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On the residues associated with the use of technical grade BHC with special reference to the occurrence and determination of three pentachlorocyclohex-1-ene isomers

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Introduction

Benzene hexachloride, BHC, is the common name used in English-speaking countries for the mixed isomers of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane. In Europe it is known under the latter name which is abbreviated to HCH.

BHC is a persistent stomach poison and contact insecticide, the activity of which is determined by the content of the gamma isomer. A serious disadvantage of technical BHC is that it taints certain crops. A notable exception, however, is rice. In spite of decreased use in favour of other insecticides, notably of the purified active principle gamma-BHC (lindane), considerable quantities of the crude technical product are still used in Africa, South America and Asia. In Europe the use of technical BHC in agriculture is negligible, but there is ample evidence that it is still widely applied on farms for hygienic treatments of stables and cattle. For this reason, the milk in various European countries often contains small amounts of BHC isomers (1, 2, 3).

The determination of BHC residues in foodstuffs is now common practice in most pesticide residue laboratories. However, little information is available about the nature and occurrence of their possible breakdown products in food. A few papers have been published on the fate of lindane in soil (4), vegetables (5, 6), and cereals (7), but published evidence about the degradation products of the other BHC isomers is scarce. It has been established that beta-BHC is enormously persistent (8) and for this reason it has the highest degree of biological accumulation (9, 10). The breakdown pattern of alpha and delta isomers is believed to be identical with that of gamma-BHC (11), but supporting analytical data are lacking.

The present authors have studied the occurrence of BHC residues in various foodstuffs. During this investigation it was found that alpha-, gamma-, and delta-BHC were often accompanied by measurable amounts of their primary dehydrochlorination products which were identified as pentachlorocyclohex-1-ene isomers. This paper describes methods for the isolation, detection and determination of these breakdown products and reports their occurrence in a number of different commodities.

Chemistry and composition of technical BHC

The synthesis, chemistry and composition of crude hexachlorocyclohexane have been described by various authors (12, 13, 14). The main constituents of the

technical product are alpha-BHC 65—70 %, beta-BHC 3—5 %, gamma-BHC 13—16 %, epsilon-BHC 1—3,5 %; and 3—5,5 % consists of other isomers plus higher chlorinated material such as hepta- and octa-chlorocyclohexane. Of the eight theoretically possible stereoisomers, only the five mentioned are to be considered major components of commercial BHC formulas. Our own investigations regarding the composition of technical BHC generally confirmed the figures reported in literature, with two notable exceptions: Japanese BHC was found to contain 10 % of the beta isomer, whereas the analysis of a sample of American origin revealed a gamma-BHC content of not less than 40 %.

In all the samples which were analysed, a considerable amount of lower chlorinated compounds was discovered. During electron capture gas chromatography on 3 % OV-17 at a temperature of 150 °C, appreciable peaks were observed that eluted well before alpha-BHC. Two peaks with short retention times (see under gas chromatography, Table 1) were readily identified as 1, 2, 4 trichloro- and 1, 2, 3, 4 tetrachlorobenzene which are known to be breakdown products of BHC (15). The other three peaks were found to have different retention times than those of 1, 3, 4, 5 tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene respectively.

Table 1

Relative retention times of chlorobenzenes, PCCH- and BHC isomers on three different columns

Compound	3 % OV-17 on Chromosorb W. Temp. 150 °C. Carrier gas flow 60 ml/min.	1,5 % OV-17 / 1,95 % QF-1 on Chromosorb G. Temp. 215 °C. Carrier gas flow 50 ml/min.	2 % DEGS + 0,5 % H ₃ PO ₄ on Chromosorb W. Temp. 170 °C. Carrier gas flow 50 ml/min.
1,2,4 Trichlorobenzene	0,05	< 0,10	< 0,10
1,2,4,5 Tetrachlorobenzene	0,12	< 0,10	< 0,10
1,2,3,4 Tetrachlorobenzene	0,16	< 0,10	0,10
Pentachlorobenzene	0,33	0,23	0,25
Hexachlorobenzene	0,87	0,46	0,41
gamma-PCCH	0,27	0,27	0,34
delta-PCCH	0,48	0,32	0,82
alpha-PCCH	0,69	0,40	1,22
alpha-BHC	1,00	0,50	1,36
gamma-BHC	1,49	0,65	2,20
beta-BHC	2,15	0,73	7,73
delta-BHC	2,56	0,88	6,84
epsilon-BHC	3,15	0,97	6,60
Aldrin	—	1,00	1,00
Absolute retention time of the reference compound in minutes	14,00	5,00	2 1/2

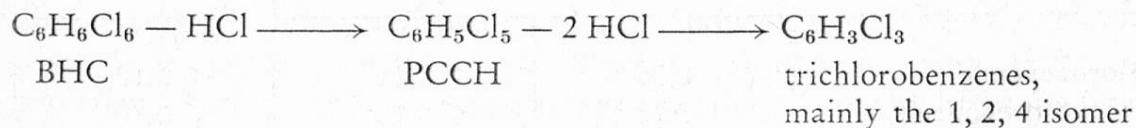
This strongly indicated that these peaks could be due to the presence of primary dehydrochlorination products of the BHC-isomers, i. e. pentachlorocyclohexenes or closely related compounds.

If technical grade BHC contains appreciable amounts of pentachlorocyclohex-1-ene isomers (PCCH's) one would expect to find them individually as slight impurities in the analytical standards of alpha-, gamma-, delta- and epsilon-BHC; the likelihood of finding a PCCH derived from beta-BHC was considered to be very small, because the structure of this isomer does not permit initial trans-dehydrochlorination (8). Upon injecting nanogram quantities of the individual isomers into the gas chromatograph under the conditions described above, alpha-, gamma-, and delta-BHC were found each to be accompanied by a peak which coincided with one of the three peaks observed on the chromatogram of the technical product.

It was, therefore, highly probable that crude BHC really contained alpha-, gamma-, and delta-PCCH, but this could only be ascertained by comparison with authentic samples of the said isomers which were, not being commercially available, prepared by dehydrochlorination of their higher chlorinated homologues.

Preparation of the PCCH isomers

The method used for the preparation of alpha-, gamma- and delta-PCCH was somewhat unusual, being derived from a study on the dehydrochlorination mechanism of chlorinated pesticides on thin layers of alumina (16). During this study it was discovered that neutral Al_2O_3 layers readily degraded a number of chlorinated hydrocarbons at slightly elevated temperature. Under these conditions BHC isomers, except beta, were dehydrochlorinated as follows:



Although this reaction went with considerable velocity, it still permitted an acceptable yield of the intermediate reaction product.

Small scale preparations were carried out by applying 1 mg of the individual isomers (except beta-BHC) as small bands on separate chromatograms. The practical $\text{Al}_2\text{O}_3\text{E}$, Merck alufoil ready-made plates were used. After heating in a stove at 60°C during 45 minutes, the chromatograms were developed twice in pentane + 1 % acetone as a mobile phase. Small strips, representing the length of run, were cut off and photochemically revealed with silver nitrate in order to detect the separated zones. The following results were observed: alpha-, gamma- and delta BHC had yielded four zones which were liberated HCl (at the application point), unchanged parent compound, a monodehydrochlorination product (PCCH) and a diffuse band situated very close to the front, probably representing 1, 2, 4 trichlorobenzene.

The chromatogram of the epsilon isomer did not show any evidence of the presence of a corresponding PCCH isomer. The only visible reaction product was trichlorobenzene, although very little of the parent compound had remained unchanged.

Apparently, loss of HCl from epsilon-BHC occurs more easily than from the other isomers. Once the first molecule of HCl was eliminated, the two other HCl molecules were lost so rapidly that no monodehydrochlorination product was observed. This behaviour indicates that epsilon-BHC is far less stable than the other BHC isomers.

The zones on the chromatograms corresponding with the monodehydrochlorination products of alpha-, gamma-, and delta-BHC observed on the strips were scraped off and the powder thus obtained was repeatedly extracted with small portions of warm benzene. The extracts of each zone were gathered in 25 ml volumetric flasks and subsequently brought up to volume. The purity of the isolated compounds was first checked by both gas and thin-layer chromatography. Both methods indicated the presence of only one compound. The concentration of the benzene solutions was determined in the following way:

2 ml aliquots were refluxed with 20 ml 0,2 N ethanolic KOH during two hours. A blank on the reagents was run simultaneously. After cooling, the reaction mixture was quantitatively transferred to a separating funnel and diluted with 125 ml of distilled water. The trichlorobenzenes formed by the alkaline treatment were extracted by shaking the dilution twice with each time 10 ml of pentane. The combined pentane extracts were washed with distilled water and the pentane was drained in a volumetric flask of 20 ml.

Electron capture gas chromatography of the pentane extracts on 3% OV-17 at 100 °C revealed the presence of 1, 3, 5; 1, 2, 4 and 1, 2, 3 trichlorobenzenes, which were quantitated by injecting standard solutions of the authentic compounds. No interference from the reagents was observed. The total trichlorobenzene content, determined in this way, permitted to calculate the concentration of each PCCH solution. It turned out that the yield of the Al_2O_3 dehydrochlorination procedure was not better than 20% for each isomer.

Although the method of preparation is already strong evidence for the identity of the isolated compounds, one cannot altogether exclude the possibility of confusion with BTC, benzene tetrachloride or tetrachlorohexadiene. However, the gas and thin-layer chromatographic characteristics of gamma-PCCH prepared as described above, were identical with those of the product obtained by mild alkaline treatment of lindane as reported by *Reed and Forgasch* (17). A large scale preparation of delta-PCCH, carried out according to *Cristol et al.* (18), yielded a very pure product that proved to be chromatographically identical with the monodehydrochlorination product obtained from the reaction of delta-BHC with alumina.

There remained some doubt on the nature of the alpha derivative. From its gas chromatographic behaviour, it was clear that it was a PCCH isomer, because a lower chlorinated homologue would have had a much shorter retention time.

See Table 1. The properties of this compound, however, differ considerably from those of gamma- and delta-PCCH. Its retention time on all columns studied was found to be much longer and it possessed a lower electron affinity. Moreover, its R_f -value in the system Al_2O_3 — pentane + 1% acetone proved to be lower than that of its parent compound. See respectively Tables 1, 2 and 3. These anomalies can probably be explained by assuming a change in its stereoisomerism during dehydrochlorination. In fact, its chromatographic and electron capturing properties indicate a beta structure. The designation of alpha-PCCH in this paper denotes, therefore, not a steric relationship with its parent compound, but describes it only as the primary dehydrochlorination product of alpha-BHC.

Reports on the preparation of alpha-PCCH could not be found in the available literature. All attempts to obtain a selective dehydrochlorination of alpha-BHC by conventional means (17, 18) were unsuccessful. It was found that it was rather stable in weak alkalis at room temperature, especially in acetone solutions. Application of stronger alkaline substances or working at higher temperature yielded only trichlorobenzenes.

Analytical methods for the determination of PCCH isomers

Extraction and clean-up

It was considered highly desirable that PCCH's should be determined simultaneously with their parent compounds in order not to complicate the routine analysis for chlorinated pesticide residues.

Accepted methods for the extraction and clean-up of chlorinated pesticides such as the AOAC official procedure (19) and the method of *Langlois et al.* (20) gave recoveries ranging from 70—90% for all three PCCH isomers. A critical step in the analysis is the evaporation of eluates, because gamma- and delta-PCCH are much more volatile than their parent compounds. Evaporation to dryness should, therefore, be avoided.

Gas chromatography

Initial work on the gas chromatography of BHC isomers and their breakdown products was performed on 3% OV-17 at 150 °C. Under these conditions excellent separations were observed for all the compounds of interest. See Table 1. The only drawback was that the retention times of the less volatile compounds such as beta- and delta-BHC were unpractically long.

It was soon found that most stationary phases used for the GLC of chlorinated pesticides were able to separate the three isomers of PCCH under normal conditions. Because of its remarkable separation characteristics, the 1,5% OV-17/1,95% QF-1 column of *Thompson et al.* (21) was considered to be most suitable. Diethylene Glycol succinate, DEGS, a polyester liquid phase (22), was selected for confirmatory purposes, because it permitted elution of the compounds in a different order from that observed on the polysiloxane columns. See Table 1.

The response of the individual isomers of BHC to the electron capture detector has been reported (23), except for that of the epsilon isomer. As is shown in Table 2, the PCCH isomers also have sufficient electron affinity to be detected at low levels, although the response of alpha-PCCH is significantly lower than that of the other isomers.

Table 2 *Relative electron capture response of BHC and PCCH isomers*

alpha-BHC	100
gamma-BHC	99
beta-BHC	39
delta-BHC	103
epsilon-BHC	50
alpha-PCCH	33
gamma-PCCH	53
delta-PCCH	68

Individual responses were calculated by measuring the surface of the peak given by 0,10 nanogram of each compound. The peak furface yielded by the same quantity of alpha-BHC was taken as a unity.

This study was performed on the above-mentioned OV-17/QF-1 mixed phase column. The apparatus used was the Perkin-Elmer F-11, equipped with a ^{63}Ni electron capture detector heated at 240 ° C.

Thin-layer chromatography

Gas chromatography on two different colums is generally considered as adequate proof for identity, but in our opinion it is still desirable to perform confirmatory analysis by thin-layer chromatography (19). Chlorinated pesticides are usually separated on thin-layers of aluminium oxide in pentane + 1 % of acetone as a mobile phase. The spots are detected by photochemical revelation with silver nitrate. This system proved also to be applicable to the TLC of PCCH isomers. See Table 3. Detection limits were often found to be lower than 50 nanograms. If the gas chromatographic results indicated very low levels in a sample, an aliquot was chromatographed without subsequent chemical revelation. The zones corresponding with the R_f -values of the compounds of interest were scraped off, eluted with warm benzene and the eluates were subjected to gas chromatography. In this way the presence of ppb levels of BHC- and PCCH isomers could be confirmed.

Table 3

R_f-values of BHC- and PCCH isomers measured on Al₂O₃ E. Merck ready-made plates when using pentane + 1 % of acetone as a mobile phase

Compound	R _f -value
alpha-BHC	0,31
alpha-PCCH	0,08
gamma-BHC	0,20
gamma-PCCH	0,48
beta-BHC	0,03
epsilon-BHC	0,12
delta-BHC	0,00
delta-PCCH	0,35

Occurrence of BHC- and PCCH isomers in different commodities

An investigation concerning the presence of BHC isomers and their primary dehydrochlorination products in different commodities from all over the world permitted the following observations:

1. Commercial insecticides with technical BHC as a major ingredient often contain varying amounts of lower chlorinated material. Trichloro- and tetrachlorobenzenes are always present in traces, but the concentration of PCCH isomers sometimes exceeds 10 per cent. This means that PCCH residues in foodstuffs are not always metabolites. Their presence may simply be due to treatment with degraded BHC formulas.
2. The distribution of the BHC isomers in various foodstuffs is rather different. Epsilon-BHC is seldom encountered, because it has the lowest persistance of all isomers mentioned. In fact, it is only to be found in vegetable material if the total BHC content is well over 0,5 ppm. In BHC-contaminated foodstuffs of animal origin it generally contributes less than 1 % of the total residue. This means that it can only be detected in grossly contaminated samples.

The presence of low levels of gamma-BHC in cereals can be explained by the general practice of protecting stored grains against pest infestations by fumigation with lindane formulas. Sometimes traces of alpha-BHC are also encountered, but delta- and beta-BHC residues have not been detected.

Beta-BHC is a typical contaminant of animal fats because it is the isomer with the highest rate of biological accumulation. It is mostly accompanied by alpha- and gamma-BHC, but virtually never by the delta isomer.

3. The BHC isomer content of milk varies considerably from one country to the other. In milk from Northern Europe where BHC contamination is generally

very low, the alpha isomer predominates to the extent of about 70 per cent, the rest being mostly gamma-BHC, although the concentration of the beta isomer is not seldom within detectable limits.

In tropical countries where technical BHC is used for the treatment of cows against ectoparasites («cattle dipping»), all four isomers turn up in milk. It is interesting to note that the level of beta-BHC is often as high as that of the alpha isomers, which is no doubt due to selective accumulation.

4. PCCH isomers have mainly been detected in milk. They were present in a significant part of the samples analysed (15%). However, these breakdown products were only detectable when the total BHC content exceeded 0,20 ppm calculated on fat basis. The concentration of alpha-PCCH in the samples varied between 15 and 30% of that of its parent compound. As for delta-PCCH, this compound could always be found when delta-BHC was present. Its mean concentration related to that of its parent compound was about 60%. Gamma-PCCH, however, could not be detected in milk, indicating that this breakdown product is more transient than alpha- and delta-PCCH. Twelve samples of human milk were also analysed for PCCH's but with negative results. Although all samples contained high levels of BHC, the beta isomer predominated to the extent of more than 90%. Sometimes small quantities of gamma- and alpha-BHC were also detected, but the delta and epsilon isomers were definitely absent.
5. BHC and PCCH residues were also determined in a small number of miscellaneous products. The results are listed in Table 5. The highest level was encountered in a sample of adeps lanae (wool fat). There is no doubt that the

Table 4
Occurrence of BHC and PCCH isomers in animal fats, vegetable oils, cow's milk and human milk

Commodity	Number of samples	Number of samples with BHC	Number of samples with alpha-PCCH	Number of samples with delta-PCCH	Number of samples with gamma-PCCH
Animal fats (beef, pork and chicken)	35	28	0	0	0
Vegetable oils and cocoa butter	37	19 mainly lindane	0	0	0
Cow's milk	200	190	31	24	0
Human milk	12	12 mostly beta-BHC	0	0	0

Table 5
BHC and PCCH isomers in miscellaneous products.
All values expressed in ppm

Product	alpha BHC	gamma BHC	beta BHC	delta BHC	epsilon BHC	alpha PCCH	gamma PCCH	delta PCCH
Vermicelli	—	1,00	—	—	—	—	0,10	—
Tomato flakes	—	2,14	—	—	—	—	0,07	—
Tomato flakes	—	2,05	—	—	—	—	0,24	—
Tomato flakes	—	0,19	—	—	—	—	0,005	—
Dehydrated mushrooms	0,12	0,16	0,016	—	—	0,08	0,076	trace
Rice straw feed stuff	0,48	0,14	0,79	0,20	0,10	0,062	0,014	0,068
Lanoline cream, cosmetic product	0,21	0,14	2,04	0,71	<0,01	<0,01	0,20	inter- ference
Adeps lanae (wool fat)	4,7	5,4	10,0	—	—	0,40	3,0	6,0

BHC residues in this raw material can be traced to the practice of sheep dipping. The presence of considerable amounts of PCCH isomers is probably due to the very slow rate of dehydrochlorination in the fat phase.

Conclusions

Although our study only concerns a small number of commodities, the majority being milk samples, it clearly shows that PCCH isomers are of significance as terminal residues of BHC. For this reason, it would be desirable that the routine determination of BHC residues include the analysis for possible present PCCH isomers. There is also a need for studies on the toxicology of these compounds.

Considering that the use of technical BHC is the cause of the widespread diffusion of highly persistent residues in foodstuffs, there seems to be sufficient reason to discontinue its application. However, this objection is not valid against the use of the purified gamma isomer under the name of lindane. Successful insecticidal applications with this compound require far smaller quantities than those used with technical BHC.

Moreover, lindane is more easily broken down than the crude technical product. In fact, residues of gamma-BHC in milk and meat are small in comparison with those of the more persistent alpha- und beta-isomers.

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Summary

The occurrence of various BHC isomers as residues in different commodities is reported. In addition, the authors found that BHC residues are not seldom accompanied by three primary dehydrochlorination products which were identified as pentachlorocyclohex-1-enes (PCCH's) derived from alpha-, gamma- and delta-BHC.

Alpha- and delta-PCCH residues were mainly found in milk samples, although they were only detectable when the total BHC content exceeded 0,20 ppm, calculated on fat basis. Gamma-PCCH has not been found in milk, but its presence was ascertained in vermicelli, dehydrated vegetables and in wool fat.

Methods for the simultaneous gas and thin-layer chromatographic determination of BHC and PCCH isomers are given. Anomalous chromatographic and electron capturing behaviour were observed in the PCCH isomer derived from alpha-BHC. It is not excluded that isomerisation occurs during the dehydrochlorination procedure. The authors presume that the derivative exhibits a beta structure.

Résumé

Les auteurs rapportent que les résidus de plusieurs isomères de l'hexachlorcyclohexane (HCH) sont présents dans différentes denrées de consommation. En outre, ils apportent la preuve qu'il n'est pas rare que ces résidus soient accompagnés par trois produits, résultant d'une première déhydrochlorination et identifiés comme étant les dérivés pentachlorocyclohex-1-ènes (PCCH) respectifs de l'alpha-, gamma- et delta-HCH.

Les résidus d'alpha- et delta-PCCH ont été trouvés principalement dans des échantillons de lait et ils furent seulement détectés quand la teneur en HCH total dépassait 0,20 ppm, calculés sur la matière grasse. Des résidus de gamma-PCCH n'ont pas été trouvés dans ces mêmes échantillons de lait, mais par contre dans des pâtes alimentaires (vermicelles), des légumes déshydratés et de la graisse de laine (Adeps lanae).

Les méthodes de dosage des isomères du HCH et du PCCH par chromatographie en phase gazeuse et sur couche mince sont décrites. On a observé que l'isomère PCCH dérivant de l'alpha-HCH se comportait de façon anormale pendant la chromatographie et vis-à-vis de la capture d'électrons. Il n'est pas exclu qu'une isomérisation se produise pendant le déhydrochlorination. Les auteurs présument que ce dérivé présente une structure beta.

Zusammenfassung

Es wird über das Vorkommen mehrerer HCH-Isomeren als Rückstände in verschiedenen Gebrauchsgütern berichtet. Die Autoren haben ferner gefunden, daß HCH-Rückstände nicht selten von drei primären Chlorwasserstoffabspaltungsprodukten begleitet sind, die als von Alpha-, Delta und Gamma-HCH abgeleitete Pentachlorcyclohex-1-ene (PCCH) identifiziert wurden.

Alpha- und Delta-PCCH-Rückstände wurden hauptsächlich in Milchproben aufgefunden, obwohl sie nur dann nachweisbar waren, wenn der Gesamtgehalt an HCH über 0,20 ppm lag (auf das Fett bezogen). Gamma-PCCH wurde nicht in Milch gefunden, seine Anwesenheit aber in Teigwaren, Trockengemüse und Wollfett festgestellt.

Methoden zur gleichzeitigen gas- und dünnsschichtchromatographischen Bestimmung der HCH- und PCCH-Isomeren werden angeführt. Anormales chromatographisches und Elektroneneinfangs-Verhalten wurden beim vom Alpha-HCH abgeleiteten PCCH-Isomer beobachtet. Es ist nicht ausgeschlossen, daß eine Isomerisation während der Chlorwasserstoffabspaltung stattfindet. Die Autoren vermuten, daß das Derivat eine Beta-Struktur aufweist.

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