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A Review of Mechanisms Which May Explain the Positive Epidemiologic Association of Organochlorines with Type 2 Diabetes

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ABSTRACT

The Stockholm Convention on Persistent Organic Pollutants eliminated the production, use, and/or emissions of persistent pollutants which include organochlorines. Banned organochlorines are resistant to biodegradation and are therefore still present in soil, air, and water. They are lipophilic and bioaccumulate up the food chain so the major source of human exposure comes from fatty food. More than 40 epidemiological studies have indicated a positive association of organochlorines with type 2 diabetes but the data are insufficient to establish causality since most studies are case-control and cross-sectional where temporal associations cannot be determined. Also, some epidemiological studies have reported negative associations where organochlorine concentrations were lower in diabetics than controls and others showed inconsistencies across different populations or geographic areas. Because cross-sectional studies are prone to such biases, prospective studies were indicated. Most of these cohort prospective studies were not population based and had such small numbers of diabetics that the odds ratios' confidence intervals were large enough to engender uncertainty. It has also been suggested that obesity or diabetes may increase the level of organochlorines (reverse causation) rather than high levels leading to disease (causation). In addition, non-monotonic dose-response relationships have been reported between organochlorines and metabolic syndrome endpoints such as impaired insulin action, triglyceride levels, and type 2 diabetes prevalence. Concerns like these reinforce the need to move away from epidemiological studies and toward mechanistic research to determine whether the association between organochlorines and type 2 diabetes is causal. To this end, a literature review was conducted searching for publications suggesting mechanisms for the positive association. The most interesting mechanisms are described in detail. In addition to describing the evidence for possible mechanisms, various concerns and suggestions are discussed.

Keywords organochlorine; type 2 diabetes; positive epidemiologic association; mechanism of action; metabolic dysregulation; insulin signaling dysregulation

Abbreviations

DDT: dichlorodiphenyl-trichloroethane

Introduction

Using data from the United States of America National Health and Nutrition Examination Survey, the 2006 cross-sectional study by Lee et al.[1] found that serum levels of persistent organic pollutants, half of them organochlorines, were associated with diabetes. Since then, more than 40 cross-sectional epidemiological studies in numerous countries have indicated a similar link.[2]

In January 2011, the National Toxicology Program of the National Institute of Environmental Health Sciences held a three-day workshop on the "Role of Environmental Chemicals in the Development of Diabetes and Obesity". The purpose of the meeting was to evaluate the strength of published evidence suggesting an association between environmental chemicals and the onset of diabetes and/or obesity. They concluded that although "the overall evidence is sufficient for a positive association of some persistent organic pollutants with type 2 diabetes. Collectively, these data are not sufficient to establish causality."[3] The attendees concluded that additional experiments were required to determine whether causality existed. In the 14 years since the workshop, numerous studies have been undertaken in this effort.

Organochlorine insecticides can be divided by their mechanisms of action. The DDT family causes repetitive neuronal firing by inhibiting the closing of sodium channels whereas the cyclodienes (aldrin, dieldrin, endrin, heptachlor, toxaphene, and endosulfan) and the hexachlorocyclohexanes (lindane) cause excessive neuronal activity by inhibiting the opening of the chloride channel of gamma-aminobutyric acid receptors.[4-5] Mirex and chlordecone (Kepone) are thought to inhibit ATPases.[4]

Although organochlorine insecticides like DDT were made first, several later organochlorines were industrial chemicals or by-products such as polychlorinated biphenyls (PCBs) and the dioxins[6] which are still being released from metal industries, shredder plants, dump sites and open burning sites of e-waste in developing countries.[7] Today the organochlorines are the most abundant of the environmental persistent organic pollutants.[8]

The history of DDT typifies the pattern of organochlorine manufacture and use. During World War 2, Japanese control of the Dutch East Indies cut off the supply of rotenone and pyrethrum availability was reduced by a failure of the chrysanthemum crop in Kenya. These events required the US Army to search for new insecticides to prevent typhus and malaria in the war zones. The insecticidal ability of DDT was discovered in 1939 by Paul Müller and patented by Müller's Swiss employer J. R. Geigy. In 1942 DDT was sent to Geigy's New York office where it was tested by the US Dept of Agriculture for possible military use.[9]

Prior to World War II, scientists at the Food and Drug Administration and the National Institutes of Health fed laboratory animals DDT and found that large amounts caused convulsions and death.[9] In 1943 long term exposure to DDT was found to cause kidney, liver and thyroid damage and sometimes death. The Food and Drug Administration warned in 1944 that small amounts of DDT in the diet were toxic and that safe chronic levels were very low.[9] Further research showed, however, that short dermal exposure to DDT led to very low toxicity levels.[4] Balancing the reduced risk of skin absorption versus the number of probable military deaths due to malaria and typhus, the army adopted DDT for use as a powder. Military production of DDT went from 0 pounds in 1942 to 193,000 per year in 1943 and increased to 3,000,000 lbs per month by June 1945.[9]

Until August 1945, the US army and the Public Health Service had controlled the use of DDT as a secret war asset. After World War II there was no federal agency with the authority to control DDT. The Department of Agriculture enforced labeling requirements, and the Food and Drug Administration could seize foods with high pesticide residues but no agency could stop a business from selling accurately labeled chemicals and DDT use in homes and farms soared.[9]

In 1947 the US Congress implemented a plan to eliminate malaria by forming the National Communicable Disease Center, which later became the Centers for Disease Control and Prevention. By 1952, 6.5 million houses had been sprayed with DDT and malaria was eradicated within the US.[10] In 1955 the World Health Assembly began a Global Malaria Eradication Campaign using both indoor and outdoor DDT spraying which eradicated malaria in the developed nations plus many Asian and Latin American countries by 1967.[11]

Reduction in human deaths from typhus and malaria led to Paul Müller receiving the Nobel prize in Physiology or Medicine in 1948.[4] Popular magazines reported these actions and touted DDT as being as great a discovery as penicillin. The general public began to believe that DDT was completely safe, not realizing that the persistence and broad activity that made DDT ideal for the armed forces could leave poisonous residues on food and kill beneficial insects.[9] Annual US DDT use rose to a high of 36,000 tons in 1959.[11]

Recognition of DDT's environmental bioaccumulation and its interference with bird reproduction resulting from eggshell thinning led to Sweden banning it in 1970, the USA in 1972, and the United Kingdom in 1986. Effects on humans did not contribute to these decisions.[11] Bans on several other persistent organochlorines followed such as the polychlorinated biphenyls, the chlorinated cyclodiene pesticides, and mirex.[11] The use and production of large quantities of DDT, polychlorinated biphenyls and other organochlorines continued unabated, however, within the Communist countries of Eastern Europe until 1989[12] and agricultural DDT use continued in China until 1983.[13]

The multinational Stockholm Convention on Persistent Organic Pollutants became effective on May 17, 2004.

Participating countries agree to eliminate the production, use, and/or emissions of persistent organic pollutants including organochlorines such as aldrin, chlordane, dieldrin, and heptachlor.[14] Although agricultural uses are prevented by the Stockholm Convention, DDT production and indoor spraying are still allowed for public health uses due to its effectiveness at preventing malaria, leishmaniasis and typhus by killing the insect vectors of these diseases. India is the largest user and manufacturer of DDT with China and the Democratic People's Republic of Korea (i.e., North Korea) manufacturing much smaller amounts. China last used DDT for insect vector control in 2003 but North Korea continues to use it both agriculturally and for vector control. India and China sell both DDT and ingredients for its formulation to African countries, where its use increased after implementation of the Stockholm Convention[15] likely due to the high rate of malaria in Africa at the time. Even as late as 2023, the African region of the World Health Organization was home to 94% of the world's malaria cases (246 million) and 95% (569,000) of malaria deaths with children under five accounting for about 76% of these deaths.[16]

Although DDT is one of the few organochlorines still in active use, there is still the possibility of exposure to other banned organochlorines since they are very resistant to biodegradation and are, therefore, still present in the soil.[17] Since organochlorines are lipophilic and bioaccumulate as one moves up the food chain, the major source of exposure for humans comes from food, especially those foods that are animal based and fat-containing such as meats, fatty fish, and dairy products.[6]

The continuing use and/or manufacture of DDT in India, China and Africa should be associated with increased diabetes according to the afore mentioned epidemiological association. According to the International Diabetes Federation in 2021, the age-adjusted comparative prevalence of diabetes was 10.6% in China, 9.6% in India but only 5.3% for the combined 48 Sub-Saharan African countries, the lowest prevalence among all of the International Diabetes Federation regions.[18]

Most epidemiological data come from case-control and cross-sectional studies where temporal associations cannot be determined.[19] Also, some epidemiological studies have reported negative associations where organochlorine concentrations were lower in diabetics than controls[20] and others showed inconsistencies across different populations or geographic areas.[21] Because cross-sectional studies are prone to such biases, prospective studies were indicated.[2] Most of these cohort prospective studies were not population based and had such small numbers of diabetics that the odds ratios' confidence intervals were large enough to engender uncertainty.[6]

It has also been suggested that obesity or diabetes may increase the level of organochlorines (reverse causation) rather than high levels of organochlorines leading to disease (causation).[22] In addition, non-monotonic dose-response relationships have been reported between organochlorines and endpoints indicative of metabolic syndrome such as impaired insulin action,[21,23] triglyceride levels,[17] and type 2 diabetes.[24]

Concerns like these reinforce the need to move away from epidemiological studies and toward more mechanistic research as a way to determine whether the epidemiological association of organochlorines with type 2 diabetes is also causal. To facilitate the mechanistic approach, this literature review attempts to determine the total number of published studies which suggest mechanisms for the positive epidemiologic association of organochlorines with type 2 diabetes and to critically examine the most interesting in greater detail.

Methods

We conducted a literature review of peer-reviewed, English language journals which described original scientific research and were available from the databases listed in Table 1. Only indexed journals were searched. The dates given next to each database indicate the date range of the papers returned.

The search query was "organochlorine(s) and diabetes". The Conditions column in Table 1 indicates the filters queried for each database using this phrase.

Each abstract was read and the following were excluded: duplications, papers on gestational and Type 1 diabetes, prenatal exposure, mixtures containing chemicals other than organochlorines and epidemiological studies which did not suggest a cause or mechanism.

The full texts of the remaining papers were read in their entirety and grouped by proposed mechanism. References listed in the selected papers may also have been obtained and read in order to obtain a more complete understanding of the mechanisms being discussed.

The number of papers returned from each database are shown in Table 1 along with the number of publications which discussed mechanisms that could explain the positive epidemiologic association of organochlorines with type 2 diabetes and were therefore included in the final review.

Table 1: Databases searched for "organochlorine(s) and diabetes". The Conditions column shows the filter used to search the listed database. The number of published studies which contained "organochlorine and diabetes" are shown in the Returns column. The Included column shows the number of the returned studies which discussed mechanisms that could explain the positive epidemiologic association of organochlorines with type 2 diabetes and were therefore included in the final review.

DATABASE	DATES	CONDITIONS	RETURNS	INCLUDED
EBSCO	2000-2025	Title	19	3
		Abstract	116	25
Pubmed	1980-2025	Title/Abstract	152	23
Cochrane Library	2014-2025	Title/Abstract/Keyword	6	0
Ebsco Discovery Service	1980-2022	Abstract	256	67
Nioshtic-2	1978	Abstract	1	0
Scopus	1973-2025	Title/Abstract/Keyword	289	76
Biomed Central	2000-2025	Search Box	113	4
Springer Nature Link	1966-2025	Search Box	633	40
Environment Complete (EBSCO)	2007-2025	Title	13	5
	2002-2025	Abstract	82	26
ScienceDirect	1973-2025	Search Box	1,273	87
Medline Plus	2005-2025	Title	23	6
	1980-2025	Abstract	137	43
Medline (EBSCO)	1980-2025	Abstract	160	50
	2005-2025	Title	25	9
		TOTALS	**3298**	**464**

Results

INSULIN SIGNALING PATHWAY DYSREGULATION

The complexity of the insulin signaling pathway means that 60 nodes could be involved in the specific mechanism of action. Alteration in expression levels of pathway nodes could change the normal signaling. As far back as 1979,[25] Mahmood et al. found that monkeys fed DDT for 100 days had increased intestinal uptake of D-glucose along with elevated sucrase and lactase enzyme activities. Microarray studies of human primary blood mononuclear cells after polychlorinated biphenyl exposure indicated changes in the insulin signaling pathway.[26] Discussion of some of the most frequently suggested causes of insulin signaling alterations follow.

Moreira et al.[27] showed that 1nM technical-grade chlordane caused increased expression of the glucose transporter gene but Park et al.[28] showed that a mixture of organochlorines containing chlordane repressed glucose transporter protein levels.

In zebrafish, organochlorine exposure plus a high fat diet significantly increased glucose levels without changing insulin levels.[29] This observation was suggested to be caused by a concomitant upregulation of expression of the glucagon receptor a gene. Organochlorine exposure and caloric restriction lowered this expression. This possible mechanism was contraindicated by research using a high fat diet in mice. Ibrahim et al.[30] used whale meat containing a high level of polychlorinated biphenyls, cyclodienes, DDT and DDT metabolites plus a high fat diet. While the mice fed only the high fat diet showed evidence of insulin resistance, those fed the high fat diet plus the whale meat laden with persistent organic pollutants had better insulin sensitivity and glucose tolerance in spite of a high accumulation of persistent organic pollutants in their adipose tissues. The Inuit of Greenland have extremely high plasma concentrations of polychlorinated biphenyls and organochlorine pesticides due to consumption of marine mammal blubber but Jørgensen et al.[31] found no association between these levels and impaired glucose tolerance, insulin resistance, or diabetes.

Additional evidence against a high fat diet plus organochlorines being the cause for the organochlorine/type 2 diabetes association comes from the testing of a high fat diet plus chronic exposure to metabolites of DDT.[32] Here the high fat diet increased the fasting expression of genes involved in hepatic glucose production (*Pepck* and *G6pase*) as expected, but high fat diet plus chronic exposure to DDT metabolites decreased their expression.

In response to xenobiotic stressors, cells may reduce insulin signaling but if this occurs excessively, insulin resistance may result with the cells becoming less sensitive and requiring increasing amounts of insulin to respond.[33] As organochlorines are xenobiotic chemicals and insulin resistance, as measured by the homeostasis model assessment of insulin resistance, is a hallmark of type 2 diabetes, this interaction is frequently suggested as the cause of the organochlorine/type 2 diabetes association but the data are contradictory. Baumert et al.[34] found that higher plasma levels of hexachlorobenzene and polychlorinated biphenyl #118 were associated with higher glucose levels during an oral glucose tolerance test for obese adolescents having a family history of type 2 diabetes but not in obese adolescents without a family history. In addition, the Baumert study found no associations, in either cohort, between lipophilic persistent organic pollutants and other measures of glucose homeostasis including homeostasis model assessment of insulin resistance, insulin area under the curve, and

glycated hemoglobin. These results differ from the homeostasis model assessment of insulin resistance values of non-diabetics aged 20 years and older in Lee et al.[35] Here an elevated homeostasis model assessment of insulin resistance was associated with serum levels of oxychlordane, trans-nonachlor, and two non-dioxin polychlorinated biphenyls and this association strengthened with increasing waist circumference. Imbeault et al.,[36] however, found that as obese humans lost weight their plasma organochlorine levels increased due to release from fat storage, contradicting the association with increasing waist circumference.

Boada et al.[37] found that women with higher serum levels of aldrin or a DDT metabolite had lower serum levels of insulin-like growth factor-1. A similar result was also found between aldrin and insulin-like growth factor-1 levels in males. Insulin-like growth factor-1 is an anabolic hormone which alters glucose and lipid metabolism.[38] Since the liver is the primary source, Boada et al.[37] suggested that the correlation could be the result of liver damage by organochlorines which then led to type 2 diabetes. Although insulin-like growth factor-1 is secreted from the liver, levels are controlled by the pituitary growth hormone.[38] Since obesity has been associated with inhibiting growth hormone activity and concomitant lower insulin-like growth factor-1 levels, Kubo et al.[38] compared obese individuals having low levels to those with normal levels for altered glucose metabolism. They found that although body fat mass and percentage were significantly higher in the low insulin-like growth factor-1 cohort, there was no difference in glucose metabolism parameters (fasting plasma glucose, homeostasis model assessment of insulin resistance, insulin levels, type 2 diabetes) suggesting that the organochlorine/insulin-like growth factor-1 relationship does not lead to type 2 diabetes via changes in glucose metabolism.

Phosphorylation of protein kinase B is involved in the control of glucose uptake, gluconeogenesis, and glycogenolysis.[39] Park et al.[28] showed that a mixture of organochlorines reduced protein kinase B expression in myotubes and suggested that this could reduce glucose uptake. This concept was contradicted by the work of Howell et al.[39] which showed that exposure to DDT metabolites did not alter protein kinase B phosphorylation in the liver, skeletal muscle, or adipose tissue of male mice.

As far back as 1977, insulin secretion from mouse pancreatic islets was shown to be inhibited by oral DDT.[40] Later Jørgensen et al.[31] found that levels of polychlorinated biphenyls and organochlorines were significantly inversely related to insulin secretion in the highly exposed Inuit population. This reduction in insulin secretion was present in the absence of glucose intolerance and insulin resistance. Similar results were seen in a Korean cohort where Lee et al.[41] found decreasing insulin secretion to be associated with increasing serum levels of organochlorine pesticides and that this relationship was stronger in insulin-sensitive individuals. In the same paper, Lee showed that rat beta cells responded differently to various organochlorines with insulin secretion being lower after exposure to DDT, trans-nonachlor and the polychlorinated biphenyl Aroclor 1254, but significantly higher following low doses of beta-hexachlorocyclohexane. However, Park et al.[42] reported that low dose beta-hexachlorocyclohexane reduced rat beta cell insulin secretion as did chlordane, DDT, heptachlor, and hexachlorobenzene. Han et al.[43] found that rats exposed to low dose pentachlorophenol showed decreases in insulin, C-peptide, and the homeostasis model assessment of beta-cell function (homeostasis model assessment of insulin resistance-beta), all of which are indicators of beta-cell dysfunction. They also saw histopathologic damage in the pancreatic islets in the low dose exposed rats. The median pentachlorophenol serum concentration of the rat low dose, 0.3 mg/kg group (584.1 ug/L), was close to the highest pentachlorophenol serum concentration (416.3 ug/L) found in their accompanying human case-control study. Ward et al.[44] reported that murine beta cells exposed to a DDT metabolite increased insulin secretion whether or not glucose was present. This work also found that glucose plus exposure to the same metabolite decreased prohormone convertase levels whereas the metabolite without glucose increased these levels. Since prohormone convertase is involved in the cleavage of insulin to its mature form, this observation suggests that the DDT metabolite may alter insulin production. Similarly, Pavlikova et al.[45] found that exposing a human pancreatic cell line to sublethal levels of DDT and its metabolite caused a reduction in expression of proteins involved in glycolysis and cytokeratin 18. A cytokeratin 18 knockout study[46] in the beta cell islets of mice caused a disruption of insulin vesicle morphology. Since insulin vesicles are necessary for nutrient-induced insulin release, a reduction of cytokeratin 18 could alter insulin production. A review by Hoyeck et al.[47] cited studies suggesting that many different persistent organic pollutants, not just organochlorines, may cause beta-cell dysfunction. This conclusion was supported by Bresson and Ruzzin[48] who showed that non-dioxin and dioxin-like polychlorinated biphenyls plus metabolites of DDT all disrupted the oxidant scavenging ability of rat insulinoma beta cells by different mechanisms but the end result was the same: levels of reactive oxygen species high enough to reduce insulin secretion by the cells, eventually leading to their destruction.

ENDOCRINE DISRUPTION

There is evidence that the actions of organochlorines may be sex dependent. Charles et al.[49] conducted a longitudinal, nested case-control study in which repeated blood samples were collected five times from the same 255 individuals from 1986 to 2016 and analyzed for organochlorines. Women had a slower elimination rate for all the organochlorines except hexachlorobenzene and the most common DDT metabolite which were similar in both sexes. In Imbeault et al.,[36] males showed an association between increasing organochlorine levels and a reduction in the level of fasting insulin, but no similar relationship was seen in women. Lee, et al.[50] saw hyperglycemia in zebrafish after exposure to an organochlorine mixture but found it happened only in females. Neither sex showed changes in insulin levels. These results may relate to the concept that many organochlorines have been classified as endocrine

disruptors which interfere with levels of natural hormones or imitate their effects.[51] Aldrin, dieldrin, endosulfan, polychlorinated biphenyls and chlordecone are all estrogen mimics while DDT, metabolites of DDT, and dioxins are both estrogen mimics and also have antiandrogenic effects.[51] Some polychlorinated biphenyls have also been reported to be antiestrogenic.[52] The organochlorines functioning as endocrine disruptors would suggest that low doses, similar to levels of natural hormones, could elicit significant effects and also lead to sex dependent responses based on the ratio of natural estrogen to estrogen mimics. Also, low doses generating significant effects support the assertion that the increasing number of type 2 diabetes cases at the present time can still be caused by organochlorines even though body burdens have declined following their banning decades ago.[53]

Estrogen receptors are expressed in many tissues associated with glucose maintenance such as adipose tissue, skeletal muscle, pancreatic beta-cells, and the liver. In a more direct association with type 2 diabetes symptomology, estrogen has also been shown to reduce blood glucose levels, while insulin resistance and activation of estrogen receptors are involved in glucose-stimulated insulin synthesis and release.[51] Interactions such as these have led to research on endocrine disruption by organochlorines as a possible explanation for their epidemiological association with type 2 diabetes.

Estrogen activates estrogen receptors alpha and beta which then bind to estrogen response elements located in promoters thereby controlling the downstream gene's expression.[54] Estrogen mimics also bind to these receptors but, due to their conformation being slightly different from the endogenous ligand estrogen, they bind less efficiently and engender a slightly lower gene expression response. Because of this lesser receptor affinity, estrogen mimics were originally not considered to be of much concern.

This opinion changed, however, when new mechanisms of interaction were discovered. In addition to the slow transcriptional changes from interaction with the alpha and beta receptors, estrogen, and its mimics, also bind to plasma membrane receptors and initiate rapid signal cascades.[55] Dieldrin, DDT metabolites, and endosulfan have been shown to alter calcium flux[56] and endosulfan has been shown to affect the activity of several kinases.[57] These changes can result in artificially increased estrogen signaling in tissues intimately involved in type 2 diabetes such as adipose tissue, skeletal muscle, pancreatic beta-cells and the liver.

OBESOGENS AND ADIPOCENTRIC MECHANISMS

When excess energy is available, body fat increases via white adipose tissue storing the neutral lipid triacylglycerol in adipocyte vacuoles and releasing fatty acids through lipolysis when energy is needed (as reviewed in[58]). In addition, adipose tissue secretes hormones active in energy homeostasis such as leptin[59] and adiponectin.[60] Preadipocyte fibroblasts undergo adipogenesis to differentiate into mature adipocytes whose size increases as additional lipid is sequestered in their vacuoles. Hyperplasia and hypertrophy lead to adipocyte dysfunction and then obesity, which is a major type 2 diabetes risk factor.[61-62] The total number of adipocytes is established in early development while their size increases in adulthood.[63]

Baillie-Hamilton[64] reported that animals gained weight following exposure to low concentrations of several chemicals including persistent organic pollutants. Grün et al.[65] suggested the "obesogen hypothesis" where obesogens are chemicals that cause obesity by disruption of energy and lipid homeostasis mainly through altering adipose tissue function and adipogenesis. Due to their long-term use, world-wide transport, resistance to degradation, bioaccumulation and ability to cause chronic internal exposure, it is suggested that persistent organic pollutants contaminate all of the world's lipids and adipose tissue making them likely candidates for obesogenic activity.[23] Many studies have examined the possibility of organochlorines having obesogenic activity which can increase the risk of type 2 diabetes.[66]

The majority of *in vitro* studies have used the 3T3-L1 fibroblast cell line that was originally isolated from a mouse embryo. These cells are used as preadipocytes and chemically stimulated to initiate differentiation into mature adipocytes.[67] In this way chemical exposure can be tested for effects on adipogenesis and adipocyte function such as lipid accumulation and biomarker production that could possibly increase type 2 diabetes risk in the future.

Howell and Mangum[67] investigated the actions of oxychlordane, dieldrin, or the most common DDT metabolite on adipogenesis, fatty acid uptake, lipolysis and adipokine/cytokine expression and release. They found that dieldrin decreased adipogenesis but the others did not. All three organochlorines increased basal free fatty acid uptake but had no effect on insulin-stimulated free fatty acid uptake or lipolysis by mature adipocytes. These findings suggest that these organochlorines may promote the adipocyte hypertrophy associated with obesity. The metabolite of DDT and dieldrin increased release of adiponectin from mature adipocytes but only the DDT metabolite increased leptin and resistin release. Since alteration of adipokine/cytokine levels has been associated with systemic inflammation and insulin resistance, the DDT metabolite and dieldrin may promote the development of these two type 2 diabetes risk factors.

Taxvig et al.[68] likewise found that the same DDT metabolite and dioxin increased leptin and adiponectin without altering adipogenesis of the 3T3-L1 cells. Unlike in Howell and Mangum,[67] however, the DDT metabolite caused no significant change in lipid accumulation. A dioxin polychlorinated biphenyl caused no change in lipid storage but the non-dioxin-like polychlorinated biphenyl #153 caused increases in lipid accumulation, adipocyte differentiation and the release of leptin and adiponectin. Taxvig et al.[68] pointed out that the importance of these results is heightened by the fact that polychlorinated biphenyl #153 is one of the most abundant polychlorinated biphenyls in both the environment and in humans.

Mangum et al.[69] reconfirmed the earlier findings of Howell and Mangum[67] by showing that a DDT metabolite induced a concentration dependent increase in neutral lipid accumulation and that these lipids were triglycerides. By using a sub-optimal differentiation cocktail, they were also able to show that the metabolite of DDT increased expression of genetic markers of adipocyte differentiation. They suggested that these changes in gene expression indicate that high levels of DDT metabolites may promote an increase in both the number of adipocytes and the amount of lipid they contain, leading to the increased visceral adiposity typical of obesity.

Kim et al.[70] found that both DDT and its most common metabolite increased triglyceride accumulation and expression of genes and proteins involved in adipocyte differentiation. Both the dioxin-like polychlorinated biphenyl #118 and the non-dioxin-like #138 decreased the number but increased the size of the lipid storage droplets in 3T3-L1 adipocytes.[71] They also increased both mRNA and protein levels of several adipocyte differentiation markers. Metformin, a drug for treating insulin resistance, was able to reverse both the lipid droplet enlargement and mitigate the marker expression changes. Using a mixture of polychlorinated biphenyl and organochlorine pesticides based on levels found in the blood of the Scandinavian population, Xie et al.[72] found that 3T3-L1 cells started forming adipocytes and storing lipids at polychlorinated biphenyl/organochlorine concentrations equivalent to one-tenth that found in human blood.

An interesting new mechanistic possibility was suggested by research using 3T3-L1 cells and reported in Piano et al.[73] Aquaglyceroporins are transmembrane channel proteins that control the adipocytic accumulation and release of glycerol. Aquaglyceroporins 3 and 7 are involved in glycerol release whereas #9 is involved in its uptake. Piano et al.[73] exposed mature 3T3-L1 adipocytes for 48 hours to 1uM of one of three non-dioxin-like polychlorinated biphenyls (#101, 153, and 180) and found that all three reduced the protein expression of aquaglyceroporin 3. Numbers 153 and 180 also repressed aquaglyceroporin 7 protein levels whereas #153 increased expression of aquaglyceroporin 9 protein levels. This reduced protein expression of aquaglyceroporins 3 and 7, but increased expression of #9, which, in turn, caused decreased release and increased uptake of glycerol. Since glycerol is important in triglyceride synthesis and lipid metabolism, this increased accumulation leads to increased adipocytic triglyceride levels, adipocyte hypertrophy, dysfunctional lipid metabolism and possibly obesity.

A few *in vitro* studies have utilized human adipose derived mesenchymal stem cells. Müllernová et al.[74] showed that a DDT metabolite decreased the amount of perilipin 2 mRNA. Perilipin 2 protein surrounds the triglyceride core of adipocyte lipid droplets and is normally increased when adipocyte differentiation occurs in the presence of lipids. Perilipin 2 protein is also thought to be involved in lipid accumulation, stabilization of lipid droplets and the maturation of adipocytes. These researchers suggested that this under expression could lead to obesity yet they mention that perilipin 2 overexpression has been linked to hepatic steatosis which can be caused by obesity and/or type 2 diabetes.

Pesta et al.[75] tested similar DDT metabolite concentrations on human adipose derived mesenchymal stem cells and reported increases in the expression of insulin signaling and lipid metabolism genes, the length of pluripotency and fatty acid synthesis. The authors stated that the metabolite unexpectedly stimulated lipid synthesis and increased expression of a gene believed to protect against triglyceride accumulation. In spite of stating that the metabolite elicited effects similar to that of insulin, the authors still suggested that these changes could promote obesity and metabolic disease progression.

More recently Howell and Young[76] used immortalized human subcutaneous preadipocytes/adipocytes to study the effects of an organochlorine pesticide mixture. The mixture ratios of DDT metabolite, trans-nonachlor, and oxychlordane were based on the ratios found in human adipose tissues. Mature human adipocytes exposed to the mix exhibited a slight increase in fatty acid uptake but significant decreases in mitochondrial membrane potential, basal and insulin stimulated glucose uptake, and cellular ATP levels. All these changes support the theory that organochlorines alter adipocyte function which in turn alters adipose tissue functioning which contributes to metabolic dysfunction.

The organochlorine mixture did not affect adipogenesis in contrast to earlier work using 3T3-L1 murine preadipocytes exposed to only the most common DDT metabolite.[69] Likewise the organochlorine mixture used in Howell and Young[76] decreased isoproterenol stimulated lipolysis in the human adipocytes whereas murine adipocytes exposed to either the DDT metabolite or oxychlordane showed no changes in lipolysis.[67] It is unclear if these differences are due to mixture effects or are species related but they emphasize the concerns with translating results from either one species to another or individual exposures to the real life situation of multiple exposures.

La Merrill and Birnbaum[77] pointed out that several rat studies using organochlorines such as hexachlorobenzene and DDT had failed to show any weight gain. They suggested that this might be due to a failure to measure fat mass and using younger rats that were less likely to be obese than middle-aged rats. The lack of weight gain was supported, however, by a later rat study that reported 12 weeks of exposure to a DDT metabolite at 100 ug/kg/day in drinking water failed to cause any weight changes.[78] The metabolite exposure increased lipolysis in visceral adipose tissue and exacerbated the metabolic syndrome risk factors of a high fat diet such as glucose intolerance and dyslipidemia. The high fat diet alone and the standard diet plus DDT metabolite treatment groups had increased adipocyte proliferation whereas the high fat diet plus metabolite treatment showed a significant decrease in the rate of proliferation. In addition, the percentage of small to medium sized

adipocytes increased in the high fat diet plus metabolite group while the percentage of large adipocytes declined. The authors suggested that these changes are indicative of adipose tissue dysfunction but this suggestion contradicts the prevailing opinion that large adipocytes promote obesity.[67,69] Body and liver weight also did not increase in mice exposed to intraperitoneal injections of either dioxin-like or non-dioxin-like polychlorinated biphenyls at 37.5 mg/kg for two, three, and five weeks.[71] However both polychlorinated biphenyls caused several symptoms associated with metabolic syndrome: reduced insulin efficiency plus increases in insulin resistance, adipose mass, adipocyte size, blood glucose level, plasma insulin level, hyperglycemia and the expression of a protein associated with lipid droplets.

La Merrill and Birnbaum[77] reported on the organochlorine cross-sectional human studies conducted up to that point. In these cross-sectional studies a metabolite of DDT and hexachlorobenzene both had negative correlations with the body mass indices of adolescents of both genders but positive correlations with the body mass indices of adult men and women. Lee[53] reported that in both cross-sectional and prospective studies serum concentrations of organochlorine pesticides and polychlorinated biphenyls were strongly linked to type 2 diabetes but obesity was not. Lee[53] reported that in one of their prospective studies concentrations of a DDT metabolite and highly-chlorinated polychlorinated biphenyls in young adults were associated with a larger body mass index 18 years later but in another of their prospective studies they found that the same DDT metabolite, less-chlorinated polychlorinated biphenyls or dioxin, predicted the future risk of abdominal obesity in elderly adults. These reports suggest that the obesogen theory (i.e., that organochlorines cause obesity which leads to type 2 diabetes) is incomplete and much more complex than is presently thought.

Additional studies support this increased complexity. Lind et al.[79] found that in 70 year old Swedes high serum concentrations of organochlorine pesticides and polychlorinated biphenyls containing five or less chlorines corresponded to higher weight gain during the previous 50 years of life. The authors also found that the opposite was true. High serum concentrations of polychlorinated biphenyls with more than five chlorines were inversely proportional to obesity. They suggested several possible mechanisms for the difference including the idea that less-chlorinated and highly-chlorinated polychlorinated biphenyls might have different effects on the rate of fat accumulation. They also noted that the half-life of highly-chlorinated polychlorinated biphenyls is two to three times longer than the five to six year half-life of the less-chlorinated variety and suggested that this could mean that the time between maximum exposure and sampling is therefore shorter for the highly-chlorinated version. This suggestion, however, would seem to contradict the obesogenic theory that higher total concentrations of polychlorinated biphenyls are correlated with obesity since the researchers found that the longer half-life, highly-chlorinated polychlorinated biphenyls were inversely proportional to obesity. Differences in half-lives as an explanation is also problematic since kinetic studies have indicated that obesity itself can lengthen the half-lives of persistent organic pollutants.[23]

Also, Charles et al.[49] reported results from a longitudinal, nested case-control study, with repeated blood samples collected from the same individuals up to five times between 1986 and 2016. Although persistent organic pollutant concentrations declined over time for both controls and subjects with type 2 diabetes, the overall decline for most persistent organic pollutants was slower for those with type 2 diabetes. The authors suggested that type 2 diabetes, or risk factors for it, lower the elimination rate of persistent organic pollutants and that this may explain the positive associations between persistent organic pollutants and type 2 diabetes. Women also had a slower rate of persistent organic pollutant decline than men and different persistent organic pollutants showed different correlations with body mass indices, indicating even more unknown complexities. One of the type 2 diabetes risk factors which could alter the rate of persistent organic pollutant elimination is lipodystrophy, an increased fat redistribution to the viscera which is genetically determined and had previously been associated with type 2 diabetes. Jia et al.[80] determined that the genetic risk of lipodystrophy was highly correlated with the levels of beta-hexachlorocyclohexane and DDT metabolite in the serum of subjects. The levels of both were also significantly associated with a higher risk of type 2 diabetes leading the authors to suggest that a genetic predisposition for visceral fat deposition could affect circulating organochlorine levels and type 2 diabetes risk.

Lim and Jee[81] found a negative correlation between the serum concentrations of non-dioxin-like polychlorinated biphenyls and adiponectin in Korean subjects with high body mass indices. Since adiponectin is involved in glucose homeostasis, the authors suggested that this association supported the idea of polychlorinated biphenyls being obesogenic and diabetogenic. However, since adiponectin expression is reduced in obesity, insulin resistance and type 2 diabetes, reverse causality cannot be ruled out.

Rolle-Kampczyk et al.[82] analyzed human adipose tissue collected from 54 surgical patients for nine persistent organic pollutants. Several organochlorines such as hexachlorobenzene, DDT metabolites and polychlorinated biphenyls were among those detected and measured. The strongest associations between persistent organic pollutants and adipose tissue macrophage infiltration were found in lean individuals. The results led the authors to suggest that certain, but not all, persistent organic pollutants may contribute to adipose tissue dysfunction, adipocyte hypertrophy, and variation in fat distribution, but not to obesity.

Other human studies contradict those above. La Merrill et al.[83] found that plasma levels of DDT and one of its metabolites in Asian Indians living in the San Francisco, California region from 2006 to 2007 were associated with increases in their body mass index, waist circumference, adiposity, and obesity. The participants with high levels were also more likely to have hepatic fat,

fatty liver, insulin insensitivity, high circulating insulin, prediabetes and type 2 diabetes. The authors stated that the DDT and metabolite levels were higher than those found in the general population of both California and the USA and, in fact, their subjects had median DDT levels 12 times higher than California farm workers. The authors said that none of the other 20 chemicals examined had positive associations with measures of adiposity (body mass index and waist circumference) and concluded that most persistent organic pollutants are not obesogens. These other 20 chemicals examined in[83] include several which other researchers have concluded to be obesogens.

The persistent organic pollutant serum concentration can be an inaccurate measure of the total body burden if the person is obese since most of their persistent organic pollutants will be sequestered within their adipose tissue stores. As described in Wolff et al.,[84] the pharmacokinetics suggests that a lean person will have more persistent organic pollutants in their serum since they have less fat to store it in. This can lead to incorrect cross-sectional associations being made.[23]

Although obesity is a well-established risk factor for both metabolic disease and type 2 diabetes, it is also known that obese people have a better prognosis than normal weight people in several chronic conditions such as cardiovascular disease, cancer, chronic kidney disease and even type 2 diabetes.[85-86] This is termed the "obesity paradox" [87] and the difference in mortality is even more pronounced in the elderly.[88-89] Evidence of this paradox was observed in a project involving participants from two US Air Force medical facilities.[90] There was a higher risk of type 2 diabetes associated with increasing DDT metabolite concentrations in older people of normal weight but a lower risk associated with increasing concentrations in those who were overweight or obese.

Lee et al.[23] posits that although persistent organic pollutants stored in adipose tissue act as a chronic internal exposure source, this sequestration reduces the serum level which is available to circulate to and damage other organs and thus the "dilution problem" discussed earlier is part of the explanation of the "obesity paradox". The authors pointed out that among the elderly obese with low serum persistent organic pollutant concentrations no paradox was observed and that they had a higher mortality rate than normal weight elderly with low serum persistent organic pollutant concentrations. Elderly obese with high serum persistent organic pollutant concentrations, however, did benefit from the paradox with a lower mortality rate than normal weight elderly with similarly high serum persistent organic pollutant concentrations.

The statement that obese elderly with high serum persistent organic pollutant concentrations benefit from the paradox with a lower mortality rate contradicts the theory suggested in [23] that since the obese have more adipose tissue they can store more persistent organic pollutants and sequestration reduces the serum level which is available to circulate to and damage other organs. . If sequestration truly reduces the serum level, the obese elderly should not have the high serum persistent organic pollutant concentrations mentioned in. [23]

NUCLEAR RECEPTOR DYSFUNCTION

Nuclear receptors function as concentration sensors of small, lipophilic ligands.[91] Ligand binding controls gene expression of signaling pathways important to homeostasis. Since many organochlorines are also small and lipophilic they can bind in place of the natural ligand and function as agonists and/or disrupt the ability of the nuclear receptors to accurately sense the levels of the natural ligand.

Thyroid nuclear receptors are important regulators of the rate of energy metabolism and, by extension, the use of energy stored as fat. Organochlorines compete with the thyroid hormones, triiodothyronine and thyroxine, for the thyroid nuclear receptor and also for the proteins which transport the thyroid hormones.[92] Also, since organochlorines induce the enzyme uridine diphosphate glucuronyltransferase which glucuronidates thyroid hormones to facilitate excretion, organochlorines also decrease the level of thyroid hormones. Since serum triiodothyronine levels and the resting metabolic rate decrease after weight loss while the level of serum organochlorines increase due to release from body fat, Pelletier et al.[92] measured these in 16 obese men before and after a 15 week stringent diet. The authors reported significant increases in plasma concentrations of hexachlorobenzene, oxychlordane, trans-nonachlor, the most common DDT metabolite and ten polychlorinated biphenyls along with significant decreases in serum triiodothyronine levels and the resting metabolic rate. Changes in the concentrations of DDT, hexachlorobenzene , and five polychlorinated biphenyls were significantly negatively associated with changes in serum triiodothyronine levels. Hexachlorobenzene and polychlorinated biphenyl #156 concentration changes were significantly negatively associated with changes in the resting metabolic rate adjusted for weight loss. The authors suggested that these associations could possibly lead to less weight loss or weight being regained at the end of the diet.

Turyk et al.[93] reported similar results for a group of Great Lakes anglers that consumed their catches. Significant negative associations were found between serum polychlorinated biphenyls and the levels of triiodothyronine, thyroxine and thyroid stimulating hormone. Thyroxine and thyroid stimulating hormone were also found to be significantly higher in a control group that did not consume fish from the Great Lakes. The authors suggested that the results could be explained by several mechanisms including increased glucuronidation, decreased binding to transthyretin, and/or increased conversion from thyroxine to triiodothyronine

Langer et al.[94] found that individuals residing in an area of Slovakia polluted with several organochlorines had increased thyroid volumes, number of thyrotropin receptors, level of thyroperoxidase antibodies and prevalence of impaired fasting glucose levels compared to individuals living in a less polluted area. The increased level of thyroperoxidase antibodies suggests an impaired immune system which could lead to hypothyroidism.[12] Hypothyroidism would decrease metabolism and could lead to obesity.

The peroxisome proliferator-activated nuclear receptors are important to lipid and glucose homeostasis.[95] There are 3 isotypes: alpha, gamma, and delta. Activation of peroxisome proliferator-activated nuclear receptor-alpha improves insulin sensitivity thereby reducing hyperglycemia, hyperinsulinemia, and insulin resistance. The gamma isotype also reduces insulin resistance but by a different mechanism. It increases fatty acid uptake in adipose tissue by inducing lipoprotein lipase and fatty acid transporters. The fatty acid uptake enhances adipose differentiation and fat storage. Peroxisome proliferator-activated nuclear receptor-delta is involved in adipogenesis, lipid metabolism and energy homeostasis.[96] Activation of the delta isotype located in adipose tissue stimulates expression of genes required for fatty acid oxidation and energy use, making it an important regulator of fat burning and thermogenesis. It is obvious that antagonism of the peroxisome proliferator-activated nuclear receptors could lead to a loss of control over fatty acids and possibly obesity.

Shi et al.[97] examined the associations between organochlorine exposure and the levels of sex hormones and methylation of genes for sex hormone receptors. They found a significant negative association between plasma levels of a DDT metabolite and beta-hexachlorocyclohexane and the level of serum testosterone in Chinese males. Organochlorine mixture levels were negatively associated with methylation levels of the androgen receptor in premenopausal women. The authors suggest that, since reduced testosterone and other endocrine disruptions are associated with a greater risk of insulin resistance, changes such as these may increase risk. They saw no statistically significant association between sex hormone methylation levels and type 2 diabetes in their own case-control study of 1,812 participants, however.

Concerns and Suggestions

STATISTICAL RIGOR

Few experimental papers included a description of the statistics used and fewer still included an explanation of the assumptions tested which led to the chosen statistical test. Also, few indicated how adjustments were made for the problem of multiple comparisons. This problematic lack of control for multiple comparisons has been noted by others.[49] Omissions such as these prevent both direct comparisons of data and evaluation of their accuracy.

ADIPOCYTE BIOLOGY

Although adipocytes are the cells used for many obesity and type 2 diabetes studies, there seems to be a lack of consensus about how they develop in humans. Some researchers state that the total number of adipocytes is determined during childhood and that adult adiposity is caused by increasing the size of these; not increasing the number of adipocytes.[63] Other researchers state that adipocytes expand in size (adipocyte hypertrophy) during early weight gain but increase in number in later stages through adipogenesis or adipocyte hyperplasia.[98] Without accurate knowledge of how adipocyte changes relate to obesity, it seems risky to claim that any adipocyte changes observed after organochlorine exposure cause obesity.

OBESOGEN RESEARCH

Gutgesell et al.[99] indicated several areas where additional work is needed. They point out that although animal and human studies have suggested an association between pesticides and increased body weight or fat mass, these have rarely been conducted along with measurement of energy intake and use. They mention the paucity of research on brown and beige adipose tissue and the fact that the observed effects on white adipose tissue may not be due to pesticide action directly on that tissue but, instead could be due to pesticide effects on an entirely different organ system. They voiced concern over whether the doses used in *in vitro* experiments accurately reflect those seen in whole organism exposures and whether the results found using the immortalized 3T3-L1 cells can be replicated in primary cells.

Conclusions

There is a positive epidemiologic correlation of organochlorines with type 2 diabetes but as statisticians point out: correlation does not indicate causality. The mechanisms described above may explain the associations observed but they do not prove that organochlorines on their own can cause type 2 diabetes. It is much more likely that they are but one of a multitude of factors (poor diet, lack of exercise, genetics, increased testing and diagnosis) that contribute to the rising incidence of type 2 diabetes. That said, it is reasonable to be concerned that high organochlorine levels, in combination with a series of other unhealthy factors, may be a factor that causes a person to become diabetic. For this reason, it may be prudent for physicians to be more aggressive in their choice of initial diabetes treatments for patients that are known or suspected of having a high risk of serious organochlorine exposure such as farmers, pesticide applicators, and chemical workers and include questions about such exposure possibilities in a patient's initial intake evaluation.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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