Polychlorinated Biphenyls. An Interpretation of their Persistence and Effects in Birds

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Commercial PCB mixtures have been fed to birds and the magnitude and nature of the residues together with some of their biochemical effects have been determined over a period of six months after the cessation of feeding the compounds.

Peakall and Lincer[1] suggest that polychlorinated biphenyls (PCBs) found in nature are derived only from highly chlorinated commercial mixtures such as Aroclor 1254. Residues of lower chlorinated mixtures have not been reported despite their predominant industrial use. This may be due either to utilisation in enclosed systems or to more rapid metabolism or excretion.

In order to demonstrate which residues remain after PCB feeding, pigeons (Columbia livia) and japanese quail (Coturnix coturnix japonica) have been fed Aroclor mixtures.

Feeding of pigeons

Twenty four separately caged feral pigeons were fed ad libitum for 28 days with wheat dressed with 500 ppm Aroclor 1242. Six control pigeons were fed wheat. At the end of the feeding period six test birds were sacrificed by cervical dislocation and the remainder returned to a diet of mixed grain and pulses, before sacrifice in batches of six at 28, 56 and 168 days.

Feeding of quail

Eighteen, four week old female japanese quail were caged in batches of six. One batch was fed ad libitum for 20 days with Aroclor 1242 dressed food at 250 ppm. another with Aroclor 1254 dressed food at 250 ppm and the remaining batch left as

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control. All birds were sacrificed 24 h after withdrawal of dressed food. This regimen allows comparison with other quail feeding experiments[2].

Methods of residue analysis

Immediately after sacrifice, breast muscle, liver, brain and omental fat samples were removed from pigeons. Similar samples were removed from quail with the substitution of heart for breast muscle. A portion of liver was taken for preparation of microsomes[2] and determination of cytochrome P_{450}[3] and microsomal protein[4]. The remaining liver and the other tissues were extracted by the method of Taylor et al[5] before gas-liquid chromatographic analysis using a 3 foot Apiezon L column[6] at 200°C.

Presentation of changes observed

The complex nature of Aroclor mixtures makes the expression of results difficult. As gross changes have taken place in our experiment we have chosen the method of Risebrough[7] from those published and compared the sum total of peak heights with a 1,1-di(4-chlorophenyl)-2,2-dichloroethylene (DDE) standard, which in turn was related to a known weight of Aroclor 1242. The mean results obtained from the batches of pigeons are shown in Fig 1 and from quail in Table 1. Regular checks made on excreta revealed no gross excretion of unchanged material.

The presentation of changes in the GLC pattern of PCB isomers is also difficult. Most workers have preferred to suggest that the patterns which they have obtained resemble one of the commercial mixtures, leading to the general conclusion that PCBs remain unaltered in the Biosphere. Since we wished to determine whether birds are able to metabolise PCBs we adopted the following method of presentation for GLC data. A single peak occurring in all chromatograms from fed birds was selected and represented arbitrarily as 100 units. The mean of peak height for each relative retention time from each batch of birds was normalised to this peak. By this method an average residue "spectrum" can be presented in the form of a line diagram. Fig 2 shows line diagrams from the livers of pigeons fed

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Aroclor 1242, and Fig 3 those from quail fed Aroclor 1254. Other tissues gave similar results.

Rate of decay of chlorinated residues

Total PCB residues immediately after withdrawal of treated food show a similar distribution between tissues to those in pigeons fed 1,1-di(4-chlorophenyl) -2,2,2-trichloroethane (DDT)[8]. Allowing for the higher feeding level in the DDT study initial PCB residues are five to ten times higher. In the muscle and fat there is a steady logarithmic decay with half-lives, by inspection, of 50 days and 125 days respectively. After an initial large drop in levels the liver residues also follow a logarithmic decay with a half-life of 140 days. These figures compare[8] with a statistically computed half-life of 28 days for DDT in all pigeon tissues (uncertainty in interpreting PCB analytical results precludes statistical analysis). PCB residues in the brain reach a maximum and then decay logarithmically with a half-life of 50 days. The high liver residue level immediately after withdrawal of food may be attributed to the rate at which liver receives freshly absorbed PCB, and the initial low brain level may reflect the operation of the blood-brain barrier[9].

Total residues in quail fed Aroclor 1242 (Table 1) are higher than those in pigeons despite a shorter feeding period at half the level. However, food consumption per unit weight of quail is two to threefold larger than that of pigeons. Residues of Aroclor 1254 are generally twice those of Aroclor 1242, suggesting that this mixture is more difficult to metabolise or excrete.

Differential removal of isomers

The standard 1242 mixture gives a pattern dominated by a few isomers of low retention time on our GLC. There is a dramatic change in pigeon liver residue patterns (Fig 2) with peaks of low relative retention times considerably reduced immediately following withdrawal of treated food and virtually absent by the end of the experiment. These peaks seem to be readily removed from the body revealing

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other minor or less electron capturing constituents with a longer biological half-life. We cannot yet be certain that all these peaks are chlorinated biphenyls present in the original mixture, some may be metabolites. Peaks with a higher retent n time are relatively enhanced over the experimental period. The patterns obtained after feeding quail Aroclor 1242 have the same general features but Aroclor 1254 feeding produces less aberration from the standard.

The conclusion that the less chlorinated isomers are more readily metabolised is also supported by two analyses on a high resolution capillary column. A standard Aroclor 1242 exhibited 44 peaks and a considerable proportion could be assigned structures[10]. An extract of fat from a pigeon sacrificed immediately after withdrawal of dressed food exhibited 29 peaks. Most of the 15 peaks of lowest retention time (mainly dichloro and trichloro isomers) were absent from the extract. Peaks of longer retention time (mainly tetrachloro or higher chlorinated isomers) were present in the pigeon and many were relatively enhanced. These results agree with previous work involving quail[11] and rats[12].

Significance of residues

The hepatic microsomal systems responsible for metabolising foreign compounds are sensitive to induction by chlorinated hydrocarbons. The significance of induction is not yet understood but it can be used as an indicator of the biological activity of a compound. Increases in microsomal protein and cytochrome P_{450} are central to microsomal induction and we have measured these to indicate the interference by PCB residues in the normal physiology of the birds (Table II & III).

Pigeon liver weights remain elevated for at least two months following withdrawal although hepatic microsomal protein returns to normal. In contrast, cytochrome P_{450} levels are still at three times the control level six months after returning to normal diet when PCB levels are approximately 2 ppm and composed mainly of higher chlorinated isomers, suggesting that extremely low levels (< 1 ppm) of these isomers cause significant elevation of microsomal enzymes. Quail fed

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either Aroclor 1242 or 1254 show similar increases which are far larger than those obtained from similar experiments involving DDT and DDE. Aroclor 1254 produces considerably more induction than 1242 supporting the suggestion above that the more highly chlorinated biphenyls are the most potent inducers.

Despite analytical and interpretive difficulties we can conclude that pigeons and quail metabolise polychlorinated biphenyls at a rate generally dependent on the amount of chlorine in the molecule. Further work in our laboratory suggests that some lower chlorinated isomers are metabolised extremely rapidly. This could account for the almost universal finding of residues of "Aroclor 1254" despite the major production of 1242. However since biological activity depends on the highly chlorinated isomers interpretation of findings may need to take this into account.

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TABLE I

Mean total PCB residues (ppm) found in quail tissue after feeding Aroclor 1242 and Aroclor 1254 at 250 ppm for 20 days.

Tissue	Aroclor 1242	Aroclor 1254
Liver	17.0 ± 2.6*	28.1 ± 7.9
Heart	3.2 ± 0.7	7.0 ± 1.1
Brain	7.8 ± 1.7	7.7 ± 2.3
Omental fat	123 ± 17	304 ± 31

* All figures are mean of six birds ± S.E.M.

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TABLE II

Mean liver weights, microsomal protein levels, cytochrome P_{450} levels and residue levels for pigeons fed Aroclor 1242 at 500 ppm for 28 days and sacrificed at 0, 28, 56 and 168 days after the withdrawal of dressed food.

Days to sacrifice	Mean liver weight g	Microsomal protein mg/g liver	Cytochrome P_{450} nmoles/g liver	Total liver residues ppm
Control	6.97 ± 0.32 *	13.70 ± 1.07	2.40 ± 0.28	0
0	10.75 ± 0.98	19.30 ± 1.80	15.47 ± 1.70	15.3 ± 1.8
28	8.78 ± 0.61	17.31 ± 1.51	11.00 ± 1.46	3.6 ± 0.5
56	9.74 ± 0.93	13.47 ± 1.72	7.48 ± 0.83	3.4 ± 0.1
168	6.50 ± 0.45	12.81 ± 1.40	6.73 ± 0.81	1.8 ± 0.2

* All figures are mean of six birds ± S.E.M

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TABLE III

Mean liver weights, microsomal protein levels , cytochrome P_{450} levels, and residue levels for quail fed Aroclor 1242 and Aroclor 1254 at 250 ppm for 20 days before sacrifice.

Compound fed	Mean liver weight g	Microsomal protein mg/g liver	Cytochrome P_{450} nmoles/g liver	Total liver residues
Control	3.82 ± 0.22 *	25.05 ± 2.27	5.42 ± 0.64	0
Aroclor 1242	4.52 ± 0.37	31.62 ± 1.27	22.18 ± 2.83	17.0 ± 2.6
Aroclor 1254	5.08 ± 0.22	42.87 ± 1.42	42.21 ± 3.31	28.1 ± 7.9

* All figures are mean of six birds ± S.E.M

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Fig. 1 Mean total levels of PCB in pigeon tissues following feeding of Aroclor 1242 at 500 ppm in diet for 28 days.

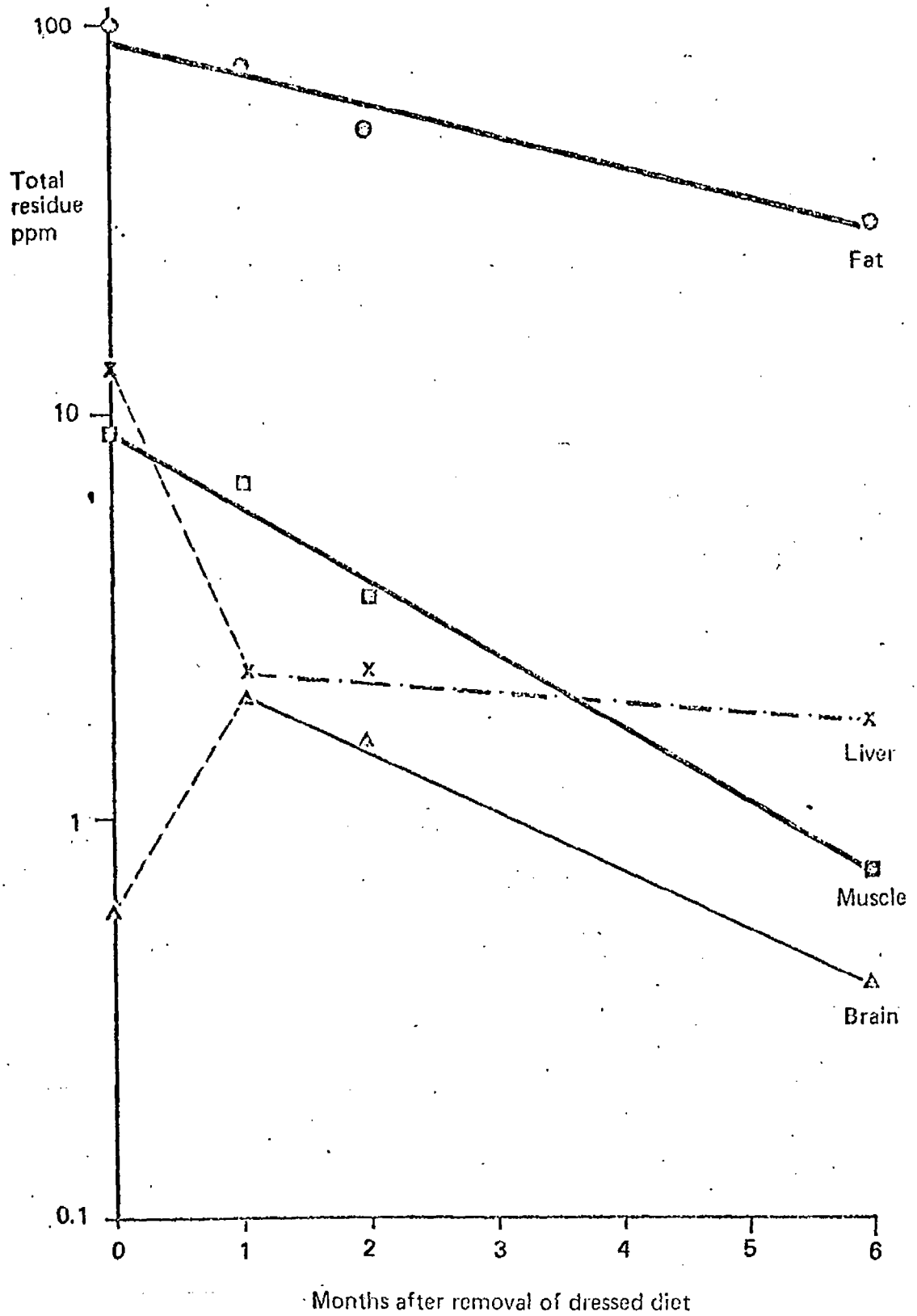

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FIG 3

G.L.C. 'Spectra', normalised to a common peak, of Aroclor 1254 standard and mean Quail liver residues following feeding at 250 ppm for 20 days.

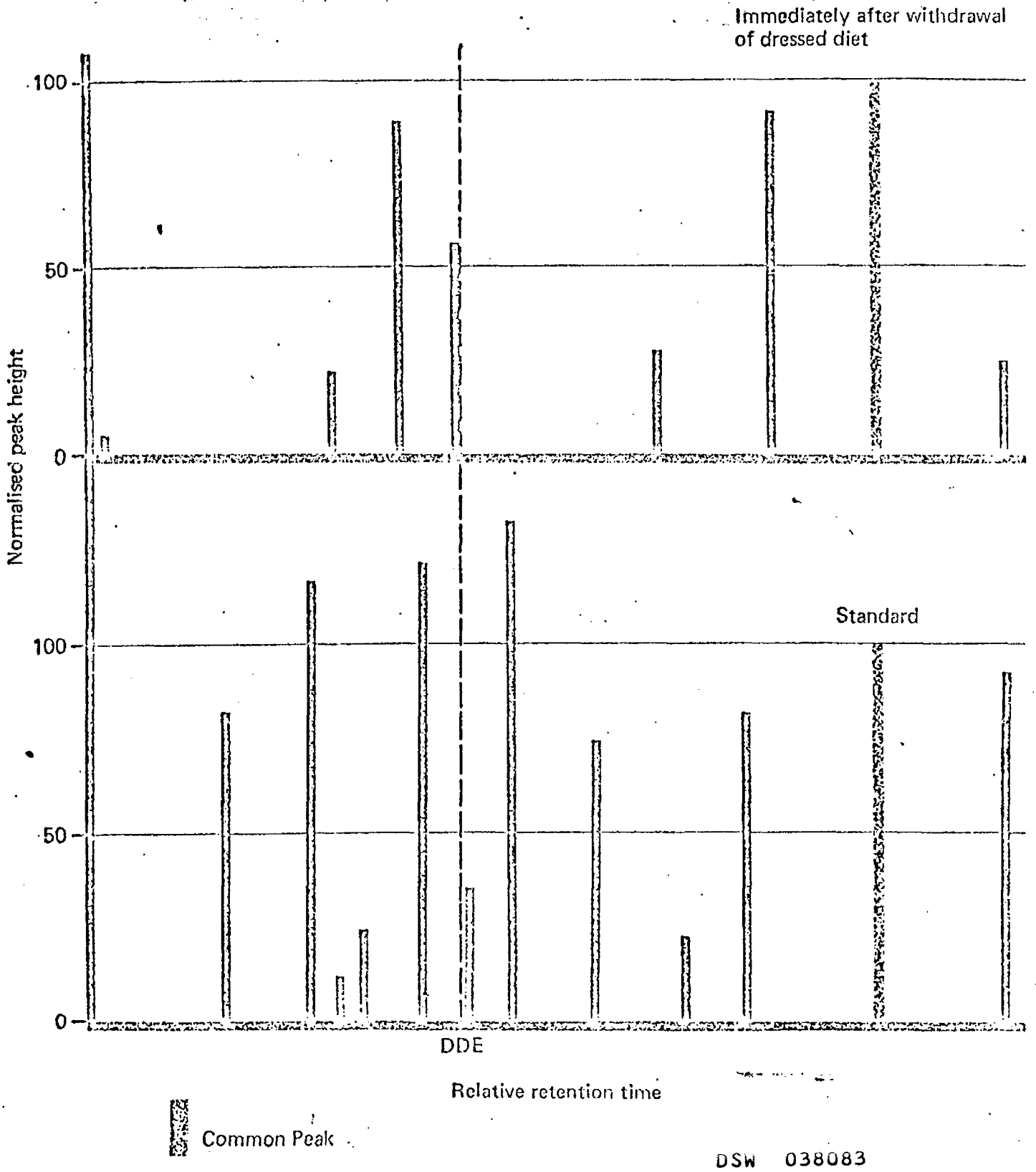

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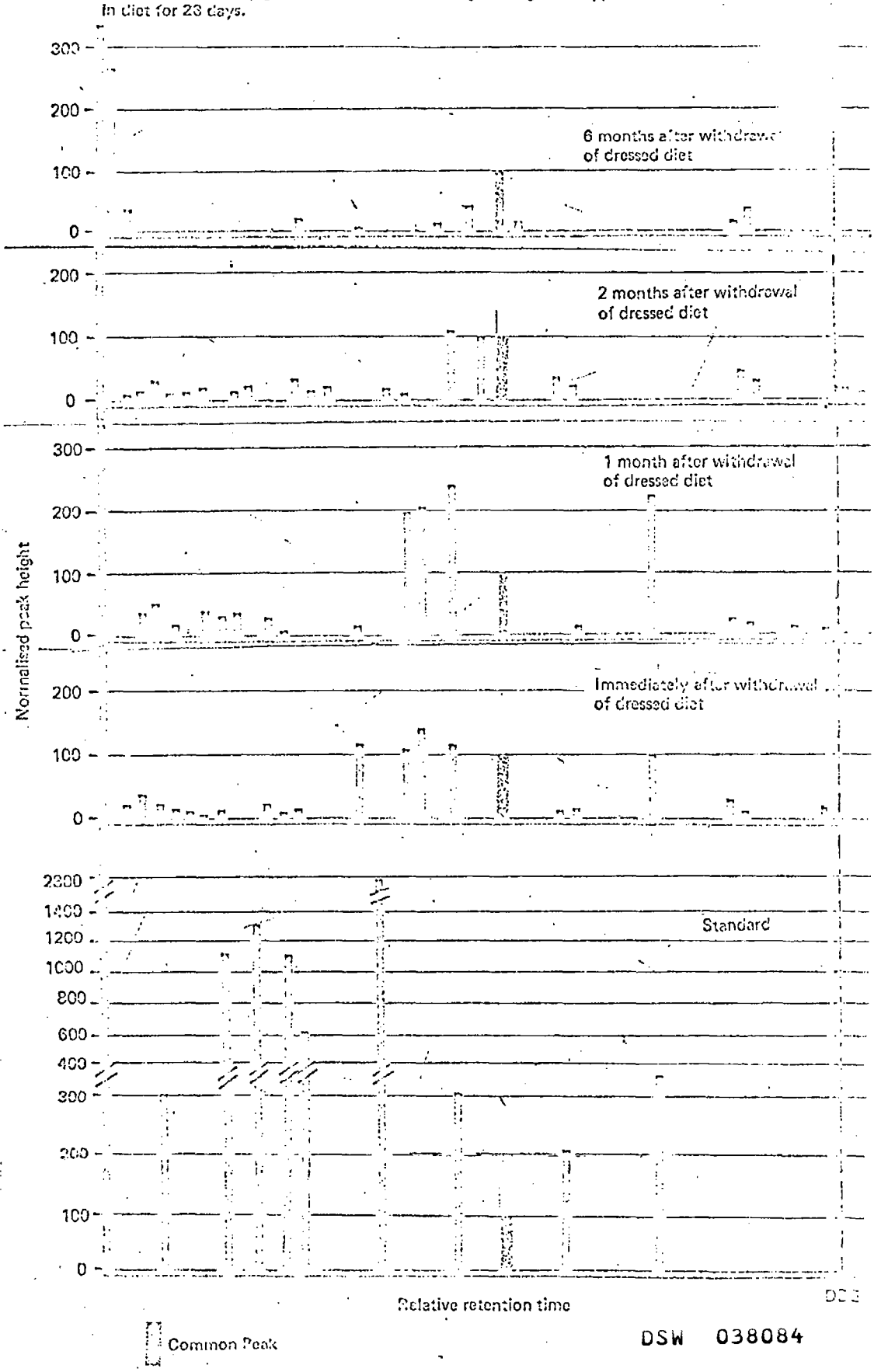
FIG 2.

G.L.C. 'Spectra', normalised to a common peak, of Aroclor 1262 standard and mean pigeon liver residues following feeding at 500 ppm in diet for 28 days.



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