Penetration of the Blood-Brain-Cerebral Spinal Fluid Barrier by DDT

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The central nervous system (CNS) is a unique organ system in that it not only receives a rich vascular supply, but, in addition, it is suspended in the cerebral spinal fluid (CSF). Basic toxicological information about any neurotoxic compound includes ascertaining the absolute and relative amounts of the compound in these three separate, but intimately related, compartments (blood, CSF, brain) at a given time after the administration of an intoxicating dose of the compound. The present study was undertaken to obtain this information for the organochlorine compound, p,p'-DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane).

The chief manifestation of DDT intoxication involves central nervous system dysfunction (1). However, the mechanism by which DDT effects these aberrations remains unknown. Before it can be elucidated, the accessibility of the compound to, and its affinity within, the CNS must be established.

There have been reports dealing with DDT concentrations in blood and brain after a single oral dose in the rat (2-4), a single intravenous dose in cats (5), and after long-term daily dosing in rats (6,7). Apparently, however, no data have been published of the concentration of DDT in CSF.

Materials and Methods

Twenty-four Sprague-Dawley weanling rats, random-bred, were housed individually and fed ground chow and water ad libitum until adulthood (90-120 days of age). At that time they were divided into three groups of equal sex distribution.

The rats were dosed orally by gastric intubation with 200, 400, or 600 mg/kg of analytical grade (99.9% pure) p,p'-DDT dissolved in warmed peanut oil (38°C). The dose was administered on a fixed volume per weight basis (0.005 ml of solution per g of body weight) according to the method of Gaines (8). Doses above the LD_{50} of DDT (8) were used to facilitate the development of neurotoxic signs.

The animals were observed until generalized convulsive activity occurred; at this time CSF was collected. The animals were anesthetized with ether and placed in a special stand (9) or their head was acutely flexed over the edge of a table, and a No. 26 gauge needle was inserted into the cisterna magnum. Although it was difficult to obtain the necessary 0.02-0.10 ml of clear, colorless fluid before blood appeared, this was essential; if the fluid contained even a few red blood cells the amount of DDT was spuriously elevated.

Bulletin of Environmental Contamination & Toxicology
Vol. 6, No. 1, 1971, published by Springer-Verlag New York Inc.

After the CSF was obtained, a 0.1-ml sample of blood was collected by cardiac puncture. The animal was then sacrificed and a 2-400 mg section of cerebral hemisphere was removed.

All samples were placed in pre-weighed glass tubes; sintered-glass hand tissue-grinding tubes were used for blood and brain and small centrifuge tubes were used for CSF. They were then extracted as follows.

CSF was extracted with 1 ml of hexane.

Two milliliters of distilled water was added to the blood sample, the mixture was extracted with 5 ml of hexane, and 1:5, 1:6, or other suitable dilutions were prepared in hexane (10).

Brain was extracted with 2.5 ml of acetonitrile, an equal volume of 2% aqueous sodium sulfate was added, and the mixture was then extracted three times with 2 ml of hexane. The extracts were combined and 1:10 or other suitable dilutions were made in hexane (11).

All analyses were performed with a Micro-Tek MT-220 gas-liquid chromatograph equipped with a Ni^{63} electron-capture detector. The 6-foot, 1/4-inch chromatographic column was packed with 1.95% QF-1 + 1.5% OV-17 coated on 80-100 mesh HP Chromosorb W. All samples were analyzed against a standard DDT solution of 133 pg per 10 µl. The temperature, voltage, and flow rates of nitrogen were adjusted to give a DDT retention time of approximately 15 min with maximal sensitivity. Picogram amounts were analyzed by this method. The peaks were sharp and symmetrical and no other significant peaks appeared. Under these conditions there was no detectable DDT in samples of CSF, blood, or brain from non-poisoned animals.

Results and Discussion

The means, ranges, and standard deviations of the p,p'-DDT concentrations are summarized in Table 1. The mean concentration in all dosed animals at the time of convulsion was 0.12 ppm in the CSF, 3.4 ppm in the blood, and 16 ppm in the brain. There was no significant sex difference in the levels attained under these experimental conditions. The mean DDT levels in these three tissues were also independent of the dosages given in this study.

All treated animals convulsed between 2 and 8 hours after dosing. Although there was no significant difference in the ultimate convulsing concentration of DDT in these three tissues, the animals given the higher doses (i.e., 600 mg/kg) developed signs of intoxication in the early part of this period (2-4 hr) and those given the lower doses (i.e., 200 mg/kg) developed signs in the later part of this period (5-8 hr).

TABLE 1

p,p'-DDT Concentrations in the Cerebral Spinal Fluid (CSF), Blood, and Brain of Rats at the Time of p,p'-DDT-Induced Convulsions[a]

p,p'-DDT Administered, mg/kg	p,p'-DDT Concentration, ppm					
	CSF		Blood		Brain	
	Male	Female	Male	Female	Male	Female
200	0.11(0.10-0.11)	0.13(0.11-0.14)	4.5(3.7-5.2)	3.7(2.8-4.6)	13(10-15)	17(16-17)
	0.12 ± 0.02		4.1 ± 1.0		15 ± 3	
400	0.17(0.15-0.18)	0.11(0.09-0.12)	4.6(3.9-5.3)	2.8(2.4-3.2)	13(12-14)	18(16-19)
	0.14 ± 0.04		3.7 ± 1.9		15 ± 3	
600	0.09(0.04-0.12)	0.14(0.12-0.16)	2.3(2.0-2.5)	3.0(2.4-3.6)	16(15-16)	18(18-19)
	0.11 ± 0.04		2.6 ± 0.6		17 ± 2	
200-600	0.12 ± 0.04	0.12 ± 0.02	3.6 ± 1.2	3.2 ± 0.8	14 ± 2	18 ± 1
	0.12 ± 0.03		3.4 ± 1.1		16 ± 3	

[a] The separate values for males and females are given as means and ranges of 4 rats; combined values are means ± SD of 8 rats, and 12 and 24 rats at all dosages.

The concentrations of DDT are expressed as ppm (μg/g) of tissue. Many investigators, e.g., Dale, et al. (2), have reported DDT levels in various body organs as ppm in tissue lipid. Although most of the DDT is held in the lipid fraction of a tissue, if that fraction constitutes varying amounts of the whole tissue, the measured concentration is then dependent upon two variables: the amount of lipid in the tissue and the amount of DDT in the lipid. Since the amount of lipid may not be constant in blood, it is perhaps preferable to express the DDT concentration as ppm of whole blood (or plasma). A slightly different argument has been advanced for expressing brain concentrations of DDT on a fresh weight basis and not on the basis of tissue lipid (3).

Nevertheless, DDT is lipophilic, it is concentrated and stored in adipose tissue, and, in general, the amount of DDT in any particular body tissue is a function of the tissue lipid content (1,12). However, other factors must also be considered, especially in a dynamic situation such as acute intoxication. Brain total lipid is about 12 g % of fresh tissue (grey matter 4.0-7.9 g %, white matter 13.9-23.1 g % (13,14), blood total lipid 650 mg % (450-900) (14,15), and spinal fluid 1-2 mg % (14,15). Thus, blood and brain have lipid concentrations approximately 400 and 8000 times that of CSF. If the concentration of DDT is solely a function of the amount of lipid in that tissue, then a similar proportional fractionation can be anticipated. But, in fact, the blood and brain DDT concentrations were only 30 and 130 times that found in the CSF. It would appear that under these experimental conditions the amount of DDT in the CSF was greater than that anticipated from its lipid content alone and/or the amount in the blood and brain was considerably less than that anticipated.

The latter possibility seems unlikely because the values reported here compare favorably to those previously reported for blood (3-5,16) and for brain (3-5). Therefore, the amount of DDT in the CSF of these animals is larger than that anticipated. The physiological significance of this is wholly speculative.

Most compounds enter the brain from the blood; in doing so they must first cross the "blood-brain barrier" (17) or the "blood-CSF barrier" (18). This concept of a "brain barrier system" (19) encompasses the partitioning of a compound in the blood, CSF, and brain compartments. It is a physiological barrier of pharmacological selection whose degree of development will depend on the species and the age of the animal (20). Compounds differ greatly in their ability to cross this "barrier" (18); in, general, the more lipid-soluble the compound, the more easily it can cross. Some compounds may exert their effect on nervous tissue via the CSF and/or the CSF may serve as a reservoir for the compound.

In clinical toxicology the concentration of a toxicant is measured in a readily accessible biological sample (e.g. blood) and then these data are extrapolated to the individual as a whole. Thus, with organochlorine compounds, attempts have been made to assess the effects in the end organ (the CNS) by measuring blood concentrations under various conditions.

In man, exposure to DDT is measured by levels in blood (21), but the latter are not necessarily related to clinical symptoms or signs. In rats, although the severity of signs of acute poisoning with DDT is directly proportional to the concentrations of the compound in their brains (2,7), concentrations in blood do not correlate with those in brain (4). This lack of correlation is in contrast to observations with dieldrin; in dogs fed a daily dose of the compound, the concentration of dieldrin in the blood and the severity of the clinical signs of intoxication were directly related (22). This apparent contradiction between two organochlorine compounds may not be real. There is a differential distribution of DDT, related to time after exposure, within the various areas of the CNS (4,5); with repeated dosing an equilibrium may be established that is not established in an acute intoxication.

Therefore, exposure can be related to concentrations in blood and these concentrations are not predictive of the concentrations in brain (at least in acute intoxications); however, the concentrations in brain are directly related to clinical signs of intoxication. Where the CSF should be fitted into this scheme is not readily apparent and in the present study a particular sign of intoxication (namely convulsions) was used as the end point. It is interesting to speculate that the CSF might subserve an important transport and/or storage role in the dynamics of DDT intoxication.

Summary

Adult male and female rats were orally dosed with 200, 400, or 600 mg/kg of p,p'-DDT. When convulsions occurred, samples of cerebral spinal fluid, blood, and brain were collected and assayed for p,p'-DDT. Means of 0.12, 3.4, and 16 ppm, respectively, were found. Neither the sex of the animal nor the dosage given affected the concentrations present in these three tissues at the time of convulsions.

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