function of xenobiotic.

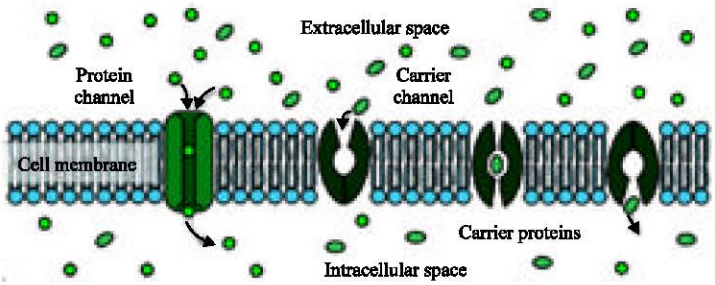

Fig. 1: Transportation of xenobiotics by facilitated diffusion protein

Identification of an Inducible Form of Cytochrome P-450 in Human Liver

The cytochromes P-450 are a family of hemoproteins, abundant in the endoplasmic reticulum of the hepatocyte, that catalyze the oxidative metabolism of many drugs, environmental chemicals and endogenous compounds (Watkins *et al*., 1985, Popper *et al*., 1979). An important characteristic of some of the forms of cytochrome P-450 is that they are inducible. For example, different forms of liver cytochrome P-450 accumulate in rats treated by a member of one of three classes of inducers (Guengerich *et al*., 1982) typified, respectively, by phenobarbital (P-450b, P-450e), 3-methylcholanthrene (P-450c, P-450d) and pregnenolone-16a-carbonitrile (PCN) (P-450p). Since, the amounts and types of cytochromes P-450 in the liver may be rate-limiting for metabolism of foreign chemicals, enzyme induction may play an important role in such clinically relevant phenomena as interactions among therapeutic drugs, Mayer *et al*., 1980) metabolic idiosyncrasy in hepatotoxic drug reactions (Davies, 1981) and inter individual differences in susceptibility to toxic effects of environmental chemicals (Kouri *et al*., 1982). There is abundant, albeit indirect, evidence that human liver also contains cytochromes P-450 that are inducible. For example, exposure of humans to inducers of animal cytochromes P-450 including such drugs as phenobarbital (Kellermann and Luyten-Kellermann, 1977), macrolide antibiotics (Ohnhaus and Park, 1979; Ohnhaus *et al*., 1983), or environmental chemicals such as organochlorine pesticides (Kolmodin-Hedman, 1974) or polychlorinated biphenyls (Alvares *et al*., 1977), accelerates the disappearance of administered substrates for the cytochromes P-450 from the blood or the appearance of metabolites of such model drugs in the breath (Henry *et al*., 1979). Such patients may also exhibit increased urinary excretion of metabolites of endogenous substrates such as 6,8-hydroxy derivatives of cortisol (Saenger *et al*., 1981). Additional evidence for liver enzyme induction in humans are proliferation of the smooth endoplasmic reticulum in hepatocytes, as judged by electron microscopic examination of liver biopsies (Pamperl *et al*., 1984), increased urinary excretion of glucaric acid (a breakdown product of a constituent of the endoplasmic reticulum) (Hunter *et al*., 1971) and increased concentration of CO-binding hemoprotein or drug oxidizing activities in liver microsomes prepared from such patients (Schoene *et al*., 1972). However, although it has been possible to purify at least six individual polypeptide forms of human liver cytochrome P-450 (Wang *et al*., 1983), there has to date been no clear evidence which, if any, of these cytochromes are inducible.

Interactions of Pesticides with the Estrogen-binding Protein in Rat

The binding of 3H-estradiol to testicular cytosol was inhibited by o,p-DDT, a DDT analog which is estrogenic in the intact female, but not by p,p-DDE which is a nonestrogen in the female. The pesticide methoxychlor, which is estrogenic in vivo in the female, failed to inhibit 3H-estradiol binding, presumably requiring metabolic activation for binding to the testicular cytosol. In fact, its di-demethylated metabolite 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), also estrogenic in vivo, caused marked suppression of 3H-estradiol binding (Bulger *et al*., 1978). Bulger *et al*. (1981) stated that by using Laboratory grade methoxychlor (99% pure), base-washed methoxychlor and a metabolite of methoxychlor, 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), were tested for their ability to compete with [3 H] estradiol-17 beta ([3 H]E2) for specific binding to the estrogen receptor from immature rat uterine cytosol. The effect of o,p-DDT on the binding of 3H-estradiol to DMBA-induced rat mammary tumor cytosolic estrogen-binding protein (EBP) was examined in vitro. Scatchard plot analysis indicated that o,p'-DDT displaced estradiol from specific 4S and 8S

proteins. As estrogens have been shown to affect the development and growth of these tumors, this experimental findings suggest that o,p-DDT may possibly influence DMBA-induced tumors in an estrogenic manner (Mason and Schulte, 1983).

Isolation of Pesticide-Binding Protein from Rat Blood

Rats were given a single oral dose of the herbicide propachlor-[1-^{14}C] (8×10^{6} d.p.m., 10.3 mg). The plasma, erythrocyte cytosol and erythrocyte ghosts (collected 90 min after dosing) contained 41, 15 and 28%, respectively, of the ^{14}C in the blood (0.5% of the ^{14}C dose). Plasma, erythrocyte cytosol and erythrocyte ghost were found to contain protein(s) 13.4 to 13.9 kDa (MW) which were associated with the ^{14}C (2.4% of ^{14}C in plasma; 51% of ^{14}C in erythrocyte cytosol and 65% of ^{14}C in erythrocyte ghosts). This ^{14}C associated with protein was extractable with methanol and was tentatively characterized by T.L.C. to be the cysteine conjugate (11%), the mercapturic acid conjugate (18%), *S*-oxide of the mercapturic acid conjugate (18%) and propachlor (25%). MW of the native 13.4-13.9 kDa protein(s) was found to be 43.5 kDa. Immunoblot or binding studies of the 13.4-13.9 kDa protein(s) showed no evidence that this protein(s) was liver or heart fatty-acid-binding-protein (FABP) or transthyretin (Larsen *et al*., 1994).

Transportation of Pesticides in Fish

A number of reports have appeared on the toxicity, uptake and tissue distribution of pesticides in a number of fishes (Guiney and Peterson, 1980; Kouudinya and Ramamurthi, 1979) studied the effect of Sevin on some hematological parameters in *Sarotherodon mossambicus*. Vijayalakshimi (1980) observed that sumithion reduced tissue respiration and oxygen consumption of the fish, *Etroplus maculatus*. Some amount of pesticides can enter the digestive system of fishes through the consumption of food chain organisms and pesticides present in water and in food material can adversely affect the processes of digestion of food material and absorption of nutrients by the intestine (Jarvinen and Tyo, 1978; Mac *et al*., 1979; Neimi and Cho, 1980). It is possible that pesticides entering the intestine of fishes either through water or food can reduce the rate of transport of nutrients. Among the three nutrients examined, the decrease in the rate of transport was maximum in case of tryptophan and the rate of uptake of fructose was more affected than that of glucose. The present study also shows that higher concentrations of Sevin decreased the rate of transport of the three nutrients to a greater extent than lower concentrations. Sastry and Siddiqui (1982) and Sastry and Sharma (1978) have also reported similar decrease in the rate of intestinal transport of glucose in *Channapunctatus* by endosulfan and quinalphos. Madge (1976) studied the absorption of amino acid and hexoses in mice treated with hexachlorobiphenyls and noted decrease in the transport rate. The decrease was attributed to either a carrier effect at the brush border membrane or impairment of cellular metabolism.

Pesticides Directly Accelerate the Rate of α-Synuclein Fibril Formation

Parkinson's disease involves intracellular deposits of α-synuclein in the form of Lewy bodies and Lewy neurites. The etiology of the disease is unknown, however, several epidemiological studies have implicated environmental factors, especially pesticides. Here we show that several pesticides, including rotenone, dieldrin and paraquat, induce a conformational change in α-synuclein and significantly accelerate the rate of formation of - α synuclein fibrils *in vitro*. They propose that the relatively hydrophobic pesticides preferentially bind to a partially folded intermediate conformation of α -synuclein, accounting for the observed conformational changes and leading to association and subsequent

fibrillation. These observations suggest one possible underlying molecular basis for Parkinsons disease. α-Synuclein, a relatively abundant brain protein of 140 amino acids and of unknown function, was first identified in association with synaptic vesicles (Maroteaux *et al*., 1988). α-Synuclein belongs to the class of proteins known as natively unfolded; i.e., the purified protein at neutral pH is substantially disordered (Uversky *et al*., 2001a). Fibrils of α -synuclein have been reported in Lewy bodies from individuals with Lewy body diseases, as well as *in vitro* (El-Agnaf *et al*., 1998; Narhi *et al*., 1999). We have recently established that the fibrillation of α-synuclein involves a critical partially folded intermediate (Uversky *et al*., 2001b). Here, we show that certain pesticides can significantly stimulate the formation of α-synuclein fibrils. Since, these agents also induce a conformational change in α-synuclein, that this partially folded conformation is a critical precursor to association and fibrillation.

In vitro Binding of Trichlorphenol, Fenvalerate and α-Endosulphan to Rat Serum Transferrin and Albumin for Biomonitoring of Pesticides Pollution

- **Objectives:** To study the changes in serum protein binding profile with pesticides and remarks any new protein bands after pesticides incubation in-vitro with rat serum as well as with individual proteins of transferrin and albumin. Animals Male albino rats obtained from animal house colony in Faculty of Agriculture (Afify *et al*., 2000)
- **Setting:** Biochemistry Department, Faculty of Agriculture, Cairo University, Cairo, Egypt
- **Main outcome measures:** Serum transferrin, albumin, prealbumin and small molecular weight proteins
- **Results:** Electrophoretic separation of the protein subunits of rat serum treated with different pesticides showed that these pesticides have high affinity to transferring, albumin as well as high molecular weight proteins. The increase in the intensity of transferrin was occurred with trichlorophenol and α-endosulphan. On the other hand, the intensity of the albumin fraction was decreased with fenvalerate, while it is markedly increased with trichlorophenol and α-endosulphan. The individual incubation of each pesticide with transferrin,, albumin or prealbumin showed that trichlorophenol and α -endosulphan was found to cause aggregation of transferring by 49.1 and 43.9%, respectively, while fenvalerate was found to cause marked disintegration of transferrin as compared to controls. The albumin fraction was significantly decreased with the three pesticides. The Pre-albumin was found to Markedly increased in its Intensity by 44.8 and 57.3% with Trichlorophenol (5 ppm) and α-endosulphan (15 ppm), respectively
- **Conclusion:** The results of the current study indicated that several protein bands have responded to pesticides treatment including the known serum proteins, transferrin, albumin, prealbumin and small molecular weigh proteins. However, some of the small molecular weights proteins have been identified as results of pesticides binding which require further characterization. Therefore, the detection of serum proteins after electrophoresis is considered a very good diagnostic parameter for biomonitoring of pesticides pollution studies (Saleh *et al*., 1996b; Afify *et al*., 1997)

Table 1 showed that scanning of electrophoretic profiles of rat serum incubated with different concentration of pesticides 5, 10, 15 and 20 ppm with trichlorophenol, fenvalerate and α-endosulphan.

Table 2 showed that scanning of electrophoretic profiles of Transferring and albumin incubated with different concentration of pesticides 5, 10 and 15 ppm.

Table 1: Scanning electrophoretic pattern of rat serum protein subunits treated with trichlorophenol, fenvalerate and α-endosulphan pesticides

	Rat serum proteins MW (kDa)												
Groups	300	200	160	100	76	70	67	55	52	45	37	35	30
Control	0.1	0.7	0.6	0.8	5.2	5.2	40.1	15.2	14.3	3.4	3.9	5.9	4.6
Trichlorophenol													
5 ppm	2.2	2.9	1.5	1.8	8.9	4.2	58.2	8.5	7.5	2.7	1.6		
10 ppm	2.8	3.3	1.3	1.5	9.6	2.3	56.7	7.9	9.5	2.2	2.9		
15 ppm	1.4	2.9	2.5	4.5	10.6	2.5	56.8	5.7	6.6	4.2	2.3		
20 ppm	2.4	3.1	2.3	2.2	15.2	3.5	56.2	4.5	6.4	5.3	2.2		
Fenvalerate													
5 ppm	0.1	0.3	0.3	0.7	12.5	21.9	38.5	10.9		3.6	3.8	4.2	3.2
10 ppm				1.2	1.4	0.6	45.9	18.1		5.8	4.8	5.5	4.3
15 ppm					10.7		45.3	26.2		6.4	4.2	3.1	4.1
20 ppm							30.6	22.1		25.9	2.4	1.8	17.2
α-Endosulphan													
5 ppm	3.5	2.8	7.2	3.1	7.5	2.9	52.8	16.5	5.6	1.1			
10 ppm	1.5	4.9	8.3	3.4	8.1	3.6	48.3	12.7	5.4	3.8			
15 ppm	2.1	4.4	8.6	3.2	8.3	3.9	52.2	9.2	4.6	3.1			
20 ppm	2.1	6.8	7.1	2.5	8.5	3.8	55.5	9.5	4.5	3.7			

Table 2: Percentage of transferrin and albumin and their protein profiles after incubation with trichlorophenol, fenvalerate and α-endosulphan pesticides

	Transferrin MW (kDa)									Albumin MW (kDa)						
Groups	76	60	55	52	48	45	35	30	67	55	52	48	45	40	35	30
Control	38.8	22.1	18.1	9.4	6.9	3.4	0.7	0.6	64.1	21.2	4.1	4.2	6.4			
Trichlorophenol																
5 ppm	48.7	13.6	12.6	6.9	13.4	4.8			37.6	44.8	4.3	6.8	6.5			
10 ppm	49.1	13.3	12	6.1	13.8	5.7			49.5	26.1	11.2	6.9	6.3			
15 ppm	46.2	14.4	18.1	7.5	10.1	3.7			49.8	18.4	15.5	9.3	7			
Fenvalerate																
5 ppm	34.8	16.7	16.6	6.6	12.6	7.3	2.3	3.1	41.6	30.6	9.2	12	6.6			
10 ppm	36.5	15.6	15.2	8.9	15.6	8.2			39.8	33.4	12.5	7.8	6.5			
15 ppm	34.1	14.1	16	8.4	17.8	9.6			49.3	30.5	5.8	2.2	12.2			
α-Endosulphan																
5 ppm	42.5	14.2	8.6	8.5	10.9	10.1	2.8	2.4	31.1	29.6	13.4	2.9	7.1	1.6	4.2	10.1
10 ppm	43.9	12.4	8.5	8.5	11.8	7.7	2.3	4.9	30.2	52.1	9.4	3.5	4.8			
15 ppm	43.6	12.6	8.6	8.4	9.1	6.6	5.7	5.4	26.9	57.3	8.2	2.8	3.5	1.3		

CONCLUSION

The aim of the present investigation was to determine if there is any changes among serum proteins which could be used as a biomarker for pesticides pollution. In addition, during the transport of the pesticides with carrier proteins in blood throughout the organs, do complex cause a destruction in macromolecules. The data of the present study revealed that the incubated pesticides have, high affinity to the proteins binding sites (Saleh *et al*., 1996b; Afify *et al*., 2000). Similar, observations have been recorded for particle mediated uptake of chlorinated pesticides by human, rat and insect lipoprotein (Shalsky and Guthrie, 1975; Larsen *et al*., 1994; Shu and Nichols, 1979; Maliwal and Guthrie, 1982) and by serum albumin and α-globulin in rat and rabbit (Shakoori *et al*., 1996; Moss and Hathway, 1964). The binding of pesticides to proteins is correlated to the binding of DNA. DNA was considered the most important leader of the genetic code in human (Hemminki, 1986) which may induce genetic, risks (Ehrenberg *et al*., 1974). Therefore, the binding of pesticides to the macromolecules of rat serum protein could be serve as biomarker in the monitoring of pesticides (Hemminki, 1986). Pahler *et al*. (1999) showed that the accumulation of some proteins such as alpha 2 macro-globulin has been implicated in the tumorigenicity of many nongenotoxic chemicals to the kidney of the male rat. These chemicals have been shown to

bind to alpha 2 macro-globulin and this binding was found to impair the renal degradation of the protein, resulting in lysosomal overload, cell death, increased cell proliferation and, presumably renal tumor formation. The present study proved that the major proteins transferrin and albumin are the main sites for the three studied pesticides. The data of incubation of the three pesticides with transferrin and albumin were showed that the destruction of transferrin and albumin with the three pesticides produced a similar but not identical protein profile and the prealbumin was found to represent the major one as recorded by Altland *et al.* (1981). Dissociation into small MW proteins has been demonstrated in case of *in vitro* incubation with the tested pesticides. These results are in agreement with the results obtained by prolonged exposure of proteins to pesticides (Nilsson *et al*., 1975). The changes in the binding of serum acute phase proteins such as transferrin and albumin with some chemicals has been used to detect or identify human breast cancer (Heys *et al*., 1998). Insecticides have been shown to bind to blood protein especially organochlorine compounds which are extensively bound to blood lipoproteins (Shalsky and Guthrie, 1975, 1977). Dutta *et al.* (1992) revealed that malathion an organophosphorus pesticide has profound effect on serum protein as other parameters. Therefore, the detection of the prealbumin as well as small MW proteins after electrophoresis is considered a very good diagnostic marker for pesticide pollution. In conclusion the induced destructed proteins by pesticides in-vivo and *in vitro* may be utilized as biomarkers reliable for pesticides monitoring (Saleh *et al*., 1996b; Afify *et al*., 2000).

Breast Milk as a Biomarker for Monitoring Human Exposure to Environmental Pollutants (this work was funded by EPA Grand # CR 818220-02-5)

Saleh *et al.* (1996a) cited that 60 million Egyptian inhabitants can be grouped into three main different community types. The urban population, living in the capital city of Cairo (15 million) and other big cities, is generally exposed to air pollutants, especially lead evolving with vehicle exhaust, petroleum and gasoline vapors, carbon monoxide and mineral dusts. In addition, the urban communities may also be exposed to toxic residues in food and drinking water which may include pesticides and other toxicants. The second large community consists of those living in rural villages who are more likely to be exposed to pesticides and other agrochemicals. The third community includes those living in remote desert and mountain areas in the western and eastern deserts, the Sinai peninsula and northern and eastern sea coasts. The population in these areas is still engaged in few agricultural activities. There are also some Bedouin tribes in the southern part of Egypt such as Bassharia who live mainly on raising herds of camels and sheep in the desert areas. In such locations, the exposure to man made chemicals and pollutants is a minimum. Therefore, it is reasonable to consider such inhabitants as a real unexposed reference group compared to the urban or rural communities. This Egyptian model structure, where clear differentiation can be drawn between rural, urban and remote desert inhabitants, is expected to be successful in reaching significant correlations between typed of human activities and levels of exposure to hazardous chemicals. Since most organochlorine insecticides are environmentally persistent and fat soluble, they may accumulate in food and be stored in high concentrations in tissues and lipid rich organs such as the adipose tissues, liver, meat and milk (Hernandez *et al*., 1993). Human milk is the most important and indispensable food for the newborn. During lactation, fat mobilization could take place from the adipose tissue and therefore, organochlorine compounds such as DDT and HCHs and their metabolites are mobilized and released mainly by breast milk.

Recent epidemiological studies indicated the commonly occurring persistent pesticides and industrial chemicals found in breast milk. These chemicals are dichlorodiphenyl

trichloroethane as dichlorodiphenyl dichloroethene dieldrin, chlordane as oxychlordane, heptachlor, polychlorinated biphenyls, polychlorinated dibenzofurans and polychlorinated dibenzodioxins. We present a worked example of the kinds of pharmacokinetic assumptions and calculations necessary for setting regulatory limits of contaminants in the food supply, calculating dose of chemical contaminants to the nursed infant, converting risks from lifetime exposure in laboratory animals to risks for short-term exposure in humans and estimating the excess cancer risk to the nursed infan (Rogan and Ragan, 1994). Thus, monitoring of such residues in mother's milk will be a good Criterion to measure their impact on general population, not only on the existing population, but also on the next generation of the newly born children. In Egypt, a few surveys have been carried out in this regard mainly in two governorates Beny Sweif and Fayoum. Although the environmental contamination by chlorinated hydrocarbon insecticides has been reduced since 1970, when these pesticides were banned, some amounts of these chemicals were recently found in human breast milk. However, these amounts were within the range of acceptable daily intakes according to the FAO/WHO guidelines (Dogheim *et al*., 1991). On the same time contamination of buffalo milk with pesticides residues of diazinone insecticide after spraying animals were conducted and results detected high level in the first day after spraying (0.586 ppm) as reported by El-Kholy *et al*. (2000). The main objectives of this research was to evaluate the Egyptian mother's milk contents of organochrlorine insecticides and the heavy metal lead (Pb) as criterion for measuring the body burden with environmental pollutants due to long term exposure.

Lead Concentration in Mother's Milk and its Hazardous Impact

Table 3 presents the mean lead concentrations in milks samples collected from the 20 different locations. According to the daily permissible level established by the WHO (Vahter and Slorach, 1990) (5.0 μg kg^{-1} body weight/day, which is equivalent to about 15.5 ppb in mother's milk), it can be seen that the mean values of lead levels were below the permissible level in the governorates of Fayoum, Matruh, Minia and Suez, Lead levels in Aswan. Beheira, Beny Sweif, Dakahlia, Gharbia Giza, Ismailia, Kaliobia, Menoufia, New Valley North-Sinai, sfarkia and Sohag were slightly higher than the WHO permissible value. Mean read revels in mother's-milk Alexandria, Assiut and Cairo were significantly higher than the permissible value. The higher levels of lead mothers milk from Cairo, Alexandria and Assiut may be attributed to heavy automobile traffic and the use of leaded gasoline in addition to contamination of drinking water from the lead drinking pipe lines. There is very little information regarding the transfer of lead via the milk from mothers who are at high risk, such as those living in big cities i.e Cairo, Arexandria and Assiut. Chronic adult exposure to lead occur mainly through the inhalation of dust and fumes and incidental ingestion of polluted food and drink and the inhalation of cigarette smoke. The threshold limit value-time weighted average (TLV-TWA) for lead, dust and fumes is 0.15 mg m^{-3}. An average of 66 μg L^{-1} mother milk from women in Cairo equivalent to 10.54 μg/kg/day while a child weighing 5.5 kg may receive at least 58 ig of lead per day. According to Mahaffei (1977), the tolerable or maximum daily intake lead from all sources for infants between birth and the age of 6 months should be as low as possible and should be less than 100 ig/day. In Table 4 the lead levels in human milk recorded in this study were compared with those of other countries from 1971-1994. By comparison with the published data from other countries, the lead levels in Egypt are between moderate lead levels found in Japan, Germany, Sweden and the united states and the high value found in Mexico, Indonesia and Thailand. The current lead level in Egypt, although higher in some cases than the recommended WHO levels still appears not to be at the level of a serious risk except in those cases where the lead level is higher than 100 ppb in Assiut, followed by Cairo and Alexandria.

Table 3: Lead concentration (μg L^{-1}) in Human's Breast Milk from Egypt

	Subject							
Location	1	2	3	4	5	6	Average	Range
Alexandria (17)	40 (1.9)	40 (1.7)	66(3.4)	42 (1.8)	68 (4.0)	40 (3.8)	49.2	40-68
Assiut (12)	4.8 (8.1)	40 (3.6)	158 (14.3)	128 (1.1)	116(3.5)	118 (3.3)	101.4	40-158
Aswan (14)	74 (0.9)	8 (2.9)	18 (1.9)	20 (0.6)	8(2.5)	16(1.3)	24.0	8-74
Beheira (3)	52 (7.6)	40 (6.2)	28 (1.6)	32 (5.6)	24(7.6)	40 (7.8)	36.0	24-52
Beny Sweif (10)	10 (4.7)	24 (6.8)	26 (6.3)	20.4 (4.3)	82(4.0)	30 (4.5)	32.0	10-82
Cairo (7)	64 (3.9)	48 (5.9)	82 (5.2)	68(3.6)	46 (5.7)	88 (2.8)	66.0	46-88
Dakahlia (2)	36 (3.9)	10 (9.4)	16 (3.0)	18 (3.4)	10(8.0)	16 (4.6)	17.6	10-36
Fayoum (9)	10 (2.0)	18 (1.7)	4 (4.2)	16 (1.4)	10(1.1)	12 (1.6)	11.6	4-18
Gharbia (1)	28 (8.0)	22 (7.8)	24 (2.1)	26 (7.5)	28 (1.9)	36 (5.2)	27.4	22-36
Giza (8)	28 (7.8)	12 (5.3)	24 (5.2)	28(3.1)	12 (1.4)	20 (1.8)	20.6	12-28
Ismailia (19)	82 (6.2)	24 (4.9)	20 (1.6)	8 (1.2)	20 (5.8)	12 (1.6	27.6	8-82
Kaliobia (6)	28 (1.8)	36 (2.7)	24 (4.6)	36 (7.1)	28 (8.5)	36 (6.5)	30.6	24-36
Matrouh (16)	12 (2.9)	6 (1.3)	8 (2.6)	8 (3.1)	12(1.2)	8 (0.3)	9.0	6-12
Minia (11)	16 (4.3)	16 (8.7)	8 (4.5)	6 (4.1)	0 (1.9)	8 (1.8)	9.0	0-16
Menoufia (4)	3.6 (4.2)	24 (1.4)	26 (7.3)	36 (2.6)	86(4.6)	8 (4.8)	36	8-86
New Valley (15)	16 (3.3)	22 (5.1)	20 (3.8)	14 (5.6)	24 (0.5)	20 (2.4)	19.4	14-24
North Sinai (18)	40 (5.1)	20 (7.5)	46 (8.3)	20 (7.2)	18 (4.9)	28 (2.9)	28.6	18-46
Sharkia (5)	22 (3.0)	26 (4.0)	42 (5.3)	24 (6.6)	32 (7.3)	36 (2.1)	30.4	22-42
Sohag (13)	14 (8.9)	24 (1.6)	20 (3.7)	36 (2.9)	10 (2.1)	26 (3.4)	21.6	10-36
Suez (20)	0	72 (1.4)	0	0	0	0	15.2	0-72

Error % are shown in parenthesis

Table 4: Lead concentration (μg L^{-1}) in breast milk reported international

Location	No. of subjects	Average	Range
Cincinnati (USA)	22	0.012	-
Connevticut (USA)	7	20.00	0-70.0
New York (USA)	10	5.00	-
Iowa (USA)	4	26.00	15.0-64.0
Washington, D.C. (USA)	20	20.00	0-50.0
Boston (USA)	100	17.00	0-72.0
Arizona (USA)	39	2.80	0.90-10.0
Bangkok (Thiland)	164	85.00	136-220
Uppsala (Sweden)	41	20.00	5.0-90.0
London (England)	28	2.40	1.9-8.6
Kuala Lumpur (Indonesia)	114	47.8	24.9-106.1
Hamburg (Germany)	10	13.2	9.1-15.5
Mexico	35	61.8	9.2-351
Japan	22	27.00	0-56.0
Styria (Austria)	64	3.40	0-20.4
Egypt (20 Cities)	120	30.6	0-158

Saleh *et al.* (1996a,b)

Chlorinated Insecticides Levels in Human Milk

The data in Table 5 shows that the main detected organochlorine insecticides and their metabolites were DDE and lindane. DDT and endosulfan I residues were also detected in some samples. Endrin was only detected in one of the samples in New vally, while aldrin was not detected in any of the samples. However, from the 60 human milk samples, 5l% of the samples were free from any detectable DDT levels. a fact which may suggest that there were no recent sources of pollution by intact DDT (Saleh *et al*., 1996a, 1999).

Hexachlorocyclohexanes (HCH lsomers)

δ-HCH (lindane) was detected in 95% of the analyzes human milk samples. The lowest levels were found in governorates between Cairo and Assiut and in Suez (0.00-10.00 ppb), while the higher levels (10.00-33.00 ppb) were found in the Delta area and in Alexandria. The higher levels could be a reflection of the use of lindane in agriculture and in the control of cattle ecto-parasites. Also, this might be due to the human consumption of large quantities

Table 5: Distribution of the main organochlorine insecticide residues in Egyptian Mother's milk

	Lindane		Endosulfane I		4,4'-DDE		4,4- DDT	
Governorate*	Average	Range	Average	Range	Average	Range	Average	Range
Greater Cairo								
Cairo (7)	5.05	2.72-8.98	0.00	0.00 -0.00	11.30	1.40-19.7	0.95	0.00-2.85
Giza (8)	4.96	2.69-6.59	0.69	0.00-2.08	12.02	1.96-19.7	0.00	0.00-0.00
Kaliobia (6)	13.87	1.21-33.20	0.00	0.00-0.00	20.62	8.95-27.3	2.04	0.00-3.87
Delta Region								
Sharkia (5)	6.57	4.15-9.78	0.00	0.00-0.00	54.50	19.7-83.3	2.43	1.7-3.64
Gharbia (1)	4.63	3.47-6.49	10.5	0.00-18.90	25.82	4.06-53.3	1.68	0.00-2.53
Behera (3)	12.82	4.47-22.80	29.98	7.34-57.90	50.95	5.74-117.0	5.51	0.00-10.6
Dakahlia (2)	19.53	13.40-24.70	6.00	0.00-18.00	41.70	7.3-67.2	4.12	0.00-9.38
Menoufia (4)	1.52	4.15-9.78	0.00	0.00-0.00	7.93	2.4-10.9	2.59	0.00-7.76
Upper Egypt								
Fayoum (9)	3.77	0.68-7.82	0.00	0.00-0.00	20.06	4059-37.4	1.84	0.00-4.61
Beny Sweif (10)	2.04	0.95-2.90	3.25	0.00-5.81	27.26	9.17-46.7	1.59	0.00-3.41
Minia (11)	3.11	0.81-6.30	8.03	0.00-20.30	16.36	6.88-21.7	4.83	0.00-14.5
Assuit (12)	10.95	0.78-31.00	1.63	0.00-4.90	30.89	3.47-71.3	5.12	3.51-7.02
Sohag (13)	12.88	6.75-21.30	4.77	0.00-14.3	29.42	2.97-77.5	5.47	0.00-13.6
Aswan (14)	12.84	4.47-28.20	7.02	2.41-12.7	8.95	3.8-18.5	2.46	0.00-7.38
Costal Areas								
Alexandria (17)	12.46	2.37-20.50	0.00	0.00-0.00	9.35	7.32-12.1	0.00	0.00-0.00
Matrouh (16)	11.30	6.41-15.00	25.83	0.00-61.8	15.11	7.04-23.0	0.00	0.00-0.00
North Sinai (18)	11.81	9.92-12.9	0.00	0.00-0.00	8.84	5.17-13.7	14.52	4.77-32.9
Ismailia (19)	3.71	1.36-5.43	0.00	0.00-0.00	19.17	14.2-25.4	0.00	0.00-0.00
Suez (20)	1.44	0.00-2.21	0.00	0.00-0.00	11.35	5.78-20.3	2.39	0.00-7.16
Desert								
New Vallery (15)	13.08	8.93-18.20	0.00	0.00-0,00	5.83	4.13-8.54	1.04	0.00-3.13
Average in Egypt	8.42	0.00-31.00	4.84	0.00-61.8	1.37	1.4-117	2.93	0.00-32.9

*No. of collection samples

of polluted fatty fish. Several studies (Kucinski, 1986) have pointed out the presence of organochlorine residues including lindane in different food stuffs (meat, dairy products. grain and drinks). These contaminated foods, which represent the basic staples for the donor mothers, may explain the source of lindane in their milk. Another studies Saleh *et al.* (1998, 1999) conducted on breast human milk proved that samples containing relatively higher levels of DDT group (DDT, DDE and DDD) showed significant effect on the level of lysozyme, lactalbumin protein bands relative to low or no residue level.

REFERENCES

Afify, A.M.R., A.A. Ragab, G.S. El-Baroty, M. Abo-Zeid and M.A. Saleh, 1997. Protein profile of humans milk of selected Egyptian pollutions. Egypt. J. Nutr., 12: 1-17.

Afify, A.M.R., S.A. Abd El-Azim and M.M. Rashid, 2000. *In-vitro* binding of trichlorophenol l, fenvalerate and α-endosulphan to rat serum transferrin and albumin for biomonitoring of pesticides pollution. Arab. J. Lab. Med., 26: 143-151.

Altland, K., S. Rauh and R. Hacker, 1981. Demonstration of human prealbumin by double one dimensional slabe gel electrophoresis. Electrophoresis, 2: 148-155.

Alvares, A.P., A. Fischbein, K.E. Anderson and A. Kappas, 1977. A alterations in drug metabolism in workers exposed to polychlorinated biphenyls. Clin. Pharmacol. Ther., 22: 140-146.

Bulger, W.H. and R.M. Muccitelli, D. Kupfer, 1978. Interactions of chlorinated hydrocarbon pesticides with the 8S estrogen-binding protein in rat testes. J. Toxicol. Environ. Health, 32: 165-177.

Bulger, W.H., R.M. Muccitelli and D. Kupfer, 1981. Interactions of methoxychlor, methoxychlor base-soluble contaminant and 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane with rat uterine estrogen receptor. Res. Commun. Chem. Pathol. Pharmacol., 33: 119-128.

Crompton, M., 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem. J., 341: 233-249.

Davies, D.S., 1981. Drug Hepatoxicity: Formation and Importance of Reactive Metabolites. In: Drug Reactions and the Liver, Davis, M., J.M. Tredger and R. Williams, (Eds.). Pittman Medical Limited, London, pp: 12-18.

Dogheim, S.M., M. El-Shafeey, M.A. Afifi and E.F. Abdel-Aleem, 1991. Levels of pesticide residues in Egyptian human milk samples and infant dietary intake. J. AOAC, 74: 89-91.

Dutta, H.M., J.V.V. Dogra, N.K. Singh, P.K. Roy and S.S.T. Nassar *et al*., 1992. Malathion induced changes in serum protein and hematological parameters of an Indian Catfish *Heteropneustes fossillis* (Bloch). Bull. Environ. Contam. Toxicol., 49: 91-97.

Ehrenberg, L., K.D. Heische, S. Osterman-Golkar and I. Wennberg, 1974. Evaluation of genetic risks of alkylating agents : Tissue doses in the mouse from air contaminated with ethylene oxide. Mutant. Res., 24: 83-103.

El-Agnaf, O.M.A., R. Jakes, M.D. Curran and A. Wallace, 1998. Effects of the mutations Ala30 to Pro and Ala53 to Thr on the physical and morphological properties of α-synuclein protein implicated in Parkinsons disease FEBS Lett., 440: 67-70.

El-Kholy, A.F., A.M.R. Afify, A.A. Ragab and G.S. El-Baroty, 2000. Contamination of buffalo milk with residues of diazinon insecticdes after spraying animals. Vet. Med. J. Giza, 48: 7-11.

Guengerich, G.A., S.T. Dannan, M.V. Wright, Martin and L.S. Kaminsky, 1982. The warfarin hydroxylase activities of the P-450 isozyme components of the various microsomal preparations. Biochemistry, 21: 6019-6030.

Guiney, P.D. and R.E. Peterson, 1980. Effect of the carbamate pesticide Sevin on the intestinal absorption of some nutrients in the teleost fish, Channa punctatus. Arch. Environ. Contam. Toxicol., 9: 667-667.

Hemminki, L., 1986. Covalent binding of styrene oxide to amino acids, human serum protein and hemoglobin. Monitorine Occupational Genotoxicants, 62: 159-168.

Henry, D., G. Sharpe, S. Chaplain, S. Cartwright, G. Kitchingman, G.D. Bell and M.J. Langman, 1979. The [14C]-aminopyrine breath test. A comparison of different forms of analysis. J. Clin. Pharmacol., 8: 539-545.

Hernandez, L.M., M.A. Fernandez, E. Hoyas, M.J. Gonzalez and J.F. Garcia, 1993. Organochlorine insecticide and polychlorinated biphenyl residues in human breast milk in Madrid. Bull. Environ. Contam. Toxicol., 50: 309-315.

Heys, S.D., K.N. Ogston, W.G. Simpson and L.G. Walker, A.W. Hutcheon, T.K. Sarkar and O. Eremin, 1998. Acute phase proteins in patients with large and locally advanced breast cancer ffeated with neo-adjuvant chemotherapy; response and survival. lnt. J. Oncol., 13: 589-594.

Hunter, J., J.D. Maxwell, M. Carrella, D.A. Stewart and R. Williams, 1971. Urinary D-glucaric acid excretion as a test for hepatic enzyme induction in man. Lancet, 297: 572-575.

Jarvinen, A.W. and R.M. Tyo, 1978. Refined criteria must be established to determine the survival requirements for aquatic life. Arch. Environ. Contain. Toxicol., 7: 409-409.

Kellermann, G. and M. Luyten-Kellermann, 1977. Phenobarbital induced drug metabolism in man. Toxicol. Applied Pharmacol., 39: 97-104.

Kolmodin-Hedman, B., 1974. Decreased Plasma Half-Lives of Antipyrine and Phenylbutazone in Workers Occupationally Exposed to Lindane and DDT. In: Drug Interactions, Morselli, P.L., S. Garattini and S.N. Cohen (Eds.). Raven Publishing, Inc., New York, pp: 249-257.

Kouri, R.E., C.E. McKinney, D.J. Slomiany, D.R. Snodgrass, N.P. Wray and T.L. McLemore, 1982. Multiplicity of mammalian microsomal cytochromes P-45. Cancer Res., 42: 5030-5037.

Kouudinya, P.R. and R. Ramamurthi, 1979. Effect of the carbamate pesticide Sevin on the intestinal absorption of some nutrients in the teleost fish, Channa Punctatus. Curt. Sci. lndia, 48: 877-877.

Kucinski, B., 1986. The quantity and quality of breast milk. Ciencia Hoje, 4: 58-62.

Larsen, G.L., K.L. Davison, J.E. Bakke and N.H. Bass, 1994. Isolation of Pesticide-Binding Protein from Rat Blood. In: Biomarker of Human Exposure to Pesticides, Saleh, M.A., J.N. Blancato and C.H. Nauman (Eds.). American Chemical Society, Washington DC., pp: 166-177.

Mac, M.J., L.W. Nicholson and C.A. McCauley, 1979. PCBs and DDE in commercial fish feeds. Prog. Fish. Culture, 41: 210-211.

Madge, D.S., 1976. Polychlorinated biphenyls and intestinal absorption of D-glucose in mice. Gen. Pharmacol.: Vascular Syst., 7: 45-48.

Mahaffei, K.R., 1977. Relation between quantities of lead ingested and health effects of lead in human. Pediatrics, 59: 448-455.

Maliwal, B.P. and F.C. Guthrie, 1982. *In-vitro* uptake and transfer of chlorinated hydrocarbons among human lipoproteins. J. Lipid Res., 23: 414-479.

Maroteaux, L., J.T. Campanelli and R.H. Scheller, 1988. α-Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J. Neurosci., 8: 2804-2815.

Mason, R.R. and G.J. Schulte, 1983. Interaction of o,p-DDT with the estrogen-binding protein (EBP) of DMBA-induced rat mammary tumors. Biochem. Pharmacol., 32: 1005-1010.

Mayer, S.E., K. Melmon and A.G. Gilman, 1980. Goodman and Gilmans the Pharmacological Basis of Therapeutics. 6th Edn., MacMillan, New York pp: 1-27.

Moss, J.A. and D.E. Hathway, 1964. Transport of organic compound in mammal. Biochem. J., 91: 384-392.

Narhi, L., S.J. Wood, S. Steavenson, Y. Jiang and G.M. Wu *et al*., 1999. Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation. J. Biol. Chem., 274: 9843-9846.

Neimi, A.J. and C.Y. Cho, 1980. Uptake of hexachlorobenzene (HCB) from feed by rainbow trout (*Salme gairdneri*). Bull. Environ. Contam. Toxicol., 24: 839-839.

Nilsson, S.F., L. Rask and P.A. Peterson, 1975. Studies on thyroid hormone-binding proteins II Binding of thyroid hormones, retinol-binding protein and fluorescent probes to prealbumin and effects of thyroxine on prealbumin subunit self-association. J. Biol. Chem., 250: 8554-8563.

Ohnhaus, E.E. and B.K. Park, 1979. Measurement of urinary 6-β-hydroxycortisol excretion as an *in vivo* parameter in the clinical assessment of the microsomal enzyme-inducing capacity of antipyrine, phenobarbitone and rifampicin). Eur. J. Clin. Pharmacol., 15: 139-145.

Ohnhaus, E.E., E. Gerber-Taras and B.K. Park, 1983. Enzyme-inducing drug combinations and their effects on liver microsomal enzyme activity in man. Eur. J. Clin. Pharmacol., 24: 247-250.

Pahler, A., K. Blumbach, J. Herbst and W. Dekant, 1999. Quantitiation of alpha 2 -ì globulin in rat kidney cytosol by capillary electrophoresis. Anal. Biochem., 267: 203-211.

Pamperl, H., W. Gradner, L. Fridrich, H. Pointner and H. Denk, 1984. Influence of long-term anticonvulsant treatment on liver ultrastructure in man. Liver, 4: 294-300.

Popper, H., M.A. Gerber, F. Schaffner and I.J. Selikoff, 1979. Environmental Hepatic Injury in Man. In: Progress in Liver Diseases, Popper, H. and F. Schaffner (Eds.). Grune and Stratton, New York, pp: 605-638.

Rogan, W.J. and N.B. Ragan, 1994. Environmental health issues environmental health perspectives supplements. Volume 102, Number S11, 1994.

Saenger, P., E. Forster and J. Kream, 1981. 6 bete hydroxycortisol: A noninvasive indicator of enzyme induction. J. Clin. Endocrinol. Metab., 52: 381-384.

Saleh, M.A., M. Abou Zeid, A.M. Zaher and F. Abdel-Rahman, 1996a. Serum Protein Profile: A Possible Biomarker for Exposure to Insecticides. In: A Biomarkers for Agrochemicals and Toxic Substances/Applications and Risk Assessment, Blancato, J., R. Brown, C. Dary and M. Saleh (Eds.). American Chemical Society, Washington, DC., pp: 106-113.

Saleh, M.B, A.M. Afify, A. Ragab, G. El-Baroty, A. Kamel and A.K.H. El-Sebae, 1996b. Breast milk as biomarker for monitoring human exposure to environmental pollutants. ACS Symp. Series, 643: 114-125.

Saleh, M., A. Abdel-Moneim and A. Kamel, 1998. Mother milk protein profile, A possible biomarker for human expsuure to persistent insecticides. J. Environ. Sci. Health, 33: 645-655.

Saleh, M.A., A. Kamel, A. Ragab, G. El-Baroty, M.R.M. Afify and J. Jones, 1999. Organochlorine insecticide residue in Egyptian mothers milk. Toxicol. Environ. Chem., 68: 429-444.

Sastry, K.V. and S.K. Sharma, 1978. Endrin toxicity on liver of *Channa punctatus* (Bloch). Indian J. Exp. Biol., 16: 372-372.

Sastry, K.V. and A.A. Siddiqui, 1982. Effect of endosulfan and aninophos on intestinal absorption of glucose in the fresh water murrel Channa Punctuatus. ToxicoI. Lett., 12: 289-289.

Schoene, B., R.A. Fleischmann, H. Remmer and H.F. Oldershausen, 1972. Determination of drug metabolizing enzymes in needle biopsies of human liver. Eur. J. Clin. Pharmacol., 4: 65-73.

Shakoori, A.R., A.L. Mughal and M.J. Igbal, 1996. Effects of sublethal doses of Fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a freshwater fish, ctenopharyngodon idella. Bull. Environ. Contam. Toxicol., 57: 487-494.

Shalsky, H.L. and F.E. Guthrie, 1975. Binding on insecticides to macromolecules in blood of rat and American cockroach. Pesticide Biochem. Physiol., 5: 27-34.

Shalsky, H.L. and F.E. Guthrie, 1977. Affinities of parathion, DDt, Dieldrine and carbaryl for acromolecules in blood of rat and American cockroach and comprtitive interaction of steroids esticide. Biochem. Physiol., 7: 289-296.

Shu, H.P. and A.V. Nichols, 1979. Benzo(a)pyrene up-take by human plasma lipoproteins *in-vitro*. Cancer Res., 39: 1224-1230.

Uversky, V.N., J. Li and A.L. Fink, 2001a. Evidence for a partially folded intermediate in α-synuclein fibril formation. J. Biol. Chem., 276: 10737-10744.

Uversky, V.N., J. Li and A.L. Fink, 2001b. Pesticides directly accelerate the rate of α-synuclein fbril formation: A possible factor in Parkinson's disease. FEBS Lett., 500: 105-108.

Vahter, M. and S. Slorach, 1990. Exposure Monitoring of Lead and Cadmium. An International Pilot Study within the WHO/UNEP Human Exposure Assessment Locations (FIEAL) Programme. WHO, Nairobi.

Vijayalakshimi, S., 1980. *In vivo* effects of Sumithion on tissue respiration and enzyme activity in the fish, Etroplus maculatus. Experientia, 36: 1280-1280.

Wang, P.P., P. Beaune, L.S. Kaminsky, G.A. Dannan, F.F. Kadlubar, D. Larrey and F.P. Guengerich, 1983. Purification and characterization of six cytochrome P-450 isozymes from human livermicrosomes. Biochemistry, 22: 5375-5383.

Watkins, P.B., S.A. Wrighton, P. Maurel, E.G. Schuetz, G. Mendez-picon, G.A. Parker and P.S. Guzelian 1985. Identification of an inducible form of cytochrome P-450 inhuman liver. Med. Sci., 82: 6310-6314.