MINIREVIEW

Vitamin A Homeostasis Endangered by Environmental Pollutants (43494)

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Polyhalogenated aromatic hydrocarbons (PHAH) constitute a group of major environmental pollutants that pose a risk to human health (1–4). chemicals have been widely used in industrialized countries and for decades were freely released into the environment. Many of these PHAH are either toxic or generate toxic derivatives during manufacturing operations or pyrolysis. In this group of chemicals, the polychlorinated dibenzo-*p*-dioxins, the polychlorinated dibenzofurans, and the polyhalogenated biphenyls have been under intense scrutiny because of their toxic effects (Fig. 1). Among the most toxic is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (3–9). Although never manufactured for commercial use, it arises as a trace by-product in various commercial synthetic processes and has become widespread in world environments (3, 4, 10–15). However, the enormous production and global industrial use of polychlorinated biphenyls (PCB) (world production, 91 million kg in 1970) and their current ubiquitous distribution place them among the major environmental pollutants (1, 10–13, 16–18); thus, they are a major threat to health. Although the manufacture and use of these toxic chemicals has now been discontinued, many PHAH continue to pollute the environment due to their inert chemical nature and to the additional generation of these compounds as by-products of commercial chemical synthesis reactions, or during waste and metal reclamation, incineration, transformer fires, gasoline combustion, and industrial use of chlorine (1, 2).

Concern over the PHAH and their deleterious effects on health is worldwide because of constant public exposure to these chemicals (2, 3, 7–11, 19). Classic examples are the accidental introduction of polybrominated biphenyls into the food chain of Michigan residents in 1973 (20–22), and the exposure to TCDD, a contaminant in the defoliant Agent Orange during the Vietnam War (23). Although there have been numerous instances of human exposure to PHAH in occupational settings (3, 4, 8, 23, 24), it was the exposure of Italian citizens to TCDD in the Seveso accident in 1976 (2) that finally instigated multifaceted, serious scientific investigations in the toxicology of PHAH, as well as initiated research approaches toward the ecological and epidemiological aspects of these environmental pollutants.

Human data on dioxins and other PHAH are limited and presently difficult to interpret (5, 24–30); it has not been possible to directly link any human health problem with exposure to these environmental contaminants (21, 25–27, reviewed in [3]). However, much can already be implied about human health protection from experimental work with laboratory animals and from an examination of fish and wildlife populations that inhabit contaminated environments. The Great Lakes represent one of the most severely contaminated aquatic environments in the world (13, 15–17, 31–33). The impact of this environment on various species, e.g., fish, turtles, birds, otter, and mink, was the subject of an international workshop in 1989 (34). Studies with wildlife convincingly demonstrate a cause-effect linkage between environmental contamination and reproductive failure and increased mortality (14, 35–46). The consensus reached at this conference was that persistent exposure to environmental PHAH poses a risk to reproductive and developmental biology of fish and wildlife. It is, therefore, realistic to be concerned that these environmental contaminants, already taken up by our

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P.S.E.B.M 1992, Vol 201: 141–153

0037-9727/92/2012-0141$3.00/0

bodies (3, 11, 31, 32, 47), e.g., via the consumption of contaminated fish or birds (3, 4, 13, 25, 31, 32), may reach toxic levels in humans as a result of bioaccumulation (9, 17, 48).

Numerous studies have been conducted on the toxicity and the possible mechanisms of action of PHAH (3-5, 49-57). Species display a wide range of symptoms and diversity in their sensitivity to the PHAH. Typical symptoms of PHAH toxicity in rodents include weight loss, thymic atrophy, enlarged liver, teratogenicity, fetotoxicity, hepatic porphyria, anemia, and mortality (4, 58-62). The avian embryo is very sensitive to many PHAH, with exposure resulting in death or a multitude of malformations (35, 63-67). In nonhuman primates, exposure to PHAH causes fetotoxicity, multiple epithelial lesions, and impairment of hematopoietic, immune, and reproductive functions (68, reviewed in [3]). In humans, the clinical syndrome associated with PHAH exposure is well characterized and includes skin lesions (chloracne), weight loss, nausea, eye and upper respiratory tract irritations, and hepatic, intestinal and nervous disorders (3, 4, 58, 60, 69, 70). Examination of human populations exposed to large doses of PHAH reveal, in addition, impaired immune function, and increased cancer rates (25, 27, 70; reviewed in Refs. 3 and 4). Developmental and behavioral abnormalities were noted in children that had been exposed *in utero* to PCB (3, 4, 28-30, 47, 70). Most importantly, there is substantial evidence that maternal PHAH exposure adversely affects human reproductive outcomes, causing an increase in spontaneous abortions, premature deliveries, and lower birth weights (25, 28-31, 71-84). On the whole, however, human data are incomplete, particularly in regard to effects on reproduction. It is, therefore, important to note that reproductive impairment is a major cause for the gradual decline of a wide variety of wildlife species, including mammals, that are exposed to environmental PHAH in the Great Lakes basin area (15, 33, 36, 38) and in contaminated environments in Europe (11, 14, 32, 46, 85), i.e., environments that many of us inhabit. The assessment of early fetal losses has been proposed as a sensitive biomarker to monitor the health risk of humans in relationship to environmental pollutants (86).

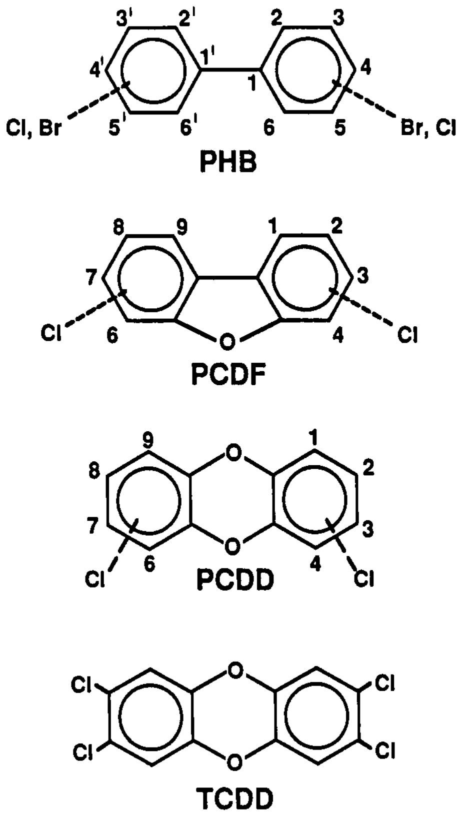


Figure 1. Chemical structures of some polyhalogenated aromatic hydrocarbons. PHB, polyhalogenated biphenyls; PCDF, polychlorinated dibenzofurans; PCDD, polychlorinated dibenzo-*p*-dioxins; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Mechanism of Toxicity of Polyhalogenated Aromatic Hydrocarbons

The mechanism of action of PHAH has been intensively investigated in the last decade. The current view holds that these structurally similar compounds exert their toxic effects through a similar molecular mechanism (4, 5, 49, 50, 52, 54). TCDD, the most toxic man-made compound known, has become the model compound for studies of the mechanism of action of the PHAH (5, 49, 51, 52). Currently, no single mechanism of action for TCDD has been identified that can explain the pleiotropic toxic effects of PHAH and the diversity of responses observed among species, strains, and tissues. The most widely accepted model for the mechanism of action of TCDD is the receptor-mediated mechanism analogous to that proposed for steroid hormone function. Many of the toxic effects of TCDD are mediated through the aryl hydrocarbon (Ah) receptor, a cytosolic protein that, upon association with TCDD or a similar ligand, translocates to the nucleus, binds to specific DNA sequences, and induces transcription of specific proteins (4, 5, 49-57, 85). The sustained pleiotropic responses that result from PHAH exposure include increased activities of mixed function oxidases (specific forms of cytochrome P-450) (52, 53, 55, 57), uridine diphosphate glucuronosyl transferases (55, 85), and aldehyde dehydrogenase (55, 85, 87), as well as other known proteins (4, 5, 85) and some newly discovered proteins (88). The best-characterized biochemical response to Ah receptor ligands is the induction of cytochrome P-450IA1. Although the involvement of the Ah receptor in the mode of action of TCDD at the molecular level is well established and progress is made in the elucidation of the details of the mechanism and in characterization of the gene products, much remains to be learned about how the initial molecular events translate into the toxic symptoms that manifest themselves in humans and animals exposed to PHAH.

Some of the lesions of PHAH-induced toxicity resemble those of vitamin A deficiency (60–62, 85, 89, 90) and have led to inquiries into the effects of PHAH on vitamin A nutritional status. Although there is controversy concerning the seriousness of PHAH as a health risk to humans, it is indisputable from numerous animal studies that these environmental contaminants severely alter vitamin A homeostasis and metabolism under conditions that most likely exist in many human populations. It was the intent of the present overview to identify, integrate, and summarize the pertinent observations concerning effects of environmental pollutants such as PHAH on vitamin A metabolism and function.

Vitamin A Homeostasis: Decreased Hepatic Storage of Vitamin A

Adequate stores of vitamin A, primarily in the liver, and adequate tissue levels are maintained by a balance between metabolic demands of the tissues and dietary intake of vitamin A; all of these aspects encompass vitamin A homeostasis and can be modified by many factors, including xenobiotics. The present overview will focus on the work that has been conducted toward an understanding of the detrimental effects of polyhalogenated aromatic hydrocarbons on vitamin A metabolism and homeostasis.

Many of the symptoms of PHAH intoxication resemble those of vitamin A deficiency (60–62, 85, 90–98), e.g., dermal changes, keratinizing epithelium in the respiratory tract and bile duct, and impaired immune, hemopoietic and reproductive functions. Administration of vitamin A concurrently with the xenobiotic partially prevents some of the symptoms (92, 99). The effects of PHAH on vitamin A status are seen in all experimentally used rodent strains (62), as well as in primates (93, 95), mink (44), and quail (100, 101).

The initial observations linking certain symptoms of PHAH pathology to a vitamin A-deficient condition, supported by the demonstration of decreased hepatic vitamin A content in exposed animals (92, 94, 102), were the beginning of investigations into the relationship between the PHAH and vitamin A status. These findings have been confirmed in many studies, either with a single dose of PHAH (61, 62, 98, 103–123), with chronic feeding of PHAH to adult rats (62, 124–127), or with chronic ingestion via mothers' milk during nursing (123).

The normal storage of vitamin A is primarily in the liver as retinyl esters. Studies on dose-related toxicity and vitamin A status demonstrate that altered hepatic vitamin A content is the most sensitive index of TCDD toxicity in rats and mice and support the idea (62, 122) that impaired vitamin A status may be linked to TCDD-induced pathogenesis. In rats consuming a vitamin A-adequate diet, an inhibition of hepatic vitamin A accumulation over a period of several months was observed after different single doses of TCDD (98): the pattern of vitamin A accumulation suggested a metabolic impairment that correlated with the remaining level of TCDD-active species in the body. It has been demonstrated repeatedly that the effects of PHAH on hepatic vitamin A content are a characteristic of all toxic PHAH; those congeners that are not toxic do not alter vitamin A homeostasis (126).

Various aspects of vitamin A nutrition have been examined to seek an explanation for the decreased hepatic vitamin A content in PHAH-exposed animals observed in all species studied. Inanition and the accompanying low dietary intake of vitamin A are not contributory causes for the low hepatic vitamin A levels observed in TCDD-exposed animals (118). Intestinal absorption and hepatic uptake of newly ingested vitamin A are also not significantly altered by TCDD exposure (62). Although there is decreased storage of newly ingested vitamin A by hepatic stellate cells, vitamin A content of parenchymal cells is not affected, which implies that TCDD specifically inhibits the transfer of vitamin A from parenchymal cells to stellate cells (62, 115).

PHAH may interfere with normal hepatic vitamin A storage by disturbing the dynamic balance between esterification of retinol in preparation for storage and hydrolysis of stored retinyl esters to mobilize retinol for release into circulation or for metabolic needs of the liver itself (Fig. 2). Acyl-CoA:retinol acyltransferase, present in many tissues, is known to be regulated by vitamin A (128–130). Hepatic activity of acylCoA:retinol acyltransferase was decreased in 3,3′,4,4′,5,5′-hexabromobiphenyl (HBB)-treated rats, but could be restored to normal levels by a short treatment of the animal with vitamin A (124). This observation suggests that the decreased acyl-CoA:retinol acyltransferase activity in chronic PHAH toxicosis is an effect, not a cause, of the decreased hepatic stores of vitamin A.

Another important enzyme to be considered is the recently discovered lecithin:retinol acyltransferase, which esterifies cytosolic retinol-binding protein (RBP)-bound retinol and is found in various tissues (131–137); it is involved in acyl-CoA-independent esterification of retinol, and has been proposed to be the major hepatic retinol ester synthase (136, 137). The activity of lecithin:retinol acyltransferase appears to be directly related to cytosolic RBP levels in tissues (137); thus, since cytosolic RBP levels appear to be directly related to the vitamin A status of animals (137, 138), reduced hepatic

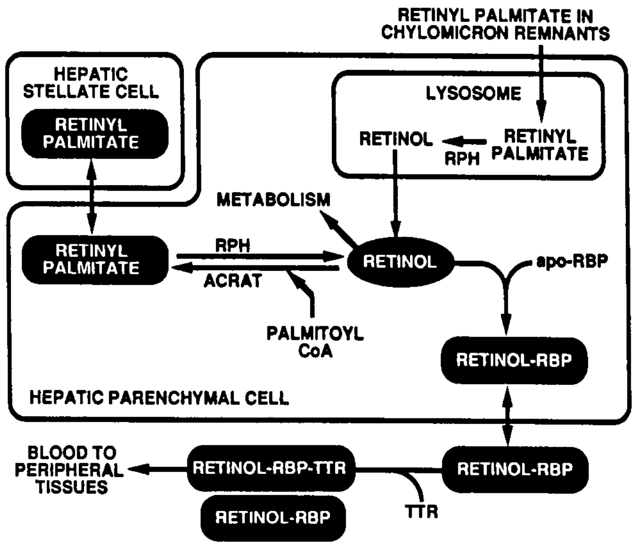


Figure 2. Hepatic uptake, storage, and release of vitamin A. Schematic representation of pathways in vitamin A storage and transport to target tissues. ARAT, acyl-CoA:retinol acyltransferase; LRAT, lecithin:retinol acyltransferase; RPH, retinyl palmitate hydrolase; TTR, transthyretin.

lecithin:retinol acyltransferase activity would be an indirect outcome of PHAH toxicosis.

It is also possible that the activity of retinyl palmitate hydrolase, the enzyme catalyzing release of retinol from its principal storage form as retinyl palmitate, is altered by PHAH. The hydrolysis of hepatic retinyl palmitate is considered to be a key reaction for the tightly regulated release of retinol into circulation (139). Enhanced hydrolysis of retinyl palmitate could account for the decrease in hepatic retinyl esters observed in PHAH toxicosis. This idea, however, is not supported by the findings that in rats treated with HBB (124), or 3,3′,4,4′-tetrachlorobiphenyl (TCB) (113), hepatic retinyl palmitate hydrolase activity was significantly decreased. Clearly, present data on enzymes involved in hepatic esterification and hydrolysis of vitamin A do not provide an explanation as to how exposure to PHAH alters hepatic vitamin A status. An imbalance in the retinyl palmitate to retinol ratio was also noted by Spear *et al.* (140) at another vitamin A storage site, the egg yolk, where vitamin A is stored as retinyl palmitate: In egg yolks from doves that had been exposed to TCB, most of the vitamin A was in the form of retinol (140), which suggests an impaired conversion of retinol to its storage form and points to a similar toxic mechanism of action at diverse vitamin A storage sites.

Another possible site of interference could be the hepatic uptake of recycled retinol-RBP or an enhanced release of retinol together with RBP into the circulation. The mobilization of liver vitamin A in the form of retinol bound to RBP and the subsequent release of this complex into the circulation is a relatively tightly regulated event (141, 142), but the regulatory signal is not known. Although initially it appeared that net mobilization of vitamin A from hepatic reserves was not affected in the vitamin A-sufficient rat (115), subsequent studies demonstrate that mobilization of hepatic vitamin A is enhanced in PHAH-intoxicated animals (62, 116, 127). We observed a significant (30–33%) decrease in hepatic retinyl esters and a corresponding increase in polar retinoids after a single, nonanorectic dose of HBB or TCDD to vitamin A-adequate rats (114, 116, 127), which indicates a loss of storage forms of vitamin A with an accompanying enhanced breakdown of vitamin A. In rats chronically treated with hexachlorobiphenyl and in which hepatic vitamin A stores had decreased to one fifth of normal, the ability of the liver to accumulate circulatory retinol was only half that of the control, whereas the plasma turnover rate of retinol in these rats was significantly increased (127). In general, studies with radiolabeled vitamin A in PHAH-exposed rats indicate mobilization of vitamin A from the liver, increased turnover of circulatory retinol, increased uptake and turnover by the kidneys, and enhanced elimination of radioactivity in urine and feces (105, 107, 117, 124, 127, 143, 144).

Alterations in Renal and Circulatory Vitamin A

Most of the abnormal vitamin A homeostasis patterns induced by PHAH are observed in all species studied (62, 98, 121), including the neonatal rat (123, 145, 146). An increase in serum and kidney vitamin A, however, is observed only in the rat (62, 98, 109, 114, 117, 121, 124, 125, 127). There is a dramatic increase in kidney retinoid content after chronic intoxication (123–125, 127) or after the administration of a single, nonanorectic dose (62, 98, 108, 109, 114, 116, 121) of polyhalogenated aromatic biphenyls to normal rats. Most of the vitamin A in these kidneys is associated with a very stable retinyl ester pool (114, 116). Normally renal vitamin A content is very low and increases only when liver vitamin A stores are nearing exhaustion (141, 147). Thus, the accumulation of renal vitamin A in the PHAH-intoxicated rat is most likely related to either a loss of hepatic stores of the vitamin, or to an altered hepatic regulatory mechanism as a result of PHAH treatment. Recent studies suggest that, in the rat, there is a sensitive feedback system between hepatic and renal vitamin A pools that is also linked to the regulation of vitamin A homeostasis in circulation (148–152). PHAH may directly affect this homeostatic balance, or affect it indirectly via a reduction of hepatic vitamin A pools. This idea is supported by the striking phenomenon that in PHAH-treated rats, the serum retinol level is significantly increased (62, 103, 105, 116, 124, 125, 127). Normally, serum vitamin A concentration is tightly regulated within a narrow homeostatic range, and is thought to be determined by the release of retinol from liver via regulation of vitamin A needs in extrahepatic tissues (141, 142, 147). Thus, an alteration in serum retinol concentration indicates a significant change in the overall dynamics of vitamin A metabolism. We have suggested that in chronic PHAH toxicosis, the normal regulatory mechanism(s) for control of serum retinol has been altered, causing a change of the "set point" for serum retinol level. The increased serum retinol concentration may be caused by an alteration in the normal homeostatic response mechanism in the liver, or by an increased positive hepatic or extrahepatic signal that up-regulates hepatic mobilization of retinol-RBP-transthyretin (TTR) (116, 127). It has been suggested that the signal is linked to peripheral tissue needs for vitamin A (142, 147, 148). Recent studies demonstrate that apoRBP, upon return from the periphery, provides a signal for release of retinol into the circulation (149–151).

In general, the response of various animal species to TCDD and other PHAH, examined in many studies in terms of vitamin A-related parameters, is comparable in most aspects (62); it is not known why the marked effects of PHAH on kidney and circulatory vitamin A are expressed only in the rat and not in the other species examined.

Metabolic Studies in Peripheral Tissues

Effects of PHAH on peripheral tissue vitamin A levels have been examined in several studies (109, 121, 127, 153, 154); variable changes in tissue vitamin A content were observed in animals with sufficient liver vitamin A stores (62, 105, 127), while inadequate vitamin A intake consistently results in a significant decrease in peripheral tissue content of vitamin A (121, 127). Spear *et al.* (140) observed decreased hepatic vitamin A, increased circulatory retinol, and increased retinol transfer into egg yolks of doves treated with TCB; in the fertile eggs laid by exposed hens, there was a loss of retinoids attributed to enhanced catabolism *in ovo* during development (140). This sequence of events most likely resulted in an insufficiency of vitamin A in the yolk and was responsible for the poor survival of the embryos. In order to gain an insight into the mechanism of PHAH action on vitamin A homeostasis, Cullum and Zile (107) employed a rat model in which the vitamin A stores were replaced with radiolabeled vitamin A. A single, nonanorectic dose of HBB rapidly and dramatically altered the steady state metabolism of vitamin A and caused an abnormal 2-fold enhancement in the metabolic output of degraded vitamin A in urine and feces. These results suggest that PHAH stimulate vitamin A degradative pathways as well as interfere with the regulatory vitamin A homeostasis mechanism(s) that is associated with dietary availability of vitamin A. Brouwer *et al.* (109, 117) employed a somewhat comparable model in examining the effect of TCB and TCDD on vitamin A status in extrahepatic tissues. These xenobiotics decreased vitamin A content of several but not all peripheral tissues. The wide variation in peripheral tissue response to PHAH may be linked to differences in hepatic vitamin A status (62). In general, in animals with adequate hepatic vitamin A stores, the effect of PHAH on peripheral tissue vitamin A content is minimal; variable results in peripheral tissue vitamin A content are obtained in animals with marginal and depleted hepatic vitamin A stores (62). Furthermore, there are significant differences among the various congeners in their specific effects, but, in general, they all disturb vitamin A homeostasis.

The mechanism whereby PHAH interfere with the regulation of vitamin A homeostasis is not known. There is existensive experimental evidence from several laboratories that both acute (107, 109, 111) and chronic (124, 125, 127) doses of PHAH dramatically increase the excretion of vitamin A breakdown products in urine and feces. Studies with radiolabeled vitamin A in PHAH-exposed animals reveal mobilization of vitamin A from the liver, increased turnover of circulatory retinol, increased uptake and turnover of the vitamin by kidneys, and an enhanced elimination of retinoid breakdown-associated radioactivity in urine and feces; the observations support the idea that both peripheral target tissue retinoids and hepatic vitamin A pools are subject to an accelerated breakdown (107, 117, 124, 127, 144). Based on results from studies with ^{3}H-labeled vitamin A under steady state vitamin A conditions (107) as well as from other studies (62, 127), it is evident that the effect of PHAH is most pronounced in the metabolic pathway that represents vitamin A function-associated metabolism, i.e., functional metabolism, rather than its other metabolic pathways, e.g., the pathways that process newly ingested vitamin A and the overflow of storage vitamin A in hypervitaminosis A (147). It is very likely that the peripheral signal for hepatic vitamin A mobilization and delivery to tissues is also increased, accounting for the increased amount of circulatory retinol in PHAH-treated rodents (109, 124, 127, 144) and birds (63). All studies clearly demonstrate that PHAH alter critical steps in vitamin A metabolism. We have proposed (111, 114, 116) that PHAH directly affect the activity of several important enzymes in vitamin A metabolic pathways; a discussion follows in the subsequent section.

Alterations in Retinoid-Specific Metabolic Pathways

Vitamin A homeostasis and metabolism have been studied extensively (141, 142, 147, 148, 155–157). All tissues that have been examined are able to metabolize vitamin A. In tissues, retinol, after delivery to the cell, can be interconverted between various intermediates, including retinyl phosphate glycosides, retinyl and retinoyl glucuronides, retinal, and retinyl esters (130, 139, 147, 155–162). Retinol can be also metabolized to polar metabolites via a P-450-dependent system in rat and human liver (147, 157, 163). Retinoic acid, a normal component of the blood plasma and tissues (164–166), is formed from retinal in a reaction catalyzed by aldehyde oxidase or dehydrogenase (167–173); this is an irreversible reaction. Reversible interconversions of retinoic acid include isomerization and glucuronidation (157, 159–161, 166). The initial step for inactivation of retinoic acid is thought to be ring hydroxylation, catalyzed by a microsomal retinoic acid-specific, P-450-dependent, mixed-function oxidase system (157, 163, 174, 175). Extensive subsequent oxidative degradation and conjugation of the molecule is responsible for the formation of highly polar vitamin A metabolites. The physiological importance and the metabolic pathways of 3,4-didehydro-retinoids (176, 177) remain to be elucidated. Vitamin A metabolic pathways are schematically represented in Figure 3. The chemical structures of important physiologically occurring retinoids are shown in Figure 4.

The sustained pleiotropic responses that result form PHAH intoxication outlined earlier include the induction of cytochrome P-450 enzymes as well as uridine diphosphate glucuronosyl transferase (UDPGT), classes of enzymes also participating in vitamin A metabolism, as discussed above. This potential relationship was evident to Innami *et al.* (94) who suggested in 1976 that PHAH-induced hydroxylation of vitamin A compounds might be a mechanism involved in the loss of hepatic vitamin A. Since ring hydroxylation and glucuronidation of retinol and retinoic acid are established reactions in vitamin A metabolism (157, 159–163, 166, 174), it was logical to examine the relationship between PHAH and those enzymes in vitamin A metabolic pathways that might be altered by PHAH. Results from several initial studies, however, demonstrated that the reduction of hepatic vitamin A stores as the result of treatment with a variety of PHAH did not correlate with the enhanced activities of aryl hydrocarbon hydroxylase (AHH) and UDPGT (104, 108, 120, 178–180); in these studies, AHH activity was assessed with benzo[a]pyrene or 7-ethoxyresorufin as substrates, and UDPGT activity was assessed with *p*-nitrophenol (PNP) as substrate. In contrast, in ring doves exposed to TCDD, liver retinol content was decreased and AHH activity increased (181). Recently, the relationship between drug-metabolizing enzyme activities and vitamin A content was reexamined in coplanar polychlorobiphenyl-induced rats; the increased activities of cytochrome P-450 toward benzo[a]pyrene and UDPGT toward PNP were found to be inversely related to hepatic vitamin A content (182).

In order to gain an insight into the effect of PHAH on specific vitamin A metabolic pathways, we examined vitamin A metabolite profiles in tissues of rats treated with a single nonanorectic dose of HBB (116). HBB caused increased metabolism of vitamin A; concurrently, there was an abnormally elevated generation of polar vitamin A metabolites suggestive of ring oxidation and side chain conjugation. These observations were followed by an examination of specific reactions in vitamin A metabolism. Spear *et al.* (110) and we (112, 114, 116, 119) have hypothesized that PHAH, via the Ah receptor, inappropriately regulate several enzymes in the vitamin A metabolic pathway; one such enzyme is UDPGT. Thunberg *et al.* (104) and Thunberg and Hakansson (180) could not demonstrate a correlation between TCDD-induced UDPGT activity toward PNP as substrate and the reduction of hepatic vitamin A stores in rat. However, we hypothesized that the isozyme involved in retinoic acid glucuronidation is not likely to be the same isozyme that catalyzes PNP glucuronidation. In our studies with a single dose of TCDD, measurements of liver and kidney microsomal

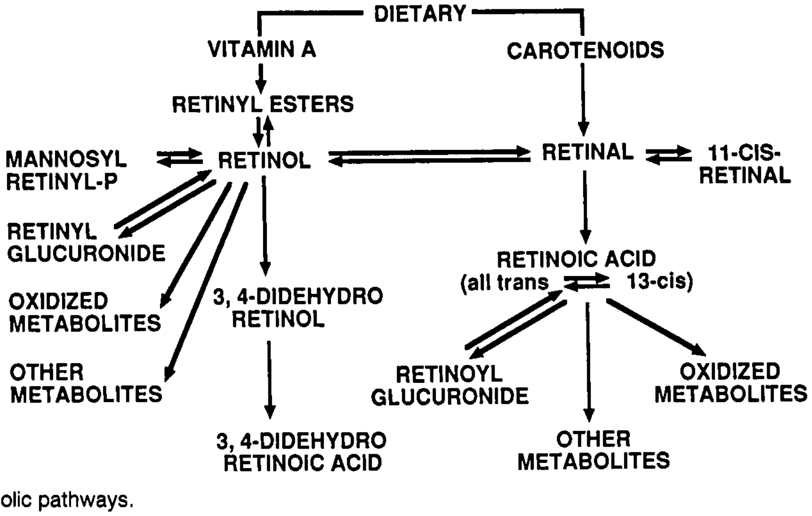
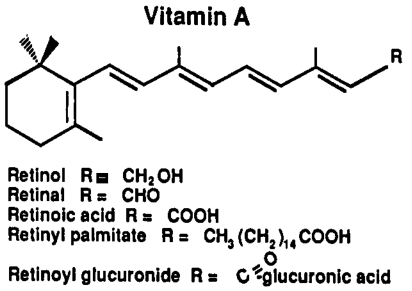


Figure 3. Vitamin A metabolic pathways.



Retinol R = CH_2OH
Retinal R = CHO
Retinoic acid R = COOH
Retinyl palmitate R = $CH_3(CH_2)_{14}COOH$
Retinoyl glucuronide R = $\overset{O}{\overset{\|}{C}}$-glucuronic acid

Figure 4. Chemical structures of important physiologically occurring retinoids. The term retinoids is currently widely used to describe both natural and synthetic vitamin A-related compounds.

UDPGT demonstrated that the enzyme activity toward all-*trans*-retinoic acid was increased 3.7-fold and 2.7-fold, respectively, 10 days after an exposure to TCDD (112, 114). Similar results (3.5-fold increase) were obtained in liver microsomes of rats exposed to HBB (110). It is thus very likely that PHAH, in addition to inducing many drug-metabolizing enzymes, also induce a separate isozyme of the UDPGT family involved specifically in retinoic acid glucuronidation. This may explain in part why, in the Gunn rat, TCDD induces vitamin A depletion even though this rat lacks the PNP-specific UDPGT (180).

Normally, retinoyl glucuronide represents only about 10% of the retinoids excreted via the biliary route (157, 159, 183). However, an increased UDPGT activity as the result of PHAH administration may accelerate the loss of vitamin A due to enhanced glucuronidation of retinoic acid and subsequent excretion of the conjugate in bile. We have observed an increased amount of retinoyl glucuronide in bile of rats after PHAH treatment (unpublished). The demonstration that a retinoic acid-specific UDPGT was induced not only in the liver, but also in the kidney (112, 114), supports the idea that this enzyme belongs to the GT-I isozyme group, which is selectively induced by both 3-methylcholanthrene and TCDD (184).

Although glucuronidation of retinoic acid is greatly enhanced by PHAH, it is not expected that the dysregulation of this pathway alone is responsible for the rapid and dramatic loss of hepatic vitamin A, since some of the biliary glucuronides will be hydrolyzed by intestinal glucuronidase and subsequently will undergo enterohepatic recirculation. It is, therefore, imperative that other steps in vitamin A metabolism are examined that might also be sensitive to PHAH.

Since retinoic acid and retinol can be metabolized by specific cytochrome P-450-associated enzymes, investigators have looked for a correlation between an enhancement in PHAH-induced AHH activity and the reduction of hepatic vitamin A stores, but have found no correlation (104, 108, 120, 178). However, as in the case with UDPGT, it may be more meaningful to examine the effects on vitamin A metabolism using vitamin A compounds as the substrates, rather than benzo[a]pyrene or 7-ethoxyresorufin, which were used in the above studies.

Following this line of reasoning, Spear *et al.* (110) and Bank *et al.* (119) examined the cytochrome P-450-catalyzed hydroxylation of the substrate retinoic acid in hepatic microsomes of HBB-induced rats and observed 2.3- and 5-fold increased activities, respectively. Although Spear *et al.* (110) consider this enhancement of minor consequence, it is important to view any single metabolic step in the context of the overall metabolic equilibrium, where certain steps might constitute critical regulatory points. The recent studies of Van Wauwe *et al.* (175) on the effect of cytochrome P-450 inhibitors on retinoic acid metabolism lend support to the hypothesis that the P-450-catalyzed oxidation of retinoic acid is an important degradative pathway of this biologically active form of vitamin A.

An important physiological pathway in vitamin A metabolism is the conversion of retinol to retinoic acid. This pathway is the main biosynthetic pathway for generation of retinoic acid and is of major interest because recent findings support the theory that most of the physiological actions of vitamin A are mediated by retinoic acid via its nuclear receptors (156, 185–188). This notion has been substantiated by demonstration of retinoic acid nuclear receptors in various tissues (189–193). It is, therefore, important to delineate the mechanisms that regulate retinoic acid biosynthesis and degradation within the target cells. In the biosynthesis reaction, retinol is first converted to retinal in a reaction catalyzed by cytosolic retinol dehydrogenase; this reaction may be influenced by the availability of cytosolic RBP (194, 195). Retinal is subsequently oxidized to retinoic acid by an isozyme of aldehyde dehydrogenase; this step is irreversible and is thought to be the rate-limiting step in retinoic acid biosynthesis (172). Recently, Shih and Hill (171) demonstrated the conversion of retinol to retinoic acid in liver microsomes catalyzed by an NADPH-requiring enzyme that is inducible by 3-methylcholanthrene. Since aldehyde dehydrogenase is among the enzymes induced by PHAH (55, 85, 87), it would be important to examine whether this retinol-metabolizing enzyme is also induced by PHAH.

Data from metabolic studies available at this time have unequivocally established that PHAH affect several enzymatic steps in retinoid metabolism. The findings provide a rationale for examining still other potentially vulnerable steps in retinoid metabolism. Our studies suggest that the effects of PHAH on vitamin A metabolism are not limited to the liver and its internal dynamic balance of various vitamin A metabolite pools, but that there is an accelerated metabolism of target tissue retinoids that results in an increased demand for vitamin A to be delivered to the target tissues and produces increased amounts of a peripheral signal that up-regulates the mobilization of hepatic vitamin A. Our studies on the regulation of circulatory retinol homeostasis point to apoRBP as a signal for the release of stored retinol (151). Circulatory apoRBP would be expected to be increased in a metabolic state where retinol is continuously removed from its carrier RBP by retinol-deficient cells. The elevated level of apoRBP in the circulation, upon reaching the liver, signals it to release more retinol. We hypothesize that an altered functional metabolism of vitamin A in all target tissues is the major cause for the dysregulation of vitamin A homeostasis and, subsequently, the depletion of hepatic vitamin A by PHAH.

Potential Role of PHAH Metabolites

In contrast to the generally similar effects on vitamin A homeostasis obtained in studies with PCB, polybrominated biphenyls, and TCDD, the administration of 3,4,3′,4′-tetrachlorobiphenyl to rodents results in a reduction of serum retinol-retinol-binding protein complex in addition to the loss of vitamin A from liver and some peripheral tissues (109, 144, 178, 179); it has been suggested that the decrease in circulatory retinol is responsible for the reduced amounts of vitamin A in some tissues, e.g., skin and lung. To explain the depression in circulatory vitamin A, Brouwer and others (85, 141, 179) have proposed that a hydroxylated metabolite of TCB interferes with plasma transport of retinol as well as of thyroxine by the RBP-TTR transport complex, which is the physiological transport protein for both retinol and thyroid hormone. There is evidence for a competition between a hydroxylated TCB metabolite and thyroxine for the thyroid hormone-binding site on TTR, the binding of the xenobiotic causing a conformational change on TTR that interferes with the binding of RBP to TTR; subsequently, there is a loss of retinol-RBP via glomerular filtration (85, 179, 196). Thus, both vitamin A and thyroid states are affected by this PHAH. This mechanism could account for the similarities in several symptoms associated with PCB toxicosis, such as dermal and epithelial lesions and impairments in energy metabolism, which are typical of both hypovitaminosis A and hypothyroidism. Brouwer (85) proposed that TCB and structurally similar planar PCB congeners bind to the Ah receptor and induce their own metabolizing enzymes that generate toxic hydroxylated metabolites from the parent compounds. The diverse and pleiotropic symptoms of PCB thus could be explained as the sum of the direct effects of the parent compounds via the Ah receptor-induced enzymes and the additional effects caused by the PCB metabolites. Studies with different PHAH in wildlife species support the concept that the toxicity of these xenobiotics cannot be explained by a single, unified mechanism and that much work remains to be done in order to understand the biochemical basis of PHAH toxicity (85, 140, 197).

Summary

Normal vitamin A function depends on adequate stores of the vitamin, a finely regulated supply of the vitamin to target tissues, and an ability of cells to generate functionally active forms of the vitamin. Both endogenous and exogenous factors can adversely affect vitamin A homeostasis. Polyhalogenated aromatic hydrocarbons are ubiquitous environmental pollutants and cause severe disturbances in vitamin A metabolism, manifested by an accelerated metabolism and breakdown of vitamin A and its metabolites and a depletion of vitamin A from the body; this sequence of events accounts for the vitamin A deficiency-like symptoms associated with PHAH intoxication. The mechanism(s) responsible for these events most likely includes altered activities of enzymes that are either directly or indirectly involved in critical vitamin A metabolic pathways. Human populations that continue to be exposed to environmental pollutants, may accumulate critical levels of polyhalogenated aromatic hydrocarbons and will be at risk for inadequate vitamin A function as well as for other health impairments that have been difficult to link to any specific causes. Therefore, it is important to seriously evaluate the similarities in physiological disturbances across species that have become apparent in studies with wildlife inhabiting polluted environments similar to ours; the relevance to human health is evident.

This review was supported by U.S. Department of Agriculture Grant 91-37200-6285. The author wishes to thank Dr. Dale Romsos for a critical review of the manuscript, Dr. Anne Sweeney for the helpful discussions concerning human reproductive outcomes, and Mary Rosner for skillful typing.

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