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Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade
Chemical Review Committee
Fifth meeting
Rome, 23–27 March 2009
Item 4 (b) (vii) of the provisional agenda*

Listing of chemicals in Annex III to the Rotterdam Convention: review of notifications of final regulatory actions to ban or severely restrict a chemical: hexachlorobenzene

Hexachlorobenzene

Note by the Secretariat

Addendum

Supporting documentation provided by Japan

The Secretariat has the honour to provide, in the annex to the present note, documentation received from Japan to support its notification of final regulatory action on hexachlorobenzene as an industrial chemical.

* UNEP/FAO/RC/CRC.5/1.

K0842869 161208

Annex

- Biodegradation and bio-accumulation data of existing chemicals (by the Chemicals Evaluation and Research Institute, Japan: CERJ)
  http://qsar.cerij,orjp/cgi-bin/DEGACC/result_head.cgi?STRID=00033&LANG=ENG
- Hexachlorobenzene: Focused Summary.
- IPCS INCHEM JMPR-Monographs and Evaluations
  http://www.inchem.org/documents/jmpr/jmpmono/v069pr20.htm

National Institute of Technology and Evaluation
独立行政法人
製品評価技術基盤機構

Biodegradation and Bioconcentration of Existing Chemical Substances under the Chemical Substances Control Law

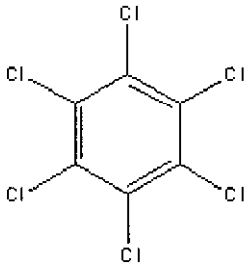
Description Biodegradability Bioconcenteration

Information on the chemical published in the Official Bulletin of Economy,Trade and Industry (Former title:The Official Bulletin of the Ministry of International Trade and Industry (published before Jan.6,2001))

Published Chemical Name	Published Date	Published result
hexachlorobenzene	1975/8/27	Chemical substance determinated to be persistent and highly bioaccumulative

Chemical Information

Structural formula



CAS Registry No.	118-74-1
Name of Chemical targeted for testing	hexachlorobenzene

Class-Reference No. in The Gazetted List	Existing Chemical Substances Name in The Gazetted List
3-76	Poly (4-6) chlorobenzene

Biodegradation

Judgement	Non-biodegradability
Test Method	MITI-I(OECD TG 301C)

Test Equipment	Test period	Chemical Concentration	Concentration of Activated Sludge
Standard type	2weeks	100ppm	30ppm

Indirect Analysis	BOD
	0%

Direct Analysis	GC
	2.5%

Bioconcentration

Judgement	High bioconcentration
Test Method	Bioconcentration test

LC50(48hr)	Species
(-)*	-

Test Equipment	Test period	Species
Standard type	8weeks	Carp(Cyprinus carpio)

	Test Concentration Set	BCF
1st Concentration area	0.5ppb	11000 - 27000
2nd Concentration area	0.05ppb	6000 - 30000

Remarks

* Minimum Detectable Concentration (MDC) of the test substance was set at two concentrations, namely, 1/10 and 1/100 of the detection limit of the test substance, determined by the analytical instrument employed, and thus no LC50 test was conducted. Therefore, the similar LC50 test was conducted at the concentrations 1000 and 10 times higher than the above-mentioned detection limit, which was used to confirm the safety of the test fish at the set concentrations.

Total Search System
for Chemical Substances
118-74-1

Return

The Japanese DNA for the PIC Convention would like to submit the following information on our notification of final regulatory action on Hexachlorobenzen.

Focused Summary
Hexachlorobenzene

1. Introduction

a) The events that led to the regulatory action

The government of Japan designated Hexachlorobenzen as Class I Specified Chemical Substances under the Chemical Substances Control Law.

b) Significance of regulatory action, eg one use or many uses, level or degree of exposure

Since Hexachlorobenzen was classified as Class I Specified Chemical Substance in August 1979, manufacture, import and use of Hexachlorobenzen have been banned.

c) An overview of the regulatory system of the notifying country if relevant

The government of Japan anticipates that persistent and highly bio-accumulative chemical substances with long-term toxicity (e.g. PCBs) may cause irreversible environmental pollution and have adverse effects on human health or the environment. In order to prevent environmental pollution and adverse effects on human health, the Chemical Substances Control Law stipulates that hazardous properties of chemicals should be checked based on the existing knowledge or by the tests which are consistent with the methods of the OECD Test Guidelines, conducted by the OECD GLP facilities. The government designates the chemical substances that are identified as not-biodegradable (persistent) and bio-accumulative as Type I Monitoring Chemical Substances. Manufacturers and Importers of Type I Monitoring Chemical Substances are required to report the quantities they manufactured and imported. If a Type I Monitoring Chemical Substance is identified as having long-term toxicity for human beings and animals at the top of food chain (higher predators), the government designates that chemical substances as Class I Specified Chemical Substances and are subject to final regulatory action (ban on manufacture, import, and use). The Ministry of Health, Labour and Welfare, the Ministry of Economy, Trade and Industry and the Ministry of the Environment are responsible for these regulations of chemical substances which are persistent, highly bio-accumulative and toxic for long time.

d) Scope of the regulatory action – precise description of the chemicals subject to the regulatory action

Aforementioned in b), it has been banned that manufacture, import and use of Hexachlorobenzen.

2. Risk Evaluation

a) Key findings of the national risk evaluation

Hexachlorobenzen is placed in the category of Class I Specified Chemical Substances based on the following judgment.

1. Biodegradation: Based on the report of the biodegradation test with microorganisms, the percentage of biodegradation is 0% and 2.5%, measured by BOD and by GC, respectively. Thereafter, Hexachlorobenzen is considered to be very persistent.

2. Bioaccumulation: Based on the report that BCF for fish exposed is 11,000-27,000 (high exposure level case) and 6,000-30,000 (low exposure level case), Hexachlorobenzen is considered to be very bio-accumulative.

3. Long-term toxicity: A laboratory animal study on rats indicates that hexachlorobenzen decreases the pregnancy rate and also decreases the survival rate and body weight of pups. Another study on hamsters suggests its carcinogenic effect in the liver, blood vessels and the thyroid.

b) Key data reviews consulted and a brief description

Biodegradation and Bioaccumulation data

http://www.safe.nite.go.jp/data/hazkizon/pk_e_kizon_disp.html?k_no=0011D

c) Reference to national studies, eg toxicological and ecotoxicity studies

No information.

d) Summary of actual (potential) human exposure and/or environmental fate

Hexachlorobenzen concentration in water, sediment, organism, and air was 5 pg/l, 1 pg/g-dry, 3.8 pg/g-wet, and 0.03 pg/m^3 in 2005. Hexachlorobenzen was detected in the water of some wells in the past.

3. Risk Reduction and Relevance to Other States

a) Estimates of the quantity of chemicals used, or imported/exported at the time of the regulatory action and if possible information on ongoing trade

Production volume for synthetic rubber and other industrial chemical products was around 500 tones per year before 1975.

b) Relevance to other States i.e. those with similar conditions of use

No information.

c) Comments on the typical use of the chemical within the notifying country, with comments on possible misuse (if appropriate)

In Japan, Hexachlorobenzen was produced for industrial use such as synthetic rubber.

FAO/PL:1969/M/17/1

WHO/FOOD ADD./70.38

1969 EVALUATIONS OF SOME PESTICIDE RESIDUES IN FOOD

THE MONOGRAPHS

Issued jointly by FAO and WHO

The content of this document is the result of the deliberations of the Joint Meeting of the FAO Working Party of Experts and the WHO Expert Group on Pesticide Residues, which met in Rome, 8 - 15 December 1969.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

Rome, 1970

HEXACHLOROBENZENE

IDENTITY

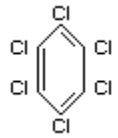
Chemical name

hexachlorobenzene

Synonyms

HCB
perchlorobenzene

Structural formula



Other relevant chemical properties

Colourless white powder or needles MP 229°C - BP. 326°C. V.P. 1.089 × 10^{-5} mm Hg at 20°C - sublimable. Insoluble in water and alcohol. Soluble in hot benzene. The technical grade used in agriculture

contains 98 percent hexachlorobenzene, 1.8 percent pentachlorobenzene together with 0.2 percent 1,2,4,5-tetrachlorobenzene.

Commercial formulations (dusts) contain 10-40 percent HCB alone or together with small quantities of lindane (0.5-1.0 percent) added to prevent insect attack on stored seed.

EVALUATION FOR ACCEPTABLE DAILY INTAKE

BIOCHEMICAL ASPECTS

Residues of hexachlorobenzene are stored in the liver and fat of birds. The biological half-life of hexachlorobenzene in the quail is about three weeks (Vos et al., 1968) (see also "Short-term studies. Quail").

When hexachlorobenzene, 400 mg/kg, was administered orally to rabbits, the compound did not appear to be metabolized, because the main portion was found in the gut contents five days after dosing, with only 6 percent appearing in the faeces. There was no significant urinary or pulmonary excretion of metabolites. Hexachlorobenzene did not form conjugated glucuronic acids, ethereal sulphates or mercapturic acids. Most of a dose (100 mg/kg) given subcutaneously was found at the site of injection after five days, with no chlorinated benzenes being found in the faeces over this period (Parke and Williams, 1960).

There is evidence that hexachlorobenzene (or a toxic metabolite of it) is excreted in milk. Two pregnant female rats were given hexachlorobenzene in their food (the dose was not stated, it was presumed to be 0.5 percent). One died, but the other was delivered normally and reared the young until they died with convulsions after seven to eight days. The mother was then given three normal week-old rats to foster, which died three to four days later with convulsions. The mother also died four weeks later with the usual symptoms of hexachlorobenzene intoxication. This study indicated that a toxic compound was excreted in the milk (De Matteis et al., 1961).

TOXICOLOGICAL STUDIES

Acute toxicity (oral)

Animal	LD_{50} mg/kg body-weight	References

Mouse	4000	Savitskii, 1964
Rat	3500	Savitskii, 1964
Guinea pig	> 1000	Melis, 1955
Rabbit	2600	Savitskii, 1964
Cat	1700	Savitskii, 1964

Short-term studies

Chicken

Hexachlorobenzene given at levels of 120-480 ppm in the diet for three months, caused no toxic effects in chickens (Melis, 1955).

Guinea-pig

A diet containing 0.5 percent of hexachlorobenzene was given to guinea-pig and to mice. These two species proved to be remarkably susceptible to hexachlorobenzene and developed very marked neurological symptoms within eight to 10 days (De Matteis et al., 1961).

Mouse

See under "Guinea-pig".

Quail

Five groups of adult Japanese quail (10 females and two males per group) were fed a mixed diet containing 0, 20, 100, 500 or 2500 ppm of hexachlorobenzene for three months. Eggs from the 100 ppm, 20 ppm and control groups were collected during three periods and incubated to determine reproduction results. At the 2500 ppm level four birds died within a week, the remainder within a month. Pre-mortem effects noted were loss of weight, drooping wings, ruffled feathers, trembling, ataxia and paralysis. On examination, bones, liver and kidney showed a red fluorescence typical of porphyria. Degeneration and necrosis of liver cells was seen. At 500 ppm all birds died within a month, showing the same symptoms and lesions seen at the higher level. At 100 ppm, the first bird died on day 20, and 10 were dead within seven weeks. An accumulation of porphyrins in liver and kidney was observed in all birds in the group. Microscopic examination showed degeneration, necrosis and local regeneration of liver cells. At 20 ppm all birds survived the three months test-period and did not

develop any visible symptoms. At autopsy one hen showed fluorescence of bones and liver in gross ultraviolet examination. Fluorescence of liver and kidney cells and swelling and regeneration of liver cells was observed in this bird. In the other hens fluorescence of kidney cells was seen under ultraviolet microscopy. Significant differences were not found in body-weight or in relative weights of liver, spleen and brain, nor was there any effect on egg production at 20 ppm. There was a significant reduction in the number of chicks hatched, however, at this level of hexachlorobenzene. It was tentatively concluded, considering the disturbance in porphyrin metabolism and the diminished reproduction results, that the no-effect level in this species is lower than 20 ppm. (A comparative test in rats indicated the quail to be much more sensitive). Residues in the liver of the birds fed hexachlorobenzene were as follows:

TABLE I

Level of hexachlorobenzene fed (ppm)	History	Number of birds	Levels of hexachlorobenzene in the liver (ppm) Average	Range
500	died	9	450	180-850
100	died	6	235	85-720
20	killed	6	36	14-94
20	killed 33 days after termination of hexachlorobenzene-feeding	6	13	5-29

Residues were also found in eggs and in the chicks after hatching. Several predatory birds, most of them found dead, had liver residues of hexachlorobenzene in the same range as those in the 20 ppm group of the quail study. It was concluded that hexachlorobenzene used as a seed-dressing, may have toxic effects on seed-eating and predatory birds (Vos et al., 1968)

Rabbit

Female rabbits were fed a diet containing 0.5 percent of hexachlorobenzene. After about six weeks an increase in urinary porphyrins was noted. Unless sacrificed earlier, the animals died in eight to 12 weeks after having demonstrated neurological symptoms. (De Matteis et al., 1961).

Rat

Groups of 10 male and 10 female rats were fed diets containing hexachlorobenzene at levels of 0, 5, 25, 125, and 625 ppm for 13 weeks. After 13 weeks five males and five females from each group were sacrificed for gross and microscopic examination. The rats left in the groups given 125 and 625 ppm were transferred to the control diet for a further two weeks, then sacrificed and examined. The rats in the 625 ppm group displayed signs of marked respiratory involvement; slight tremors were also observed in several animals and the females exhibited small sores in the skin of the head or neck. None of the other groups showed these effects. Growth of male rats at the highest level fed was reduced. Survival was not affected at any dose-level. Total leukocyte counts were elevated during the test in the group fed 625 ppm. Organ-weight data showed an increase at 625 ppm, in thyroid, liver, spleen and adrenals, and in male rats at 125 ppm, in liver. Microscopic examination showed consistent effects in the liver (lobular distortion, nuclear and cytoplasmic variations, focal loss of cell outline, mitotic activity, and focal cellular necrosis) of the rats at 125 and 625 ppm. Changes of a less distinct and less consistent nature were observed in thyroid, kidneys, adrenals and bone-marrow of animals in these two groups. No significant effects were observed in organs and tissues of the rats fed at 5 or 25 ppm of hexachlorobenzene (Weir, 1962).

Female rats were fed a standard died containing 0.2 percent hexachlorobenzene. After one week, weight-loss and general debility was noted in some rats. Within three weeks a few rats showed increased urinary excretion of porphyrins, but this manifestation did not become general until the seventh or eighth week. Rats with well established porphyria were exposed continuously to ultraviolet light. If an area of about 25 cm^2 was plucked or shaved before exposure, most of the

animals died within three to 14 days, but a few rats survived three to four months of this treatment. Rats on the hexachlorobenzene diet that were not shaved or plucked tolerated the ultraviolet light exposure well. After receiving hexachlorobenzene in the diet for six to eight

months, the rats were returned to a normal diet. Urinary porphyrin excretion decreased considerably over the first four months but was still above normal levels nine months after hexachlorobenzene has been removed from the diet (Pearson and Malkinson, 1965).

A diet containing 0.2 percent of hexachlorobenzene was fed to 26 male rats. Groups of two or three animals were killed at weekly intervals over a period of one to 12 weeks. Retardation of weight-gain occurred in the second and third week of the experiment. Evidence of a localized toxic effect was confined to the liver. An increase in liver-weight reached a maximum from the fifth to the ninth week and then the weight of the organ declined. Degenerative changes in the liver similar to those reported in earlier studies occurred. Porphyrinuria and pathological amounts of porphyrin in liver, bones and marrow were observed (Campbell, 1963).

Rats were given hexachlorobenzene at a level of 2 percent in the diet. After appearing normal for the first 10-12 year, clinical and biochemical signs of porphyria then appeared rapidly. There was a considerable loss in body-weight, the mean for 13 rats being 25 percent of the initial weight. Cutaneous eruptions on the head, back and feet occurred. Generalized tremor was observed. Porphyrinuria was seen after two weeks of feeding hexachlorobenzene. Many rats died after three to four weeks. Adenosine-5-monophosphoric acid, 20 mg daily, was given by intramuscular injection to some of the rats, starting on the 13th day of the experiment. By the 28th day, there was a striking difference between most of the adenosine-5-monophosphoric acid-treated and the other rats. Loss of weight was diminished, no new cutaneous eruptions appeared and those that had existed healed completely. There was a reduction in the urinary excretion of porphyrins. Despite these improvements, however, some of the rats treated with adenosine-5-monophosphoric acid died towards the end of the fourth week of feeding hexachlorobenzene (Gajdos and Gajdos-Torök, 1961a, 1961b, 1961c).

A group of 33 male rats were fed a diet containing 0.2 percent of

hexachlorobenzene. Within the first month 13 rats died, exhibiting terminal tremor, ataxia, weakness and paralysis. In the remaining rats, a significant increase in urinary excretion of porphyrins and porphyrin precursors was first noted after two to eight weeks of hexachlorobenzene administration. In rats in which hexachlorobenzene was discontinued shortly after peak excretory values were reached, there was a return to normal excretion values within a week. However, maintenance of high levels of porphyrin excretion by continued feeding of hexachlorobenzene for two to three weeks resulted in an apparently irreversible porphyric state, in which marked porphyrinuria continued in spite of removal of hexachlorobenzene from the diet. Hepatomegaly was common in the porphyric rats. Histological studies showed liver-cell degeneration (Ockner and Schmid, 1961).

Long-term studies

No information available.

OBSERVATIONS IN MAN

Several published reports have described an outbreak of a cutaneous type of porphyria that began in southeastern Turkey in 1955. The total number of cases over a five-year period has been estimated at 3000-5000. Most of these were in children. The clinical manifestations consisted of blistering and epidermolysis of the skin in areas exposed to sunlight, particularly the face and hands. The lesions healed poorly. Hyperpigmentation was invariable, usually accompanied by marked hypertrichosis which was not limited to the exposed skin areas. The urine contained large quantities of porphyrins. Weight loss and hepatomegaly were frequently present. Neurological symptoms did not appear to be evident but abdominal pain was reported in some cases and liver enlargement was present in 35 percent of the cases surveyed. In many cases bone and joint changes occurred, in some patients there was osteoporosis of the bones of the extremities and interphalangeal arthritis. The outbreak was traced to the consumption of wheat, intended for planting, that had been treated with hexachlorobenzene. It was estimated that the amount of hexachlorobenzene ingested by the persons affected was from 50 to 200 mg/day for a relatively long period before the disease became apparent. After stopping the consumption of bread made from hexachlorobenzene-treated wheat, the acute skin manifestations disappeared in about 20 to 30 days. Urinary findings reverted to normal in most patients. Relapses during the

summer months were often seen. However one to two years after the termination of other symptoms of porphyria the joint lesions were found to be still present. It was concluded that the disease was the result of a metabolic disorder caused by interference with porphyrin metabolism in the liver by hexachlorobenzene. Upon recognition of the situation, the use of hexachlorobenzene as a fungicide was discontinued in 1959. Subsequently the disease gradually disappeared (Cam and Nigogosyan, 1963; Cetingil and Ozen, 1960; Dogramaci, 1961, 1962, 1964; Dogramaci et al., 1962a, 1962b, Schmid, 1960, Wray et al., 1962).

COMMENT

Evidence is presented that hexachlorobenzene is a highly toxic compound. There is insufficient information on metabolism especially in relation to the toxic compound excreted in milk. Use effect on the bone-marrow gives rise to serious concern. In addition, no long-term studies or studies on reproduction are available and there is little information on the effect in mammalian species other than the rat. For those reasons no acceptable daily intake can be established. However, unintentional residues have been found in a variety of food commodities in many countries. The Meeting therefore agreed to consider the short-term study in rats as a basis for establishing a tentative negligible daily intake using an extremely high safety

factor. It was stressed, however, that the use of this compound is highly undesirable and a search for a more suitable substitute is strongly recommended. In addition, extreme precautions should be taken to prevent treated seeds and seed-grains from being consumed by humans or farm animals.

TOXICOLOGICAL EVALUATION

Level causing no toxicological effect

Rat: 25 ppm in the diet, equivalent to 1.25 mg/kg body-weight/day

ESTIMATE OF TENTATIVE NEGLIGIBLE DAILY INTAKE

0 - 0.0006 mg/kg body-weight.

RESIDUES IN FOOD AND THEIR EVALUATION

USE PATTERN

Pre-harvest treatments

Seed wheat is treated with HCB to destroy seed-borne and soil-borne spores of Bunt fungi (Tilletia spp.) and to ensure freedom from Bunt in the subsequent crop. HCB has proved outstanding for this purpose and has been used widely since being first introduced in 1945. (Costa 1952, Holton and Purdy 1957, Meagher 1953, Purdy 1961). Wheat infected with "Bunt" or "Stinking Smut" has a bad appearance, an unpleasant smell and is unsuitable for milling. "Bunt" can be regarded as a most important and common disease of wheat.

Wheat crops become infected after sowing of seed, which has live Bunt spores on its surface. These spores germinate and enter the young plant, growing inside it throughout the season. At harvest a black mass of spores replaces the starch of the grain whilst the skin of the grain remains unaffected. During harvest these "Bunt Balls" are broken, thus releasing myriads of spores over the clean grain, which if sown without treatment, will lead to heavily infected crops.

HCB is usually applied in the form of dust containing 10-40 percent a.i. but in Canada liquid preparations are also used (Houghton 1969). Formulations are coloured to assist in application and to distinguish treated seed from other grain. Usually, a blue pigment is used but sometimes carbon black or a red dyestuff is added to reduce the risk of treated seed being used for food. In Turkey where treated seed was used for food with disastrous consequences it was proposed that a denaturant with a strong taste or smell be added as well as colour.

In Australia and various other countries, wheat is selected for seed at harvest time for the next season and after grading to remove small or broken grains, fungicide is added automatically by machinery at the rate of 1-2 ozs 30 percent HCB dust per bushel (330 ppm). The treated

seed is stored in labelled jute bags until required for planting some months later. There is no evidence of significant contamination of commercial grain or animal feeds during the process of applying the HCB dust. The use of untreated seed wheat invariably leads to heavy losses from fungus diseases (Bunt or Stinking Smut) and it is therefore standard practice to treat sufficient seed for the

anticipated needs of the next season. It is exceptional for farmers to buy seed: they normally produce and save for their own requirements.

Farmers are directed not to use treated seed wheat for animal feed or to allow it to become mixed with commercial grain. Although such directions are normally observed, the presence of 1 bushel of seed wheat in 10,000 bushels of commercial grain is sufficient to lead to significant residues in eggs from hens receiving such grain in their ration. Contaminated grain sacks, machinery, storage premises, transport and dust from commercial grain all contribute to the residues found in animal products.

In Canada, seed wheat is usually treated just before planting. Although some dusts are used, aqueous suspensions suitable for application as a coarse spray are favoured. As an alternative, "drill-box" treatments have been developed whereby the fungicide dust is added to the seed wheat in the feed box of the seed drill where it is distributed by simple agitation. Such drill-box treatments are less effective but appear capable of reducing cross contamination of other grain, (Wallace 1966).

Statistics on the production or use of HCB are not available for countries other than Australia. In Australia 12 million bushels of seed wheat are treated annually with HCB dust, requiring 200 tons of technical HCB. A smaller proportion of the total crop is probably treated in U.S.A., Canada, U.K. and some European countries but there is apparently an extensive use in Turkey, Italy, Spain, Netherlands, Germany, France and some Eastern European countries.

RESIDUES RESULTING FROM SUPERVISED TRIALS

At the time of the original introduction of HCB as a seed dressing and for many years afterwards, the analytical methods were not capable of detecting the level of residues found in commercial grain or animal products. The only trial results available are those from Australia.

In animals

Craig (1959) fed HCB treated wheat to poultry at the rate of 1´ oz per bird per day for six months. At the end of this period the fat of the birds contained more than 300 ppm HCB. Craig and Dwyer (1961) fed seed

wheat containing 660 ppm HCB to aged sheep at the rate of 4 oz and 12 oz per head per day. After four months those receiving 4 oz per day (80 mg HCB/day) had 330 ppm in the fat but none in liver or kidney. Those on 12 oz per day (240 mg HCB/day) had 880 ppm in fat and 200 ppm in liver and kidney.

Gardiner and Armstrong (1960) fed two pigs on HCB treated wheat containing 660 ppm HCB for 71 days during which each consumed 329 lbs of wheat. Animals gained 50-98 lbs in weight and at slaughter fat contained 1000 ppm of HCB.

Watts (1968) reports trials where HCB residues were determined in eggs and body fat of poultry receiving HCB treated wheat. Three groups of chickens were fed wheat containing 340 ppm HCB for seven, fourteen and twenty-eight days before being given untreated grain for twenty-eight days. The maximum amount of HCB found in egg yolk was 76, 167 and 146 ppm respectively. The maximum amount in body fat of the same chickens was 178, 349 and 528 ppm respectively. Controls fed on grain believed to be free of residues showed 0.84 ppm in egg yolk and 3.3 ppm in fat. It was found that the untreated controls received 0.02 ppm HCB in their rations.

Later trials reported by Watts (1968a) showed that four groups of chickens fed rations containing 0.02 ppm, 0.08 ppm, 0.7 ppm and 7 ppm HCB for two months showed maximum residues of HCB in egg yolk of 0.2 ppm, 0.3 ppm, 2 ppm and 15 ppm respectively. Body fat from the same chickens contained up to 0.7, 0.7, 5 and 29 ppm of HCB. Half of the birds receiving the above rations were given treated grain after one month. At the end of the second month the HCB residues in egg yolk ranged from 0.18 to 0.2, 0.2 to 0.25, 0.7 to 1.2 and 6 to 12 ppm respectively. The body fat of these birds contained HCB ranging from 0.6 to 0.7, 0.7 to 0.9, 1.5 to 2.6, 16 to 24 ppm respectively. It was concluded that residues decline only slowly when birds which have previously ingested HCB receive untreated feed for one month.

De Vos et al. (1968) as a result of identifying HCB in tissues of wild birds in the Netherlands carried out semichronic toxicity tests on Japanese quail and measured the HCB residues in liver, blood and fat.

Whilst exceptionally high levels were found in birds receiving toxic concentrations in their diet, birds fed on diets containing 20 ppm of HCB showed HCB residues in the fat of 350 to 520 ppm after three months. The author concluded that the half-life of HCB in the quail is about three weeks.

Wit (1969) reports no HCB in an extensive survey of animal fats examined in the Netherlands but he notes that HCB would be obscured by and reported as alpha BHC.

In plants

Johns (1969) reports results from six samples of wheat grown from HCB treated seed in different soils. Residues range from 0.003 ppm to 0.062 ppm. Two samples of wheat grown from seed that had not been treated with HCB showed 0.001 ppm and 0.003 ppm but it is not known whether HCB treated wheat was grown in the name soil in previous years.

Smith (1969) reported trials where wheat treated with 416 ppm HCB was planted and the resulting grain analysed. Residues of 0.0033 ppm were found. Untreated seed yielded grains containing 0.0022 ppm HCB. Soils from twelve wheat farms were analysed and all showed traces of HCB ranging from 0.001 to 0.02 ppm though nine were below 0.003 ppm.

Old (1969) reports investigations with wheat containing 0.05 ppm HCB, to determine the effect of milling on the distribution of HCB residues in the various milled fractions, and the HCB residues in bread made from the flour fraction. Using wheat containing 0.05 ppm HCB it was found that residues were distributed in all main milling fractions. The total residues recovered were equivalent to 0.049 ppm. Of this 42 percent was recovered in the bran, 36 percent in the pollard and 22 percent in the flour. The flour which was found to contain 0.015 ppm HCB produced bread containing 0.0024 ppm HCB. Residues in bread were thus only 16 percent of the residues in the flour used for the making of bread. Further confirmation of the loss during baking was obtained from flour containing 0.006 ppm HCB which produced broad containing 0.0014 ppm HCB or 23 percent of the amount in the flour used.

There is extensive evidence from the literature that HCB is volatile

in water vapour even at low temperatures. It is assumed that the loss during baking in due to steam volatilisation.

FATE OF RESIDUES

As far as can be gauged the HCB applied to seeds in not broken down by physical, chemical or biological agencies but is simply dissipated and diluted in the soil and atmosphere.

Because of the extreme stability of the molecule, solubility in fatty tissue and the ease with which animals extract and concentrate residues of HCB from their feed into their fatty tissues residues are readily detectable in animal products.

The inadvertent contamination of commercial grain and animal feeds with traces of HCB, though undesirable, appears hard to eliminate.

Evidence of residues in food in commerce or at consumption

A considerable amount of the date available results from surveys conducted to determine the level and source of HCB residues in eggs, meat fat and dairy produce from individual farms. Except in isolated instances where farmers have not observed instructions not to feed treated seed grain to domestic animals, the contamination invariably results from the inadvertent feeding of grain or milling fractions containing low levels of HCB residues. It is not possible for farmers to know that such animal rations are contaminated and in view of the slow rate of excretion the retention of residues in fatty tissues of such animals presents serious economic and administrative problems.

The following table given results of an extensive survey of HCB residues in animal products calculated on a fat basis.

TABLE II

HCB residues in Animal Products

Commodity	No. of Samples	No. with HCB	Less than 0.1 ppm	0.1 to 0.25	0.25 to 0.5	Over 0.5 to 1.0	ppm. Over 1.0

Beef Fat	2,029	14	12	1	1	0	0		
Mutton Fat	833	20	2	8	9	1	0		
Butter	514	15	8	5	0	2	0		
Cheese	977	4	3	0	1	0	0		
Frozen liquid whole egg	393	227	81	106	25	11	4		
Frozen liquid egg yolk	154	90	8	42	17	19	4		

A survey of commercial wheat samples carried out in Australia revealed that HCB residues can be found in a significant proportion of the samples in amounts ranging from traces (less than 0.005 ppm) to a maximum of 0.05 ppm.

In the United Kingdom positive evidence of the presence of HCB has been obtained in several shipments of imported wheat. Three samples from one shipment contained 0.67, 1.3 and 0.48 ppm HCB. In two other shipments the combined HCB and alpha BHC was less than 1 ppm.

Surveys of cheese imported into Australia reveal HCB residues in a significant proportion of samples from many countries. More than 10 percent of such imports showed residues in excess of 0.1 ppm, with many samples ranging from 0.3 to 0.9 ppm.

Examinations of food products imported into U.S.A. (Duggan, 1969) showed a very low incidence of HCB residues for 1967 and 1968. One and cheese in 1968). However, in 1969 the incidence increased, HCB being reported in 170 samples of the 1,866 examined. The increase probably reflects improvements in analytical technique more than an increase in the incidence and level of contamination.

The majority of these findings were in manufactured dairy produce with HCB being reported in 149 of 608 samples mainly of cheese. The range of values for HCB in the positive sample was from a trace to 0.81 ppm on a fat basis. The average of 608 samples was 0.01 ppm. Processed foods, other than dairy produce, showed 12 positive findings in 345 samples examined. The range of positive findings in these commodities was from a trace to 0.23 ppm.

One sample of butter was found to contain the following residues (on a fat basis):-

BHC	-	0.24 ppm
lindane	-	0.32 ppm
HCB	-	0.37 ppm

It is obvious that rather more than average care must be taken in resolving such mixtures of residues which can easily become confused by certain GLC analytical procedures.

HCB residues have been found in small proportion of the samples of domestic food production. In 1967, HCB was reported in 12 samples out of approximately 20,000 examined. In 1968 it was reported in only one sample out of approximately 10,000 examined and in 1969 in 10 samples out of approximately the name number tested. The level in positive findings varied from a trace (less than 0.01 ppm) to 0.5 ppm.

METHODS OF RESIDUE ANALYSIS

On foods

Most chlorinated pesticides can be successfully isolated from fatty substrates by methods based on the acetonitrile-hexane partition of Mills (1959). However, HCB 10 exceptionally non-polar, and is distributed preferentially into the hydrocarbon phase. Its partition ratio by experiment using equal volumes of the two solvents in approximately 0.75 to hexane at 20°C. In three counter-current separations with equal volumes, recovery would be about 10 percent. Increasing the volume of acetonitrile to increase extraction of the pesticide would also increase extraction of unwanted fatty matter.

Methods using direct extraction with adsorption cleanup, but without partition, give acceptable recoveries of HCB from eggs, Onley and Mills (1962), butterfat, Moats (1963), Langlois et al. (1963) and meat fat. A method using elution through florisil with methylene chloride-hexane is now in use in the Australian Commonwealth Laboratories (Taylor 1969, direct communication).

In wheat

The analytical problem here is somewhat different, as the significance of the presence of HCB in grain for consumption is related to the potential for concentration of residues in animals from low levels in the wheat, as well as to that used for direct human consumption. Thus the levels of interest are much lower than those in animal products.

Extraction of HCB from wheat was achieved by cracking in a blendor, refluxing with "hexane" (Shell X4) and filtration through anhydrous sodium sulphate. Beside the chlorinated residue, this extract contains fat-soluble plant extractives. Passage through a column of activated florisil cleans the solution sufficiently for determination by GLC and confirmation by, TLC, if the levels are comparatively gross, (over 0.1 ppm). However, when the concentration is 0.01 ppm or less, the ratio of interfering substances to pesticide becomes large enough to require further cleanup if TLC is to be used. Alternatively, other means of confirmation are required.

Investigation has therefore followed two lines: to Improve cleanup and to devise other confirmatory tests.

Cleanup

Hexachlorobenzene is stable to concentrated sulphuric acid. A hexane solution of residual substrate and HCB, after florisil treatment, was washed with the acid several times until no further darkening occurred. The hexane layer was washed with water until neutral, and dried with anhydrous sodium sulphate. The infra-red spectrum of the residue after removal of solvent appeared to be that of a hydrocarbon.
Gas chromatography produced meaningful peaks at retention times corresponding to HCB, but resolution by TLC was prevented by the streaking effect of the oily residue. This cleanup in inadequate for low levels.

Attempts at separation of HCB from hydrocarbon material on alumina and on activated carbon columns with a variety of eluting solvents did not result in sufficiently clean products.

Refluxing of wheat extracts containing HCB with alcoholic potash resulted in varying degrees of breakdown of the pesticide as well as substrate. Although this precluded alkaline cleanup, it suggested possibilities for confirmation of identity of HCB (vide infra).

Steam distillation of standard solutions of HCB was found to give high recovery. Wheat extract previously partly cleaned by treatment with sulphuric acid was therefore distilled from a large (twenty-fold) excess of water, the vapour rising through a Vigreux column and descending through a water-cooled condenser before collection under

hexane. The hexane extract from 20 ml of aqueous distillate produced

clear spots on TLC plates for both hexachlorobenzene and "benzene hexachloride" the former at a level of 0.02 ppm (referred to the original wheat). Recovery tests by adding known amounts of HCB produced similar results, with recoveries close to 100 percent.

In spite of the high sensitivity of this method it is recommended that the sensitivity be developed still further to provide for the need to determine residues in cereal products at levels below 0.01 ppm.

Confirmation

Ideally, confirmation of identity should rest upon two or more methods using properties as widely different as possible. For chlorinated pesticides, chromatography by GLC and TIC are usually considered suitable. However cleanup difficulty, together with small residues, may preclude the use of TLC. It is acceptable, then, to use GLC on columns of such different stationary phases that the substance has substantially different retention characteristics, use being made of different combinations of stationary phase-solute interactions.

Experimentally, the combination of retention values on a silicone (low-polar) and polyester (polar) columns were found to provide useful confirmations of the presence of HCB.

A number of experiments were carried out to make use of the degradation of HCB by alkali. Both alcoholic potash and sodium methylate were used, the latter being more dependably effective. When small traces of the substance, of the order of 0.1 ppm or less, are present, the pattern of degradation is consistent, in that two major products appear in both TLC and GLC, one corresponding in retention values with HCB, the other being lower. However, when greater amounts of HCB are treated, the result is often obscured by the appearance of several other products. The reaction is not, therefore, a reliable confirmatory test for all samples.

A gas-chromatographic column commonly used in estimation of multiple chlorinated pesticide residues contains the mixture of DC200 silicone and QFI fluosilicone proposed by Burke and Holswade (1966). This column does not separate HCB and the alpha isomer of "BHC", the latter being the major constituent used in estimation of "BHC". In addition, alpha-BHC does not produce a strong reaction on the silver nitrate

impregnated Thin layer used. Thus BHC may not be detected if in low concentrations (at 0.1 ppm levels).

To distinguish and confirm these compounds, a stationary phase of a more polar nature may be used. Separate peaks are produced on Reoplex (poly propylene glycol adipate), XE_{60} (nitrile silicone) and Zonyl E7 (fluoroalkyl pyromellitic ester). Therefore an extract showing a peak of HCB/BHC on the Burke column may be reanalysed on one of these columns to distinguish between the two compounds.

The polar phases mentioned are, however, not suitable for all the chlorinated hydrocarbons under investigation. On some, DDT is degraded or in indistinguishable from DDD. On others, DDE and Dieldrin are not separated. Work is currently on hand to select a column suitable for separating all the chlorinated hydrocarbons for which goods are at present analysed.

NATIONAL TOLERANCES

No national tolerances are known.

Australia, Canada and the U.S.A. approve the use of HCB seed dressings on the basis that the seeds are not used for food or feed for animals.

APPRAISAL

HCB has been used extensively as a fungicide to control Bunt (Tilletia caries, T. tritici and T. foetida) in wheat since 1945. There are many reports of its action on other seed borne and soil borne fungi of cereals and as seed treatment for onions but these uses do not appear to be of great importance.

HCB formulations for seed treatment are sold in most wheat growing countries. To date the only alternative materials are organomercurial compounds. The rate of application is usually within the range of 1-3 ozs. of 30 percent dust per bushel of wheat seed equivalent to 330-990 ppm on the seed.

HCB is a comparatively pure material containing 98 percent hexachlorobenzene. The impurities are tetrachlorobenzene and pentachlorobenzene.

The data available to the meeting were obtained from the published literature and by correspondence with more than 100 authors,

administrators and manufacturers throughout the world. In spite of the wide search there is only limited information on residues in food commodities in international trade or following good agricultural practices, other than from Australia. One reason for this is that when examining food commodities by several GLC analytical procedures HCB has been obscured or confused with BHC.

All available data indicates that HCB is not metabolized to any significant extent by plants or animals and that it persists in soil and in animal tissues for long periods. There is an indication that HCB might be translocated from treated seed into plant material and subsequent grain but the level of contamination from this source is insignificant.

Contamination of commercial grain from soil, seed, machinery and bags may be minimized by strict attention to agricultural practices but it is difficult to avoid all traces of HCB in commercial grain.

Animals feeding on grain or milling fractions containing traces of HCB, concentrate and store the residues in body fat, and excrete significant quantities in milk and eggs. The residue level in such animal products ranges from ten to one hundred times the concentration in feed.

The literature includes several methods of residue analysis which measure HCB in grain and animal products by GLC but the results can be confused as and by isomers of BHC. The sensitivity for the determination of HCB is 0.005 ppm but special extraction and cleanup procedures are necessary to ensure reasonable recovery from plant products and especially from animal fats. However, no referee method has been evaluated.

In view of the importance of HCB seed dressings in countries where Bunt is endemic, and the lack of suitable alternatives, the Joint Meeting recognized that recommendations for administrative action levels were required.

RECOMMENDATIONS FOR TOLERANCES, TEMPORARY TOLERANCES OR PRACTICAL RESIDUE LIMITS

TEMPORARY PRACTICAL RESIDUE LIMITS (effective to 1973)

The following temporary practical residue limits are to apply to raw agricultural commodities moving in commerce unless otherwise indicated:

Raw cereal (wheat)	0.05 ppm

Cereal products from wheat	0.01 ppm
Fat of cattle, sheep, goats, pigs and poultry	1.0 ppm
Milk	0.012 ppm
Milk products	0.3 ppm
Eggs (shellfree basis)	1.0 ppm

FURTHER WORK OR INFORMATION

REQUIRED (before an acceptable daily intake or tolerances can be established)

1. Metabolic studies in animals including identification of the toxic product or products excreted in milk.

2. Short-term studies in non-rodent mammalian species and long-term studies especially in relation to the effect on bone-marrow.

3. Reproduction studies in rats.

REQUIRED before 30 June 1973 (for 1973 review of Practical Residue Limits)

1. Data from countries other than Australia on residues in raw agricultural commodities.

2. Data from surveys of residues in commodities moving in international trade.

DESIRABLE

1. Further information on analytical procedures capable of distinguishing HCB from BHC isomers suitable for use in laboratories engaged in general regulatory work.

2. Collaborative studies on analytical methods capable of recovering and identifying HCB residues in the above food commodities.

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See Also:
Toxicological Abbreviations
Hexachlorobenzene (EHC 195, 1997)
Hexachlorobenzene (HSG 107, 1998)
Hexachlorobenzene (ICSC)
Hexachlorobenzene (PDS)
Hexachlorobenzene (PIM 256)
Hexachlorobenzene (WHO Pesticide Residues Series 4)
Hexachlorobenzene (IARC Summary & Evaluation, Supplement7, 1987)

Hexachlorobenzene (IARC Summary & Evaluation, Volume 20, 1979)
Hexachlorobenzene (IARC Summary & Evaluation, Volume 79, 2001)