IN THE SERVICE OF SCIENCE

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POISON! - LKB HELPS TO MAKE A SAFER WORLD

Swedish firm produces instrument that detects a previously unobserved poison in fish, fowl and mammal

The Nature of the Poison

The poison that has been isolated is a hitherto unobserved chlorinated hydrocarbon having eight chlorines in the molecule. This polychlorinated biphenyl (PCB) was isolated in residue analysis carried out with the LKB Combined Gas Chromatograph-Mass Spectrometer, LKB 9000.

100 ng of residue from 20 mg of fat, taken from a Sea Eagle found dead in the Stockholm archipelago, was used in the analysis which was made by Sören Jensen, of the Institute of Analytical Chemistry of the University of Stockholm, who has been engaged on this type of research for some considerable time. The dramatic importance of this discovery can best be understood by studying the main characteristics of PCB:

1. Very high stability. Capable of being boiled with nitric acid without destruction.
2. Has hardly any metabolism in living organisms. Can have a steady build-up in the human body and traces have been found in both adults and children.
3. If more than four chlorines are present PCB is non-inflammable.

As well as isolating a poison this research has drawn attention to the need for close co-operation in two parallel lines of investigation. The study of the persistency and toxic effects of herbicides, pesticides and related chemicals, which for some time now has caused general concern throughout the world, and the quest for improved methods of assessing the pollution of industrial waste.

How the Substance was Identified

Initial research seemed to indicate that this, then unknown, poison occured as a result of the use of insecticides; the usual organochlorines present in insecticide research were detected pp^1-DDT, pp^1-DDE and pp^1-TDE and certain unknown compounds

were thought to be metabolites of the insecticides. The location of the poisons in fish, in birds and in pine needles appeared to strengthen this theory. Further investigations however, involving electron capture techniques, gave indications that this supposition was incorrect.

It was, at this stage, decided to use the LKB 9000 Combined Gas Chromatograph-Mass Spectrometer to obtain more definite knowledge of the chemical nature of the unknown compounds, their molecular weight, and number of chlorines for instance, as this was known to be the only method currently available which could give the highest degree of positive identification. Analysis had so far produced a chromatogram from which it was possible to estimate that the Eagle sample, previously mentioned, contained DDT and DDE in extractable fat at a concentration up to 13 g per kg, and the unknown compounds in more or less the same quantities. Many of the retention times however were not in agreement with those for any known pesticides and a high proportion of unknown peaks were present. The purified sample from the Sea Eagle was injected on the LKB 9000 and the mass spectrograms which were obtained of the unknown peaks showed the mass numbers to be as follows: 426, 392, 358 and 324. The molecular difference in each case being 34 mass units thereby showing a family relationship between the unknowns.

Chlorine exists as a mixture of two isotopes with atomic weights 35 and 37 in proportions of 75:25. If the molecule has one chlorine, two peaks will be obtained, one for Cl_{35} and one for Cl_{37}. If there are two chlorines it is possible to have one peak with only Cl_{35}, one with both Cl_{35} and Cl_{37} and one with two Cl_{37} and so on, so the relationship between the peaks on the mass spectrogram were found to be:

Molecular weight:	324	358	392	426
Chlorine content:	5	6	7	8

Having obtained this information it was possible to calculate the molecular weight of the parent hydrocarbon (PHC) and this was found to be 154. The most probable formula for a compound having carbon and hydrogen and giving the molecular weight of 154 is $C_{12}H_{10}$ and this can only be satisfied when the parent hydrocarbon is biphenyl and the unknown polychlorinated biphenyl.

Once this fact had been established an approach to industry elicited the answer that PCB was fairly extensively used, and when a sample was obtained and run through the LKB Combined Gas Chromatograph - Mass Spectrometer it was found that the gas chromatograph gave the same retention time for both the Eagle sample and the industrial sample, and the mass spectrometer gave exactly matching peaks, thus providing absolute verification of the previous analyses.

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To cross check on the results and to more fully evaluate their significance, the fea-

[illegible] Eagle (circa 1860) from a Stockholm [illegible]

lyzed and no trace of poison of any kind was discovered. This research was continued

with other dated specimens and the first trace of DDT and its metabolized [illegible] was discovered in a specimen circa 1948/49 and PCB in 1942

[illegible] been mentioned [illegible] that traces of PCB were found on pine needles which seemed to indicate wind-blown insecticide, but this is undoubtedly due to the fact that where PCB has 40% or more chlorines it is not consumed when industrial waste is burnt but is thrown out in the smoke and air-borne over considerable distances

The effect of this poison on man is not yet fully known. In 1936 tests showed that 93 out of 24 men employed in manufacturing PCB suffered from an acne, which appeared 6 to 8 months after handling the substance, and in 1937 it was possibly responsible for the death of three young workers. Tests so far carried out on animals indicate that in all cases the liver degenerates and that generally the skin and the liver are the parts of the body susceptable to the poison.

Current Research

Jefferies and Walker of the Monks Wood Experimental Station in England have recently carried out experiments to determine the survival and reproduction of Bengalese finches and have found a high proportion contain organochlorines. They are investigating the possibility of using the residue content of the liver as an indicator of the dose consumed.

Simmons and Tatton of the Laboratory of the Government Chemist, London, England have investigated organochlorine residues in human gallstones and have found them present in 28 patients from 2 hospitals.

These two lines of investigation have been concerned with insecticide and pesticides but they do serve to show the wide field in which traces of organochlorines occur

Some twelve O.E.C.D. nations, among whom are the Scandinavian countries, U.S.A., Great Britain, France and Holland are co-operating in research into the prevalence of this virulent and dangerous substance.

LKB

LKB-Produkter AB is fully sensible of the dedication of the scientists taking part in this work in which it has had the honour and privelege to play an important role by providing an instrument, the LKB 9000 Combined Gas Chromatograph-Mass Spectrometer, to give them their first break-through by providing positive identification of PCB

[illegible] Security Department