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COMPARATIVE TOXICOLOGY OF CHLORDECONE (KEPONE) IN HUMANS AND EXPERIMENTAL ANIMALS

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OVERVIEW

In July, 1975, a 33-year old male chemical worker from a small factory in Hopewell, Virginia was examined by his family physician. He complained of headache, tremors, and irritability. It was not the first time he had sought medical attention for these problems. In the past, no specific diagnosis had been made and tranquilizers had been prescribed. On this occasion, however, the doctor, after having taken a careful occupational history, sent a sample of blood to the Communicable Disease Center to be analyzed for Kepone^{R}, the only product made by the patient's employer, Life Science Products Corporation. The results revealed the presence of enormous amounts of chlordecone,[1] the generic name for Kepone^{R}. This index case spawned immediate epidemiologic investigations by State and Federal officials. Uncovered was an ecodisaster of spectacular proportions (1–3). Because the factory failed to follow good practices for industrial hygiene and disposal of hazardous wastes, over half of the 133 employees in the factory and many residents of the immediate vicinity had evidence of chlordecone intoxication (4). Moreover, it was learned that Allied Chemical Corporation, the manufacturer of chlordecone for the preceding decade, had illegally discharged chlordecone into the nearby James River. This resulted in

[1] 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd]-pentalen-2-one.

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extensive contamination of the waters and marine life throughout the Tidewater Region of Virginia with this persistent chemical (5, 6). The now world-famous "Kepone Episode" has become a classic paradigm of the unprecedented challenges presented to the political, regulatory, legal, and scientific communities by this new form of epidemic in the chemical era.

Over 30 of the most severely affected workers were examined at the Medical College of Virginia and were found to have toxic manifestations involving primarily the nervous system (tremors), the liver (hepatosplenomegaly), and the testes (presumed sterility). Previous human poisoning with chlordecone had not been documented. Like poisonings by other organochlorine pesticides, there were no established antidotes available and recommended therapy was directed toward relief of symptoms. Unfortunately, the available information regarding the toxicology or pharmacokinetics of chlordecone in mammals was meager indeed. In fact, there were no critically evaluated methods available for measurement of chlordecone in samples of biological material. Confronted with this challenging problem in clinical toxicology, a group of us at the Medical College of Virginia first developed improved methods for chlordecone analysis (7), and then measured the distribution, excretion, and metabolism of chlordecone in the patients and also in experimental animals (8). The results showed that chlordecone was cleared from the blood by the liver, was excreted in bile into the intestine, and was eliminated chiefly in the stool (9). However, most of the chlordecone which appeared in the feces entered the intestinal lumen not in bile, but rather via a nonbiliary pathway, probably from the gut itself (10). A key observation was that most of the chlordecone in the lumen of the gut is reabsorbed with only a minor fraction appearing in the stool. This prompted us to carry out a controlled therapeutic trial of cholestyramine, a nonabsorbable anion exchange resin that binds chlordecone in vitro. When given to the patients, cholestyramine increased fecal excretion of chlordecone and accelerated the disappearance of chlordecone from blood and other tissues (9). When the efficacy of cholestyramine had been established in man as well as in chlordecone-treated rats (11), all patients were placed on cholestyramine treatment until chlordecone had been eliminated from the body. In most cases, removal of the chemical from the tissues was accompanied by disappearance of clinical manifestations of toxicity. The nervous system disorders resolved, the liver returned to normal size, and sperm counts became normal. Because chlordecone is a liver carcinogen in rats and mice (12), the prospects for development of this (or other) chronic illness in these workers or in area residents exposed to small amounts of chlordecone in the environment remain uncertain. This question is further complicated by the fact that chlordecone does not appear to undergo biotransformation in rats or mice, whereas in humans, substantial amounts of

chlordecone are converted in the liver to a reduced form, chlordecone alcohol.[2] As yet, little is known about the toxicologic or pharmacokinetic properties of this chemically stable metabolite of chlordecone.

Chlordecone has become unique among environmental chemicals in having been extensively studied in humans. Hence, a review of these studies provides useful information in comparative toxicology and metabolism of environmental agents. Moreover, there is growing evidence that chlordecone may be a valuable probe for investigating such important physiologic processes as the metabolism of high-density lipoproteins, hypertrophy of the endoplasmic reticulum of the hepatocyte, the formation of bile, and hormonal effects mediated by estrogen receptors. For these reasons, it seems pertinent to summarize the current information on chlordecone toxicology and pharmacokinetics in humans and other mammals and in birds. Due to limitations in space, studies of aquatic species (5, 6) have not been included.

CHEMISTRY AND ANALYTICAL METHODS

Chlordecone ($C_{10}Cl_{10}O$) is an odorless, colorless, crystalline solid, synthesized by dimerization of hexachlorocyclo-pentadiene in the presence of sulfur trioxide followed by hydrolysis to form the ketone. The structure of chlordecone differs from its analogue, mirex ($C_{10}Cl_{12}$), in that two bridge head chlorine atoms have been replaced by the carbonyl group (Figure 1). Anhydrous chlordecone has a molecular weight of 491, but the molecule is readily hydrated (one to four molecules of H_2O) with an attendant increase in molecular weight. The hydrate forms a gem-diol (dialcohol) in place of the ketone (Figure 1), and this imparts enhanced water solubility to chlordecone as compared with mirex and properties of a weak acid.

Most of the published methods for chlordecone analysis rely on extracting samples with organic solvents, purifying the extracts with Florisil column chromatography or gel permeation chromatography, and measuring the extracted chlordecone by gas-liquid chromatography (13, 14). Methods developed for biological analysis involve acidification prior to extraction by organic solvents. In some instances, the organic phase is re-extracted with alkali (7) or is washed repeatedly with sulfuric acid to remove substances that interfere with analysis of chlordecone by gas-liquid chromatography (15). Lixiviation of chlordecone is variable among different tissues or excreta or, indeed, among samples from a given tissue and, therefore, it is important to use either [^{14}C]-chlordecone (7) or monohydro-chlordecone[3]

[2]1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd]-pentalen-2-ol.

[3]1a,3,3a,4,5,5,5a,5b,6-nonachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd]-pentalen-2-one.

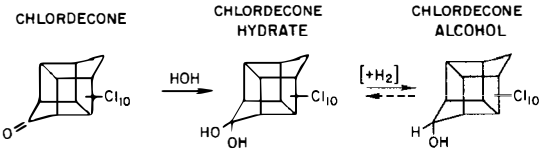

Figure 1 Structures of chlordecone, chlordecone hydrate, and chlordecone alcohol.

(15) as an internal standard to monitor recovery. Because chlordecone alcohol is not extracted from tissues or excreta in parallel with chlordecone, a second internal standard such as monohydro-chlordecone alcohol[4] must be used to monitor recovery of chlordecone alcohol (15).

TOXICOLOGY OF CHLORDECONE

General Toxic Effects

There are no reports of death in humans exposed to chlordecone. Chlordecone-poisoned workers lacked such constitutional symptoms as fever, chills, sweating, or fatigue. However, in 10 of 23 cases, there was prominent weight loss (as much as 60 pounds in four months) despite a normal appetite. The susceptibility of laboratory animals to the lethal effects of a single oral dose of chlordecone is similar between males and females (16, 17), but varies among species. The LD_{50} for rabbits (71 mg/kg) is lower than that for rats (126 mg/kg) (16, 17), dogs (250 mg/kg) (16), or chicks (480 mg/kg) (18). All rats receiving diets of 50 ppm of chlordecone or more died within six months. Mice receiving diets containing 80 ppm of chlordecone or higher died within 32 days (19). When mice were given daily doses of 50, 25, or 10 mg/kg, 90% of the animals died within 5, 9, or 24 days, respectively (20). The acute and cumulative LD_{50} for chlordecone in mice are in the same range. The mechanism of death in these studies was not ascertained. Chronic dermal contact with chlordecone produced minimal toxicity in rats (17). In two (16, 21) out of three (22) studies of adult rats maintained for many months on diets containing more than 10 ppm chlordecone, significant weight loss was noted. Depressed growth has also been observed in pregnant rats given as little as 2 mg/kg/day of chlordecone (23), in mice fed 40 ppm chlordecone (19), in mice treated with 10 mg/kg daily (24), in laying hens fed 75 ppm chlordecone (25), and in quail fed 300 ppm chlordecone (26). "Hypermetabolism," manifested by increased food and oxygen consumption, was reported in rats (16) and mice (20).

[4]1a,3,3a,4,5,5,5a,5b,6-nonachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd]-pentalen-2-ol.

Neuromuscular Toxicity

The *sine qua non* of chlordecone intoxication in humans is an irregular, nonpurposive waking tremor (rate, 6–8 Hz) involving the extremities, head, and trunk (27). Seizures were not observed. Also present in the chlordecone poisoned workers was opsoclonus, an unusual oculomotor disorder consisting of chaotic eye movements causing blurred vision. In some patients who complained of headaches, spinal fluid pressure was elevated, and three patients had frank pseudo-tumor cerebri due apparently to impaired capacity to absorb cerebrospinal fluid (28). Neuropsychiatric abnormalities included irritability, memory disturbances, exaggerated startle response and, in one case, visual and auditory hallucinations (27). No evidence for dysfunction of muscle or peripheral nerves was adduced, and muscle biopsies in five patients were histologically normal. There is speculation that the mesencephalic reticular formation may be the site of action of chlordecone in the central nervous system, although this idea lacks direct evidence (27). From the absence of distinct clinical signs of cerebellar involvement, it is unlikely that chlordecone poisoning caused parenchymatous degeneration of the cerebellar cortex as has been reported in dogs fed another organochlorine pesticide, dichlorodiphenyldichloroethane (DDT) (29). This conclusion is supported by the fact that upon removal of chlordecone from the body, toxic manifestations involving the central nervous system disappeared. An apparent threshold for disappearance of neurotoxicity was associated with a decline of blood levels of chlordecone into the range of 1000 to 100 ng/ml.

The prompt appearance of tremor in rats (16, 21, 22, 30–35), chicks (18), Japanese quail (26), and mice (20) receiving chlordecone has been well documented. Also, like our patients, chlordecone-treated rats (22, 30, 32, 33) and mice (20) exhibited hyperexcitability and an exaggerated startle response. These rats were especially susceptible to chemically evoked seizures (20). A strict departure from our experience with chlordecone poisoned workers is the observation of severe muscle weakness following administration of a single oral dose of chlordecone to chicks (18) or rats (30). Although muscle weakness has been ascribed to effects of chlordecone as a competetive inhibition of muscle lactate dehydrogenase (36), the myopathic effects of chlordecone in rats appeared to be permanent, increasing at a time when there was both a decline in chlordecone levels in muscle and a disappearance of neurotoxic manifestations (30).

The mechanism of chlordecone neurotoxicity has been attributed to inhibition of mitochondrial and synaptisomal membrane bound Na^+, K^+ ATPase(s). Research groups at the University of Mississippi have worked

sedulously to document this property of chlordecone using various tissue sources for the enzyme. These include: brain from untreated or chlordecone-treated rats (34, 37, 38), mice (24, 39), and catfish (40); muscle from the heart (41) or extremities (37) in rodents; liver from chlordecone-treated rats (21), or isolated perfused rat liver preparations (42, 43). The extent of inhibition of Na^+, K^+ ATPase and oligomycin-sensitive Mg^{2+} ATPase activities in brain synaptosomes prepared from chlordecone-treated rodents was directly proportional to the concentration of chlordecone in the brain (24) and was proportional also to the degree of tremor produced in the animal. Furthermore, inhibition of this enzyme activity in vitro was blocked in a dose-dependent fashion by added chlordecone (34, 37–39). Added chlordecone produced a greater inhibition of the enzyme than did equal amounts of mirex, a compound that produces no tremors in vivo (38), and yet is structurally similar to chlordecone. Finally, the inhibition of ATPase activity can be reversed if bound chlordecone is removed by washing (34) or by addition of anti-chlordecone antibodies (44, 45). This research group believes that neurotoxicity may be due to inhibition of synaptosomal membrane ATPase(s) by chlordecone which would then result in blocked cellular uptake and storage of such neurotransmitters as catecholamines (34, 41) or γ-aminobutyric acid (24). An alternative explanation is that by inhibiting mitochondrial ATPase(s), chlordecone may decrease the availability of cellular energy for neurotransmitter uptake. Others have suggested that chlordecone inhibits a step in rat brain mitochondrial energy production prior to the Mg^{2+} ATPase reaction (46). This results in loss of the capacity of mitochondria to maintain normal rates of Ca^{++} uptake, leading to an increase in cytoplasmic Ca^{++}. Depolarization of the neuronal membrane would ensue, promoting release of neurotransmitters (46, 47). Since the toxic manifestations of chlordecone bear little resemblance to those of cyanide, a classical mitochondrial poison, nonspecific loss of cellular energy seems an unconvincing explanation for chlordecone toxicity. On the other hand, competitive, reversible inhibition of ATPase linked processes by chlordecone could explain (*a*) the reversibility of neurotoxicity associated with decreasing concentrations of chlordecone in human tissues (9, 27); (*b*) the inhibition of cerebrospinal fluid uptake in humans which is thought to be dependent upon Na^+, K^+ ATPase (28); and (*c*), the decrease in brain content of neurotransmitters in experimental animals treated with chlordecone (24).

Liver Toxicity

In most of the chlordecone-poisoned workers, the liver and spleen were enlarged, chemical tests of liver function including clearance of sulfobromophthalein (BSP) from plasma were normal, and histologic examina-

tion of liver biopsies revealed nonspecific findings including minimal fatty metamorphosis, focal proliferation of sinusoidal cells, hyperglycogenation of nuclei, increased residual bodies, branched mitochondria with paracrystalline inclusions, and accumulation of smooth endoplasmic reticulum (48). The last observation suggests that the hepatic microsomal drug metabolizing system (cytochrome P-450-dependent monooxygenase system) was induced. In line with this finding, workers displayed an accelerated clearance of antipyrene from the blood as compared to normal subjects (48). Furthermore, the workers had abnormally high levels of glucaric acid (48), a substance which is reported to be a derivative of hepatic endoplasmic reticulum (49). However, none of the workers had elevated serum γ-glutamyl transpeptidase activity, an enzyme proposed as a marker for induction of hepatic drug metabolizing enzymes in man (50, 51). There was no clinical evidence for porphyrinogenic effects as has been reported for other environmental chemicals in man (52). It should be noted that these effects of chlordecone on hepatic functions associated with the endoplasmic reticulum may reflect an adaptive response of the liver rather than "hepatotoxicity" *per se.*

Similar to the effects of chlordecone in human liver, treatment of rats (16, 22, 23, 53), quail (26, 54, 55), mice (19, 23), or dogs (16) with chlordecone increased the size of the liver relative to total body weight. Also similar were histopathologic examinations of the livers from chlordecone-treated rats (16, 22, 56), quail (26, 57, 58), or mice (19, 59) which revealed only such nonspecific changes as fatty infiltration, pleocytosis, and focal lymphoid aggregates. There was no evidence of fibrosis, cholestasis, or significant hepatocellular necrosis.

The hepatic drug metabolizing system is induced in rats treated with commercially available chlordecone. Chlordecone treatment increased the concentration of the drug binding hemoprotein, microsomal cytochrome P-450 (35, 53, 60–63), the level of NADPH-cytochrome C reductase activity (60, 61), and the activity of cytochrome P-450 dependent oxidations of warfarin (35), pentobarbital (60, 61), aniline (60, 61), hexobarbital (61), aminopyrine (61, 62), ethylmorphine (61), and *p*-nitroanisole (62). Pentobarbital sleeping time was reduced by chlordecone pretreatment of rats (61). It has been concluded that chlordecone resembles the prototype inducer, phenobarbital, because of the profile of its effects on the hepatic microsomal monooxygenases, and because chlordecone binds to cytochrome P-450 to produce a "type I" spectrum (64). However, it should be noted that commercially available chlordecone contains an impurity that is reported to be solely responsible for the "phenobarbital-like" induction of aryl hydrocarbon hydroxylase activity in genetically responsive mice (65). To resolve the question of induction of cytochrome P-450 by chlordecone,

we administered a single oral dose of highly purified chlordecone (>99.9%, based on gas-liquid chromatography/mass spectrometry) to male rats (40 mg/kg) or gerbils (20 mg/kg) and found that the concentration of hepatic microsomal cytochrome P-450 increased an average of 150% and 259%, respectively (unpublished observations). Further studies will be needed to establish precisely the identity of the molecular form of cytochrome P-450 induced by chlordecone.

Clearance of organic anions (BSP) by the liver was unimpaired in chlordecone poisoned workers and in chlordecone-treated rats with comparable hepatic concentrations of the pesticide (ca. 1000 μg/gm). Inexplicably, however, these rats exhibited a reduced capacity to excrete imipramine metabolites (21, 43, 66) or phenolphthalein (21) in bile. These observations appear to contradict the generally accepted idea that aside from bile salts, most organic anions including BSP share a common pathway for biliary excretion (67). Impairment of the biliary excretion of organic anions by chlordecone was localized to a step subsequent to their hepatic uptake and metabolism, possibly transfer from the hepatocyte into the bile canaliculus (21, 43). The extent of impaired biliary excretion of model compounds was directly related to the content of chlordecone in the liver (31). Finally, this effect was fully reversible if exposure to chlordecone was stopped, thus permitting the concentration of chlordecone in the liver to fall (31). The proposed explanation for these observations is that chlordecone blocks Mg^{2+}-ATPase activity (42, 68) and, hence, decreases hepatic mitochondrial energy production. This, in turn, interferes with biliary transport, an energy-dependent process (21, 43, 66). However, this hypothesis does not account for the striking selectivity of the hepatobiliary effects of chlordecone. For example, chlordecone fails to impair excretion of an endogenous organic anion; namely, bilirubin. Moreover, chlordecone actually stimulates bile (volume) production, a process which is believed to be dependent on Na^+, K^+ ATPase and which is sensitive to general inhibitors of cellular energy production (69). Indeed, apparently overlooked in the interpretation of the hepatobiliary effects of chlordecone is the evident inconsistency between its choleretic properties and the fact that chlordecone is a potent inhibitor of Na^+, K^+ ATPase from many tissues (38, 39, 41) including liver canalicular membranes (H. Mehendale, personal communication). Testimony to the potency of chlordecone as an inhibitor is its capacity to displace the classical ATPase inhibitor, ouabain, from binding to the enzyme (39). Since Na^+, K^+ is pivotal to all current theories of bile production (69), the paradoxical choleretic properties of chlordecone should be actively investigated.

A fascinating effect of chlordecone on rodent liver is potentiation of the hepatotoxicity of chloromethanes. For obvious reasons, this could not be

evaluated in humans. When animals were pretreated with chlordecone and then given a single small dose of $CHCl_3$ (mice) (59, 70) or CCl_4 (rats) (71–73), there was a dramatic enhancement of hepatotoxicity as manifested by elevation of bilirubin and of liver-derived enzyme activities in the serum, by characteristic histopathologic changes, and by decreased biliary excretion of organic anions. The effect of chlordecone pretreatment appears to be specific and potent. For example, in one set of experiments, groups of rats were given either mirex, photomirex, or chlordecone at doses which resulted in comparable concentrations of these chemicals in the liver. These doses produced neither hepatotoxic effects (73, 74) nor changes in liver glutathione concentrations (70, 72). Of the three compounds, only chlordecone potentiated the hepatotoxic effects of CCl_4 (73, 74). Moreover, by comparison with equal doses of phenobarbital, chlordecone was 100 times more potent in fostering the liver toxicity of CCl_4 (73). The mechanism of the interaction between chlordecone and CCl_4 is unknown. The available evidence suggests that chlordecone does not enhance CCl_4-induced lipid peroxidation as measured by diene conjugates (72). One suggestion is that chlordecone may induce a special form of cytochrome P-450 which preferentially catalyzes activation of $CHCl_3$ to reactive metabolites that bind covalently to cellular macromolecules (70). However, it was recently demonstrated that rats could be given a single small dose of chlordecone (5 mg/kg) which potentiated CCl_4 hepatotoxicity, and yet failed to induce the hepatic monooxygenase system, at least as measured by pentobarbital sleeping time (72). Moreover, this dose of chlordecone gave no increase in the formation of covalently bound CCl_4 metabolites (72). Based on reports that aliphatic ketones enhance haloalkane hepatotoxicity, it has been proposed that the effect of chlordecone may be explained by its ketonic structure (59). However, this theory ignores the fact that chlordecone, being extremely deliquesent, readily forms chlordecone hydrate (75–77) and undoubtedly exists in the form of a gem-diol in biological systems (Figure 1). Although it is difficult to reconcile all of the reported data on chlordecone potentiation of CCl_4 hepatotoxicity, this intriguing toxic interaction is best explained at present as an effect of chlordecone to render the liver especially susceptible to attack by these classical hepatotoxins.

Toxic Effects on the Endocrine and Reproductive Systems

There were no overt clinical manifestations of endocrinologic toxicity among 28 of the chlordecone poisoned workers except for complaints of decreased libido in seven patients. All of the patients had normal serum levels of follicle stimulating hormone, leutinizing hormone, and testosterone (78). Nevertheless, only eight patients had normal sperm counts, and in only one of these eight was the concentration of chlordecone in blood

greater than 1000 ng/ml (9). In all cases, low or absent sperm numbers were associated with abnormally low percentages of motile sperm. Histologic examinations of testicular biopsies in two patients revealed arrest of sperm maturation (78), a finding that suggests a potentially reversible lesion. In 12 of 13 cases where it was possible to make repeated examinations of sperm in a given individual over several years, there was an increase in the concentration of motile sperm coinciding with a fall in blood levels of chlordecone (9). Several years have elapsed since the chlordecone was removed from body tissues in these men. Although no comprehensive study of reproductive function has been carried out, several individuals successfully fathered children. No information is available regarding the effects of chlordecone on reproductive function in women.

Blocked or impaired reproductive function in chlordecone-treated birds and rodents has been abundantly well documented. Chronic consumption of diets containing even small amounts of chlordecone by bobwhite quail (1 to 25 ppm) (79), by pheasants (5 to 10 ppm) (79), by laying hens (25), or by Japanese quail (200 ppm) (80–82) reduced the number of eggs produced. Moreover, these eggs were smaller than normal (80, 81), had reduced eggshell thickness and strength (80, 81), hatched less frequently (25, 80, 81), and produced defective chicks with decreased survival rates (25). Similarly, feeding chlordecone to inbred mice (5 or 10 ppm) (19, 83) or rats (25 ppm) (22) reduced the number and size of the litters produced. The defect was primarily associated with females (19, 43) and could be reversed within several months upon resumption of a normal diet (19, 43). Overt teratogenic effects are observed at higher doses of chlordecone. An increased number of the offspring exhibited a variety of toxic manifestations. These were low fetal weight (23, 84), reduced postpartum survival (84), developmental abnormalities involving the skeletal, ureterogenital, and central nervous systems (23), and in multigeneration studies, decreased reproductive capacity as adults (83).

The concensus view of all these investigations is that chlordecone impairs reproduction by mimicking the effects of excessive estrogens. For example, testicular atrophy was reported in chlordecone-treated rats (16) and quail (54). Gross and histologic examinations of testes taken from chlordecone-treated quail (26, 54, 57) showed a congeries of findings (reduced size, decreased spermatogenesis, and abnormalities of the semeniferous tubules) similar to those produced by estrogen administration to birds. Chlordecone treatment also produces a variety of "estrogen-like" effects on the female reproductive system. In immature quail (54, 57) or chicks (85), chlordecone treatment caused premature development of the oviduct manifested grossly by an increase in oviduct weight, and histologically by the presence of cilia, cellular proliferation and differentiation, formation of tubular glands, and

secretory activity (54, 57). Examination of the treated quail ovaries revealed fewer eggs and impaired follicular development (26, 54, 57). In immature rats, chlordecone produced an increase in uterine growth, a persistent vaginal estrous, anovultion, and the disappearance of corpus leutea from the ovaries (26, 86). The ultramicroscopic changes in the cells lining the oviduct, vagina, and uterus of these rats was characteristic of those produced by estrogenic steroid hormones.

It has been suggested that the estrogenic effects of chlordecone may be exerted indirectly through the hypothalamic pituitary system (26) by prolonging release of follicle stimulating hormone and preventing release of leutinizing hormones (19). However, it should be noted that these studies were carried out prior to the advent of sensitive and specific immunochemical techniques for measuring pituitary hormones. There is now strong evidence that chlordecone exerts its estrogenic effects directly. When incubated with explants of chick oviduct in vitro, chlordecone, like estradiol, induced *de novo* synthesis of specific egg white proteins and increased the number of specific mRNA sequences encoding for these proteins (85). These effects could be prevented by tamoxifen, an antiestrogen that competitively inhibits binding of estradiol to its specific cytoplasmic receptor (85). Moreover, there was competition between estrogen and several preparations of highly purified chlordecone for binding to nuclear (55, 85) and cytoplasmic (87, 88) estradiol receptors. Chlordecone stimulated transfer of the cytoplasmic estradiol receptor into the nucleus and stimulated synthesis of the progesterone receptor (85, 87). These are two processes for which the estrogen receptor is thought to be indispensable. Despite the fact that chlordecone was found to be 10,000 times weaker than estradiol in its biologic effects or in its affinity for the estrogen receptor (85, 87), it is possible that by persisting in tissues at high concentrations, chlordecone may produce local adverse estrogenic effects on the reproductive system (87). This hypothesis would account for the selectivity and reversibility of estrogen-like effects of chlordecone on the testes observed in the poisoned workers.

There was no evidence for adrenal dysfunction in men. Histologic examinations of adrenals from chlordecone-treated rats revealed cortical hyperplasia (33). Epinephrine cells of the adrenal medulla contained decreased levels of epinephrine, and overall catecholamine content of the organ was reduced (33).

Other Toxicity

The workers complained of pleuritic chest pains and migratory arthralgias (27), but there has been no objective clinical evidence of cardiopulmonary or rheumatologic dysfunction. Serum concentrations of cholesterol and

triglycerides were normal. Also normal were hematologic tests and repeated tests of renal function including urinalysis and measurements of urea nitrogen and creatinine concentrations in serum. In contrast, in rats treated with chlordecone, serum cholesterol and triglyceride concentrations were lower than in controls, while the fraction of serum cholesterol associated with high-density lipoproteins was increased (53). Another interesting departure from the experience with human toxicity is that rats receiving as little as 1 ppm chlordecone in the diet for one month showed mild histopathologic changes in the proximal tubules of the kidneys (56). Rats receiving 5 ppm chlordecone developed abnormally high proteinuria within six months and glomerulosclerosis after one year (16). These rats also had a decreased hematocrit (16), a finding noted for chlordecone-treated quail (26).

Carcinogenicity

The National Cancer Institute sponsored a carcinogenesis bioassay of technical grade chlordecone (>98% purity) in Osborne-Mendel rats and B6C3F1 mice (12). The initial doses were found to be too high and had to be reduced during the study because of excessive mortality. The four groups of animals—male rats, female rats, male mice, and female mice—ultimately received a time weighted "low" (8, 18, 20, or 20 ppm, respectively) or "high" (24, 26, 23, or 40 ppm, respectively) dose for 80 weeks. This was followed by resumption of a normal diet for either 32 weeks (rats) or 10 weeks (mice). There were no hepatocellular carcinomas in control rats, whereas the incidences in high dose rats were 7% for males and 22% for females, both significantly increased. The incidences of hepatocellular carcinomas in female mice receiving the low dose (52%) or high dose (47%) groups were significantly higher than in controls (0 of 40 mice). Male mice receiving the low dose (81%) or high dose (88%) had a significantly higher incidence of liver tumors as compared to matched controls (32%). Moreover, the time to detection of the first hepatocellular carcinoma was significantly shortened in all groups of treated mice. Metastases of the hepatocellular carcinomas were not observed, and there were no significant increases in extrahepatic tumors. Despite the possible defects in the design and conduct of this study, it may be concluded that chlordecone is a liver carcinogen in rats and mice. Reuber, in reinterpreting the histologic material available from this bioassay and from studies of rats fed 1 ppm (16) or 5–25 ppm (16) of chlordecone for 12–24 months, has reached the same conclusion (89, 90).

The mechanism underlying production of liver cancer by chlordecone is unknown. Although most agents that are carcinogenic in rodents also produce mutations in short-term tests in animals or in vitro, chlordecone, like some other organochlorine pesticides, proved to be negative when

mutagenicity was assayed in Salmonella (Ames Test) [(91); P. Phibbs, personal communication]. Chlordecone also proved to be negative as a mutagen when tested for enhancement of unscheduled DNA synthesis in primary cultures of adult rat hepatocytes (92, 93). A current concept of experimental liver carcinogenesis in rats is that of a multistage process. First, preneoplastic hepatocytes are formed by exposure of the liver to a "true" or "complete" carcinogen ("initiator"). However, these cells may remain dormant unless the liver is exposed to a second agent (e.g. phenobarbital) which, while not necessarily carcinogenic by itself, enhances development of the preneoplastic cells into malignancies ("promotion") (94, 95). Further work is now needed to learn whether chlordecone is a promotor and, if so, which of its prominent effects on the liver cell (see previous section), if any, are related to carcinogenesis.

PHARMACOKINETICS OF CHLORDECONE IN MAN AND LABORATORY ANIMALS

Absorption

Chlordecone is readily absorbed (>90%) from the gastrointestinal tract. This has been established in rats consuming chlordecone in maternal milk (96) or when given as a single dose of 40 mg/kg in corn oil by gastric intubation (8, 11). The absorbed chlordecone rapidly establishes an equilibrium of distribution among most tissues. This equilibrium was achieved within 24–48 hours following a single dose to rats regardless of whether chlordecone was given intravenously, by gastric intubation, or by intraperitoneal injection (97). A similar pattern of tissue distribution was found in mice fed a diet containing 40 ppm of chlordecone for many months (19). Quantitative measurements of chlordecone absorption through the skin or by inhalation have not been reported.

Distribution

From inspecting the distribution of chlordecone in the tissues of factory workers, it is evident that there was an unusually high concentration of chlordecone in blood as compared to that in adipose tissue, the ratio being 1:7 (Table 1) (9). This ratio is similar to that reported in rats two weeks (8, 11) or 26 weeks (8) following a single dose of chlordecone. The high blood to fat ratio for the distribution of chlordecone contrasts markedly to the partitioning of DDT (98), polybrominated biphenyls (99), and other lipophilic environmental chemicals (100, 101) which are hundreds of times less concentrated in blood than in fat. It is generally believed that the coefficient of distribution of hydrophobic chemicals among tissues including blood parallels the lipid content of the tissues (102). Hence, the disproportionately

high concentration of chlordecone in blood might be explained by the fact that chlordecone hydrate may be more water soluble than other organochlorine pesticides. However, from unsuccessful attempts to detect unbound chlordecone in plasma, we have concluded that the "free" plasma chlordecone, if present at all, is less than 1% of total chlordecone in blood (9, 103). Therefore, we tested the hypothesis that chlordecone may be specifically associated with plasma proteins in preference to blood lipids (103, 104). Indeed, we found that when chlordecone was incubated with human, rat, or pig plasma, more than 75% of the chemical was associated with the albumin plus high-density lipoprotein fractions, whereas these same fractions from human plasma contained only 24% of an endogenous, hydrophobic substance; namely, cholesterol (103). Moreoever, of the total chlordecone or cholesterol associated with human lipoproteins, the high-density lipoprotein fraction prepared either by ultracentrifugation or by heparin-manganese precipitation followed by agarose gel electrophoresis contained 53% of the chlordecone, but only 22% of the cholesterol. Whereas most organochlorine pesticides are associated with the lipid rich fractions of plasma through nonspecific, hydrophobic interactions (reviewed in 104), specific binding of chlordecone by albumin and high-density lipoproteins offers an attractive explanation for the enormous amounts of this hydrophobic chemical in such an aqueous fluid as blood.

A second distinctive feature of chlordecone distribution in both man (9) and rats (8, 11) is that among all sampled tissues, the highest concentration is not found in body fat, although this might be expected based on the tissue distribution of most lipophilic chemicals. The highest concentration of chlordecone is found in the liver. Indeed, rats given a single oral dose of chlordecone established within 48 hours a stable partition ratio of chlordecone between fat and blood, 13:1, whereas the ratio of the initial concentrations of chlordecone in liver and blood was 28:1. The fat to blood concentration ratio remained unchanged for 26 weeks thereafter despite the fact that the concentration of chlordecone in fat declined by a factor of 362

Table 1 Distribution of chlordecone in man

Tissue	No. of patients	Chlordecone concentration range (μg/g)	Partition Tissue/Blood	Partition Range
Whole blood	32	0.6–32.0	1.0	4.6–31.0
Liver	10	13.3–173.0	15.0	3.8–12.2
Subcutaneous fat	29	1.7–62.1	6.7	1.8–4.5
Muscle	5	1.2–11.3	2.9	1.8–4.5
Gallbladder bile	6	2.5–30.0	2.5	1.4–4.1

(8). In contrast, the concentration of chlordecone in liver declined less rapidly during this study (a factor of only 65) so that the liver to blood concentration ratio rose progressively during the 26-week study to a final value of 119:1. This finding was confirmed in mice chronically fed chlordecone (19) and in the fetuses of pregnant chlordecone-treated rats (96). In both studies, the highest concentration of chlordecone among all sampled tissues was found in the liver. There is no explanation for the preferential sequestration of chlordecone in liver. By analogy with many chemicals bound by albumin, chlordecone might be expected to be bound by ligandin or by other cytoplasmic binding proteins in the hepatocyte (105). However, when homogenates of livers obtained from chlordecone-treated rats were fractionated by differential centrifugation, less than 2% of the total chlordecone was recovered in the cytosolic fraction (97). Hence, it may be concluded that the disproportionate accumulation of body chlordecone in the liver is due to binding of chlordecone by constitutents of liver membranes, presumably proteins or lipids. Although it is premature to posit specific chlordecone binding constituents, a detailed investigation of the subcellular distribution and binding of chlordecone in the liver should be undertaken, especially because the liver is a prime target organ for the toxic effects of chlordecone.

Excretion

The major route of elimination of chlordecone is in the stool. This has been confirmed in humans (9), rats (8), gerbils (106), mice, and monkeys (107). Only minimal amounts of chlordecone appeared in the urine of these species, even when [^{14}C]-chlordecone tracer studies were carried out (8, 106). There were negligible amounts of chlordecone in human sweat, sebum, or saliva (9), or in the breath of rats (8). An exception to this generalization may be lactating women. Chlordecone has been detected in samples of breast milk from women living in Southern states of the United States (108). The environmental source of chlordecone in this region is presumed due to extensive use of mirex which may contain chlordecone as a contaminant or which may undergo photodegradation to form chlordecone (109). Chlordecone promptly (4 days) appeared in the milk of cows started on chlordecone-containing diets ranging from 0.25 to 5.0 ppm (110). After 60 days, the levels of chlordecone in the milk of the cows on these diets rose to 20 and 320 ng/ml, respectively, and then declined to barely detectable levels after resumption of a normal diet for 83 days (110). It has been estimated that female rats may excrete as much as 52% of an acutely administered dose of chlordecone in milk during the lactation period (96). This may be compared to male rats which excreted 33% of a single dose in stool during a similar interval (8).

We studied the kinetics of chlordecone elimination in the factory workers at a time when their exposure to chlordecone had ceased. The concentration of chlordecone in the blood declined at a log linear rate (9). In 22 workers in whom multiple serial examinations of blood and fat were made for two years, the average half-life of chlordecone in blood (153 days) and in fat (125 days) were similar. Moreover, in simultaneously obtained samples of blood and fat, the ratio of chlordecone concentrations was found to be 1:7 despite a 250-fold range in fat concentrations (9). From these important findings, it may be concluded that, unlike most organochlorine pesticides which rapidly disappear from blood only to be redistributed to fat (98, 100, 101, 111), chlordecone rapidly achieves equilibrium between blood and other tissues including the body's main reservoir, adipose tissue (9). This, in turn, suggests that to calculate a reliable estimate of total body content of chlordecone in a given person at anytime during or after contact with the pesticide using the coefficients of distribution listed in Table 1, one need only measure the blood concentration of chlordecone. In rats, similar to our findings in man, chlordecone disappeared from the blood, from individually sampled tissues (with the exception of the liver as described above in "Distribution") (8), and from the total carcass (11) at equal rates. However, whereas in two studies of humans (9, 112) there was no correlation between the concentration of chlordecone in blood and its half-life in a given individual (apparent first order kinetics), in rats, the half-life for chlordecone in blood was 8.5 days for the first 4 weeks, 24 days for the next 8 weeks, and 45 days for the last 14 weeks.

Pathways of Intestinal Excretion

There is now strong evidence that chlordecone enters the intestine via biliary excretion and also via a novel, nonbiliary pathway. Most organochlorine pesticides are poorly excreted in bile. In contrast, substantial amounts of chlordecone representing as much as 1% of total body content are excreted in human bile when this fluid is collected either indirectly by duodenal intubation (9), or directly from a surgically implanted T-tube (10). Even higher fractional amounts of chlordecone ranging from 2–4% are excreted in the bile in gerbils (106), hamsters (106), monkeys (107), and rats (8). Although the mechanism of chlordecone excretion in bile in unknown, it has been observed that biliary excretion of chlordecone is linked to the bile salt dependent fraction of bile flow (97), and that chlordecone is solubilized in bile by associating with mixed bile salt micelles (B. F. Scharschmidt, personal communication). Perhaps a difference between the solubility of chlordecone and mirex in bile accounts for the observation that the biliary excretion of chlordecone in rats is 10–20 times greater than that of mirex when comparable amounts of each chemical are in the liver (73).

It seemed logical that as a practical consequence of the unusually high

rates of biliary excretion of chlordecone, the pesticide would be cleared from the body more rapidly than would other hydrophobic chemicals. However, in man, only 5–10% of the total amount of biliary chlordecone entering the upper intestine appeared in the stool (9). We made the assumption that the biliary chlordecone was being reabsorbed from the intestine and recirculated to the liver. Accordingly, we tested the hypothesis that oral administration of cholestyramine, a nonabsorbable anion exchange resin that binds chlordecone in vitro (11), would interrupt the "enterohepatic recirculation" of chlordecone by binding the pesticide in the intestine, preventing its reabsorption, and augmenting its excretion in stool. Consistent with this interpretation, a randomized, controlled clinical trial of cholestyramine feeding to the workers demonstrated unequivocally that cholestyramine increased fecal excretion of chlordecone (9) and significantly shortened the half-life of chlordecone in blood (80 days) and in fat (64 days) (94) as compared to the half-lives either prior to treatment or in a concurrent control group treated with placebo (blood, 139 days) (9). Removal of chlordecone from the body by cholestyramine therapy was accompanied by amelioration of the signs and symptoms of chlordecone toxicity (9).

In rats given a single dose of chlordecone, the amount of pesticide appearing in the stool was 100% of that in the bile, in contrast to the value of 5–10% we found in humans (11). Thus, it appeared as if rats, unlike humans, lacked the capability of reabsorbing biliary chlordecone in the intestine (11). However, when the chlordecone-treated rats were fed cholestyramine, there was a prompt increase in the excretion of chlordecone in the stool and a decrease in the total content of chlordecone in the carcass (11). There was also a decrease in the concentration of chlordecone in each tissue examined individually (11). The increased fecal excretion of chlordecone in cholestyramine fed rats was not due to increased biliary excretion of chlordecone (11). However, we noted that the amount of chlordecone in the stool of cholestyramine fed rats was more than 200% of that appearing in bile (11). This observation suggested that chlordecone entered the intestine from a *nonbiliary* source. The first solid evidence for the existence of a quantitatively important nonbiliary pathway for entry of chlordecone into the intestine was obtained in a unique experiment in a chlordecone poisoned patient who had a T-tube surgically implanted in his common bile duct following cholecystectomy (10). When all bile was diverted from entering the intestine, chlordecone continued to appear in the bile at the same concentration. However, this maneuver failed to stop the excretion of chlordecone in stool as would be expected if biliary chlordecone were the only important source of fecal chlordecone (10). Indeed, with the biliary stream completely diverted from entering the intestine, fecal excretion of

chlordecone actually increased 8 times when compared to the fecal excretion of chlordecone when the diverted bile (and biliary chlordecone) was continuously infused into the duodenum (enterohepatic circulation intact). The eightfold increase in fecal excretion of chlordecone produced by diverting the bile was greater than the fivefold increase produced by treating this patient with cholestyramine at a time when the diverted bile was being continuously infused into the duodenum (10). To confirm the results of this experiment, we subjected chlordecone-treated rats to complete diversion of bile through a surgically externalized bile fistula. This produced no decrease in fecal excretion of chlordecone when compared to a preceding control period when bile was allowed to enter the intestine. This established that in rats as in humans, chlordecone enters the intestine from a nonbiliary source. However, unlike man, bile diversion in chlordecone-treated rats failed to significantly *increase* fecal excretion of chlordecone (10). Administration of cholestyramine to bile-diverted rats increased fecal excretion of chlordecone by twofold, a value similar to that produced in unoperated controls (10). It appears unlikely that the sloughing of chlordecone-laden intestinal cells can account for the nonbiliary excretion of chlordecone, based on a comparison of the rates of chlordecone excretion in stool with the total amount of pesticide contained in the excised small and large intestines (10).

To summarize, chlordecone enters the intestine primarily from a nonbiliary source, probably transmucosal transport in the gut itself, by a process that in man, but not in the rat, is inhibited by the presence of bile. Enhanced fecal excretion of chlordecone by cholestyramine may be due to direct binding of chlordecone in the intestinal lumen. In man, cholestyramine may also bind bile constituents (e.g. bile salts) and, in so doing, release inhibition of the nonbiliary pathway. Recent computer assisted modeling of chlordecone pharmacokinetics suggest that even with cholestyramine therapy, the nonbiliary pathway would be capable of excreting six times more chlordecone if better sorbents were available (113). Liquid paraffin (114), algae (115), and sucrose polyester (116) are among the sorbents currently under investigation. Since it appears that other environmental chemicals including mirex, dieldrin, and polychlorinated biphenyls (11) may be excreted by the nonbiliary pathway, a better understanding of the comparative physiology of intestinal transport of such chemicals could lead to the rational design of practical means for enhancing the elimination of slowly excreted lipophilic toxins (117).

Metabolism

It was commonly believed that chlordecone was not subject to metabolism in animals (118). We found that small amounts of the chlordecone in human bile appeared to be glucuronide conjugates (<10%) (15). Furthermore, as

much as 35% of the chlordecone in some human bile samples was present as an unidentified metabolite that can be converted to chlordecone only by acid hydrolysis under harsh conditions (15). However, the major chlordecone metabolite is a stable, reduced form of chlordecone, termed chlordecone alcohol, which was first isolated from human stool (119). This metabolite, although present in human stool in four times greater concentrations than chlordecone (10), had escaped notice for several years. This was because chlordecone alcohol is at least 10 times more lipophilic than chlordecone (K. Triebwasser, unpublished observation) and, therefore, the metabolite is poorly recovered when an alkali wash is used to "clean up" stool extracts (7). This can be overcome by using an acid washing procedure (15). There is strong evidence that the site of bioreduction of chlordecone is human liver. When bile was diverted from the intestine through a T-tube in one patient, chlordecone alcohol as well as chlordecone continued to appear in the bile, while chlordecone alcohol disappeared from the stool (10). However, in the same fecal samples, the concentration of chlordecone was increased (10). The concentration of chlordecone alcohol in four human bile samples averaged three times higher than that of chlordecone (15). Chlordecone alcohol in bile is largely conjugated with glucuronic acid (94% releasable by β-glucuronidase), whereas chlordecone alcohol in stool is unconjugated ($<$1% released by β-glucuronidase or sulfatase) (15). Thus, hepatic bioreduction of chlordecone followed by glucuronide conjugation of the alcohol metabolite is the major metabolic pathway of chlordecone in man.

Studies of comparative metabolism of chlordecone among several species disclosed that chlordecone alcohol was absent in the stool, bile, or liver of chlordecone-treated rats, guinea pigs, or hamsters (106), and in the stool of mice (107). In contrast, when Mongolian gerbils were given a single oral dose of chlordecone, chlordecone alcohol appeared in stool and in bile within 24–48 hours (106). Indeed, eight days after the dose, the biliary excretion of chlordecone alcohol was twice that of chlordecone (106), a ratio similar to that observed in humans (15). Also similar to the results in man, the bulk of the chlordecone alcohol in gerbil bile was present as a glucuronide conjugate. Hence, the gerbil should be useful as a practical model of human chlordecone metabolism. Indeed, in preliminary studies, we found that when cytosolic extracts of gerbil liver homogenate were incubated with chlordecone in the presence of NADPH, chlordecone alcohol was formed (106). It is known that the liver in many species of animals including rats and mice contains aldoketo reductase activities (120). Therefore, it would appear that chlordecone reduction is mediated by a substrate- and species-specific liver reductase. The toxicity of chlordecone alcohol has not been studied, although, like chlordecone, this metabolite can inhibit Na^+, K^+ ATPase (40). Preliminary single dose pharmacokinetic studies in

rats and gerbils suggested that chlordecone alcohol was excreted exclusively in stool at a rapid rate for three days with very little of the metabolite being excreted thereafter (106). In both species, some of the administered chlordecone alcohol was converted to chlordecone, possibly in extrahepatic tissues (106).

CONCLUSIONS

The "Kepone Episode" has provided a unique opportunity in the field of comparative toxicology. Contrary to the classical investigative approach to such inter-species comparison, we were able to make the key toxicological or pharmacokinetic findings first in humans and subsequently to confirm these in animals. We have learned that there are striking similarities between man and experimental animals in the target organs (central nervous system, liver, reproductive organs) of chlordecone toxicity, although classical toxicology studies in rats produced several "false positive" findings with respect to human toxicity. The distribution and route of excretion of the pesticide were similar in man and rats. Just as striking are the differences between humans and rats in chlordecone metabolism and in the intestinal physiology of chlordecone transport. Our experience emphasizes the utility of aggressive clinical investigations of the effects of environmental chemicals, especially in the face of industrial disasters or area-wide contaminations. Clinicians and laboratory scientists should be encouraged to join forces so that the scientific value of such "accidents" is not squandered.

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