Influence of vitamin A on hexachlorocyclohexane (HCH) toxicity in the rat

Pius Joseph, Thimmappa Shivanandappa, and Mandayam K. Krishnakumari

Toxicology Unit, Infestation Control and Protectants Discipline, Central Food Technological Research Institute, Mysore, India

The influence of vitamin A on the acute oral toxicity of hexachlorocyclohexane (HCH) was investigated in male albino rats. Rats were fed a synthetic diet free of vitamin A or containing vitamin A at 2000 or 10^5 I.U./kg for 12 weeks. The hepatic vitamin A contents of the three diet groups of rats were markedly different at the end of the week 9, when very mild signs of deficiency were noticed only in the rats fed the vitamin A-free diet. The acute oral toxicity of HCH determined at the end of week 9 of feeding on the basis of lethal dose 10, lethal dose 50, and lethal dose 90 values, and signs of toxicity and serum and liver levels of the marker enzymes of toxicity revealed greater susceptibility of the vitamin A-deficient rats to HCH toxicity. Assay of the activities of hepatic xenobiotic metabolizing enzymes showed slight-to-moderate decreases in specific activities of cytochrome P-450, N-demethylase, β-glucuronidase, and glucuronyl transferase in the vitamin A-deficient rats, though the activity of glutathione S-transferase was slightly higher in them compared with those of the supplemented rats. The results suggest the possibility that the greater susceptibility of the vitamin A-deficient rats to HCH toxicity could be due to the impaired detoxification of HCH, as inferred from the reduced activities of the hepatic microsomal enzymes of detoxification in these rats. Also, vitamin A supplementation, particularly in excess, but not at hypervitaminotic levels, is protective in the rats against HCH toxicity, possibly due to the more efficient detoxification of the chemical.

Keywords: hexachlorocyclohexane; vitamin A; acute oral toxicity; xenobiotic metabolizing enzymes; rats

Introduction

Technical hexachlorocyclohexane (HCH, $C_6H_6Cl_6$), a mixture of several stereoisomers is the most widely used organochlorine insecticide (OCI) in developing countries such as India.[1] HCH is characterized by very low acute and high chronic toxicity in mammals and its acute oral LD_{50} value reported in rats varies from 600–3000 mg/kg body weight.[2] This insecticide is neurotoxic in action and the symptoms of toxicity are those of central nervous system convulsions, ataxia, paralysis, and death. Also, HCH is toxic to the liver, kidney, adrenal, and testes in rats.[3–6]

Of the several factors that influence the response of animals to xenobiotic toxicity, the role of nutrition is less well understood. Several nutrients belonging to carbohydrates, proteins, lipids, vitamins, and minerals have been shown to influence the response of animals to chemical toxicity, primarily through their effects on the activities of the hepatic xenobiotic metabolizing enzymes (XMEs).[7] It has been reported that the activities of several XMEs are lowered during vitamin A-deficiency conditions in animals.[8,9] Also, many of the OCIs for which residues are detected very often in human and animal tissues are shown to cause secondary deficiency of vitamin A by virtue of its depletion in the body.[10] In spite of very close relationships existing between vitamin A and pesticide toxicity, not much information is available on the influence of dietary vitamin A status on the toxicity of insecticides in animals. Recently, we[11] reported that the deficiency

This work was supported by the award of a research fellowship to Pius Joseph by the Council of Scientific and Industrial Research, India.

Address reprint requests to Pius Joseph at the Department of Environmental and Occupational Health, College of Public Health, University of South Florida, 13201 Bruce B. Downs Boulevard, Tampa, FL 33612, USA

Received August 16, 1991; accepted December 16, 1991.

of vitamin A in rats accentuates the male reproductive toxicity of HCH, while its supplementation at normal as well as in excess levels, but not at hypervitaminotic levels is protective against the toxicity. To understand the biochemical mechanism(s) underlying such an interaction between vitamin A and OCIs, the present study was undertaken. The acute oral toxicity of HCH, a highly persistent and the most widely used OCI in developing countries, was investigated in male albino rats supplemented with different dietary levels of vitamin A. Also, the activities of some of the important hepatic XMEs were assessed as a cause for the possible modification of HCH toxicity in the rats.

Materials and methods

Animals and diets

Twenty-one-day-old, male albino rats *(Rattus norvegicus albinicus)* of CFT-Wistar strain were divided at random into the following three diet groups: a) vitamin A-free diet (V_0); b) vitamin A-supplemented diet at the recommended dietary level, i.e., 2000 I.U./kg diet (V_{2000}); and c) vitamin A-supplemented diet in excess, but not at a hypervitaminotic level i.e., 10^5 I.U./kg diet ($V_{10}5$).

The rats were caged individually in metallic cages under standard laboratory conditions and they had free access to the diets and tap water. All the ingredients used in preparing the synthetic diet *(Table 1)* with the exception of casein were free of vitamin A. Casein used for preparation of the diets was made vitamin A free by refluxing in ethyl alcohol, washing in diethyl ether, and drying at 60° C for 12 hours. For vitamin A-supplemented diets, vitamin A acetate (HiMedia Laboratories Ltd., Bombay, India) was dissolved in unrefined groundnut oil (vitamin A free) and mixed well with the diets. To the vitamin A-free diet, ground nut oil, equivalent in amount to that used to dissolve vitamin A acetate in the vitamin A-supplemented diets was added separately. Rats were fed the diets for a maximum period of 12 weeks by noting their daily food intake and weekly body-weight gain.

Hepatic vitamin A estimation

At the end of week 9 of feeding, four rats from each diet group were sacrificed under ether anesthesia. Livers removed immediately after killing the rats were kept frozen and used for vitamin A estimation according to the method of Olson.[15]

Toxicity studies of HCH

The technical grade of HCH (Hindustan Insecticides Ltd., India) used in this study had the following individual isomer composition as determined by gas chromatography: α, 72.8%; β, 5%; γ, 12.6%; δ, 7.95%; and ε, in traces. Groups of rats from the three diet groups were withdrawn for the various toxicity studies of HCH at the end of week 9 of feeding. For determining the acute oral LD_{10}, LD_{50}, and LD_{90} values of HCH, graded doses of the chemical ranging from 0–4000 mg/kg body weight, suspended in groundnut oil were orally intubated to groups of six rats per dose from each diet group. Control rats received unrefined groundnut oil alone at the volume corresponding to the highest dose group. The control, as well as the HCH-intubated rats, were fed the diets for 2 weeks and mortality recorded when it occurred. Another set of six rats of each diet group were HCH-intubated with an acute oral dose of 3000 mg/kg body weight (approximately the LD_{50} of HCH in the $V_{10}5$ rats, *Table 2* details) and the rats were observed for the onset, intensity, and duration of toxicity signs during the 24 hours following the intubation. Evaluation of the intensity of signs of toxicity was done by individuals who did not know to which diet groups the rats belonged *(Figure 1)*. In a separate experiment, four rats of each diet group were intubated with a single oral dose (1000 mg/kg) of HCH. Twenty-four hours after the intubation, the control and the HCH-intubated rats were killed by ether anesthesia and blood collected directly from the heart. A known weight of the liver taken immediately from the central lobe was homogenized in 0.25 M ice-cold sucrose using a Potter-Elvehjem tissue homogenizer to get a 10% (wt/vol) homogenate. Sera from the clotted blood samples and the clear supernatants obtained by centrifuging the liver homogenates at 4000*g* for 20 min at 0–4° C were used for assay of the following enzymes by procedures described elsewhere:[3] a) glutamate oxaloacetate transaminase (GOT); b) glutamate pyruvate transaminase (GPT); c) alkaline phosphatase (ALP); d) lactate dehydrogenase (LDH); and e) β-glucuronidase (β-GLR).

The total protein in liver homogenates was estimated by the method of Lowry *et al.*[16]

Assay of hepatic xenobiotic metabolizing enzymes (XMEs)

At the end of week 9, activities of the selected hepatic XMEs were determined in four rats from each of the three diet groups that did not receive any HCH. The subsequent procedures to isolate hepatic microsomes and cytosol were done essentially as described by Alphons *et al.*[17] Cytochrome

Table 1 Composition of synthetic diet[12]

Materials	g/kg diet	% level
Devitaminised casein	200.00	20.00
Vitaminised starch*	10.00	1.00
Salt mixture†	20.00	2.00
Ground-nut oil	50.00	5.00
Corn starch	717.00	71.70
Choline chloride	2.00	0.20
Vitamin oil‡	1.00 mL	0.10

*Mixture of the water soluble vitamins.[13]
†The salt mixture was prepared as per the composition described by Hubbel *et al.*[14]
‡Mixture of vitamins D and E at final concentrations of 1 and 100 mg/kg diet.

Table 2 Acute oral LD_{10}, LD_{50}, and LD_{90} values* (mg/kg body weight) of HCH in male albino rats fed with vitamin A-free and -supplemented diets for 9 weeks

Diet group	LD_{10}	LD_{50}	LD_{90}	Probit regression equation
V_0	566	1338	3163	$= -5.72 + 3.4\ x$
V_{2000}	1264	2428	4654	$= -10.30 + 4.52\ x$
$V_{10}5$	1841	2869	4459	$= -18.01 + 6.66\ x$

*Six rats of each diet group were used per dose of HCH administered.

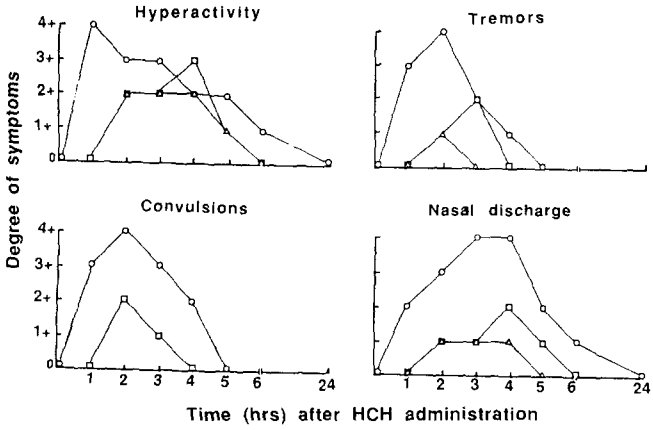

Figure 1 Time-course of symptoms of HCH toxicity (3000 mg/kg body weight, acute oral dose) in male albino rats fed with vitamin A-free and -supplemented diets for 9 weeks. ○: V_0; □: V_{2000}; △: $V_{10}5$; 0: nil; 1+: mild; 2+: moderate; 3+: high; 4+: very high.

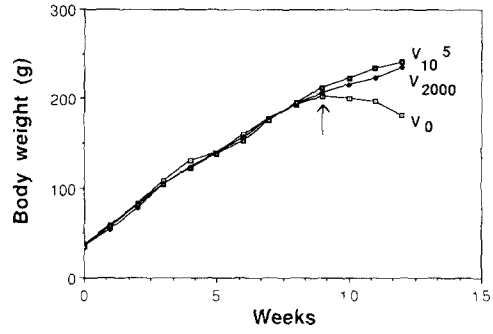

Figure 2 Weekly body weight of male albino rats fed with vitamin A-free and -supplemented diets for 12 weeks. (Arrow indicates the time at which the first symptoms of deficiency appeared in the vitamin A-free–diet fed rats).

P-450 (cyt. P-450) contents were determined by optical difference spectroscopy.[18] Using aniline as the substrate, activity of aryl hydroxylase (AH) was assayed.[19] Activity of N-demethylase (DM) was assayed with p-dimethylaminobenzaldehyde as the substrate,[20] while that of β-GLR was assayed using phenolphthalein as the substrate.[21] p-Nitrophenol was used as the acceptor for assaying the activity of uridine diphosphate (UDP) glucuronyl transferase (GT) by the method of Isselbaccher *et al*,[22] and glutathione S-transferase (GST) activity in the cytosol was assayed using 1-chloro-2,4-dinitrobenzene as the substrate.[23,24]

Statistical analysis of the data

LD_{10}, LD_{50}, and LD_{90} values of HCH were calculated from the mortality data by probit analysis.[25] The significance of differences in enzyme activities between the control and experimental groups was assessed by Student *t* test.[26]

Results

Food intake and growth

Rats of the three diet groups exhibited uniform food intake (data not presented) and growth *(Figure 2)* until the end of the week 8. However, during week 9, slight reductions in food intake and body-weight gain were noticed in the V_0 rats alone. In the following weeks, subsequent to the appearance of severe signs of vitamin A deficiency such as soiling and loss of fur, and hemorrhaging around the eyelids and nostrils, most of these rats succumbed to death. No sign of hypervitaminosis A was noticed in the rats supplemented with vitamin A even at 10^5 I.U./kg diet until the end of week 12 of feeding.

Hepatic vitamin A content

Hepatic vitamin A content of the three diet groups of rats showed significant differences *(Table 3)* at the end of week 9 of feeding. In the V_0 rats, the vitamin A content was only 1.97 μg/g liver, while in the V_{2000} and $V_{10}5$ rats they were 34 and 179 times more, respectively, when compared with the deficient rats.

Acute oral LD_{10}, LD_{50} and LD_{90} values of HCH

Acute oral LD_{10}, LD_{50}, and LD_{90} values of HCH varied markedly among rats of the three diet groups *(Table 2)*. Rats of the V_0 group had the lowest values for LD_{10}, LD_{50}, and LD_{90}. The LD_{50} value of HCH in the V_0 rats (1338 mg/kg body weight) was ≤50% compared to those of their vitamin A-supplemented counterparts. Of the two vitamin A-supplemented groups, the $V_{10}5$ rats showed relatively higher lethal dose values for HCH.

Table 3 Hepatic vitamin A contents and XMEs of male albino rats fed with vitamin A-free and -supplemented diets (Values are mean ± S.E. of four rats in each diet group)

Diet group	Vitamin A (μg/g)	Cyt. P-450	AH	DM	β-GLR	GT	GST
V_0	1.97 ± 0.85	0.44 ± 0.10	0.52 ± 0.04	2.03 ± 0.21	11.52 ± 0.79	4.12 ± 0.28	855.01 ± 99.99
V_{2000}	66.28 ± 4.38[c]	0.52 ± 0.02	0.53 ± 0.03	2.77 ± 0.47	13.11 ± 1.06	5.13 ± 0.50	644.02 ± 39.25
$V_{10}5$	353.24 ± 10.81[c]	0.67 ± 0.06	0.59 ± 0.03	3.45 ± 0.23[b]	14.41 ± 1.23	6.74 ± 0.56[b]	650.75 ± 38.87

P values: b < 0.01; c < 0.001.
Cyt. P-450 content is expressed as nmoles/mg protein while all other enzyme activities are expressed as nmoles/min/mg protein

Time-course of signs of toxicity

The common neurotoxic signs evoked by HCH in the rats were hyperactivity, convulsions, tremors, nasal discharge, salivation, paralysis, and death. The signs were earlier in onset, more prolonged in duration, and higher in intensity in the V_0 rats compared with the vitamin A-supplemented rats *(Figure 1)*. Most of the signs made their appearances in the V_0 rats at high intensities in the very first hour itself and they were prolonged up to 24 hours. However, in the vitamin A-supplemented rats, most of the signs that appeared after 1 hour of the intubation were mild in nature and disappeared by the hour 6.

Three of six rats died in the V_0 group during the observation period and the first death was within 1 hour after the HCH intubation. Only one rat died in the V_{2000} group and the death was at the hour 12 after the HCH intubation. No death was noted in the $V_{10}5$ group during the observation period of 24 hours.

Serum and liver biochemistry

All the serum enzymes that were assayed exhibited marked alterations of activities in the V_0 rats following HCH intubation. Activities of GOT, GPT, ALP, and β-GLR were increased significantly in these rats, while that of LDH showed a decrease that was not statistically significant *(Table 4)*. Activities of serum GOT and GPT of these rats were elevated by 150% and 142%, respectively, compared with their controls. Changes in serum levels of only GPT and ALP were statistically significant in the V_{2000} + HCH rats, while in the $V_{10}5$ + HCH rats, none of the enzymes showed any statistically significant changes in their activities compared with the respective controls.

The alterations in activities of liver enzymes *(Table 5)* of the HCH-intubated rats were not as conspicuous as those of the serum enzymes. In the V_0 + HCH rats, GOT, GPT, and LDH activities were significantly decreased, while those of ALP and β-GLR were elevated

Table 4 Serum enzymes of vitamin A-deficient and -supplemented male albino rats 24 hours after an acute oral dose of HCH (1000 mg/kg body weight) (Values are mean ± S.E. of four rats in each diet group)

Diet group	GOT*	GPT†	ALP‡	LDH§	β-GLR¶
V_0	8.58 ± 0.95	3.97 ± 0.61	10.97 ± 1.31	0.24 ± 0.005	0.07 ± 0.002
V_0 + HCH	21.50 ± 5.07[c]	9.61 ± 1.19[b]	17.33 ± 1.42[a]	0.22 ± 0.003	0.11 ± 0.004[c]
V_{2000}	8.28 ± 0.30	2.86 ± 0.28	12.80 ± 1.31	0.26 ± 0.002	0.07 ± 0.003
V_{2000} + HCH	10.35 ± 1.06	4.46 ± 0.56[a]	17.05 ± 0.94[a]	0.24 ± 0.013	0.08 ± 0.004
$V_{10}5$	8.68 ± 0.70	4.34 ± 0.56	12.22 ± 0.37	0.26 ± 0.011	0.07 ± 0.004
$V_{10}5$ + HCH	9.31 ± 0.30	4.48 ± 0.42	14.55 ± 0.69	0.25 ± 0.019	0.08 ± 0.004

P values: a < 0.05; b < 0.01; c < 0.001.
*μmoles of oxaloacetate $min.^{-1}mL^{-1}$
†μmoles of pyruvate $min.^{-1}mL^{-1}$
‡μmoles of p-nitrophenol $min.^{-1}mL^{-1}$
§μmoles of $NADH_2$ $min.^{-1}mL^{-1}$
¶μmoles of phenolphthalein $min.^{-1}mL^{-1}$

Table 5 Liver enzymes of vitamin A-deficient and -supplemented male albino rats 24 hours after an acute oral dose of HCH (1000 mg/kg body weight) (Values are mean ± S.E. of four rats in each group)

Diet group	GOT*	GPT†	ALP‡	LDH§	β-GLR¶
V_0	0.40 ± 0.01	1.86 ± 0.05	4.22 ± 0.45	24.42 ± 0.54	2.52 ± 0.22
V_0 + HCH	0.28 ± 0.01[c]	1.47 ± 0.04[b]	5.10 ± 0.41	21.82 ± 0.31[b]	3.17 ± 0.22
V_{2000}	0.38 ± 0.01	2.26 ± 0.07	5.31 ± 0.15	26.03 ± 0.28	2.09 ± 0.44
V_{2000} + HCH	0.34 ± 0.01[a]	2.15 ± 0.10	6.02 ± 0.26	23.51 ± 0.13[b]	2.37 ± 0.30
$V_{10}5$	0.47 ± 0.01	2.39 ± 0.13	6.56 ± 0.60	26.03 ± 0.11	2.03 ± 0.14
$V_{10}5$ + HCH	0.47 ± 0.01	2.25 ± 0.13	7.58 ± 0.48	25.51 ± 0.22	2.33 ± 0.14

P values: a < 0.05; b < 0.01; c < 0.001
*μmoles of oxaloacetate $min.^{-1}$ mg $protein^{-1}$
†μmoles of pyruvate min^{-1} mg $protein^{-1}$
‡μmoles of p-nitrophenol $min.^{-1}$ mg $protein^{-1}$
§μmoles of $NADH_2$ $min.^{-1}$ mg $protein^{-1}$
¶μmoles of phenolphthalein $min.^{-1}$ mg $protein^{-1}$

slightly. Except for slight, but statistically significant, reductions in activities of GOT and LDH in the V_{2000} + HCH rats, all other enzyme activities were comparable to those of their respective controls in the HCH-intubated rats belonging to both the vitamin A-supplemented groups.

Hepatic XMEs

The activities of hepatic XMEs, with the exception of AH, were different among the control rats belonging to the three diet groups *(Table 3)*. These differences were statistically significant only in the case of DM and GT in the $V_{10}5$ rats compared with those of the V_0 rats. Activities of all the enzymes except those of GST were lower in the V_0 rats compared with their vitamin A-supplemented counterparts. However, activity of GST was higher in the V_0 rats. Between the two vitamin A-supplemented groups of rats, a diet-related difference in enzyme activities (higher in the $V_{10}5$ group) was noticed, except for GST. Also, the total protein content of the microsomes and cytosol of the V_0 rats was slightly less compared with those of the vitamin A-supplemented rats (data not shown).

Discussion

Vitamin A is an essential micronutrient that cannot be synthesized by animals. Though the normal requirement of vitamin A for rats is 2000 I.U./kg diet,[27] its supplementation, even at 50 times the normal requirement, was tolerated by the rats without any sign of hypervitaminosis over a period of 12 weeks. Dietary deprivation of vitamin A leads to its depletion in the body of animals, resulting in the onset of signs of deficiency and ultimately death. The feeding period required to induce vitamin A deficiency in rats varies depending on their strain.[8,9,28] The 9-week period observed for the Wistar strain of rats in our study is in agreement with previous reports.[9,29] The reduced hepatic content of vitamin A observed in the vitamin A-deficient rats is also supported by earlier reports.[8,9]

LD_{50} is the most accepted method to evaluate the toxicity of a chemical.[30] A wide variation exists in the acute oral LD_{50} value reported for technical HCH in rats, ranging from 600–3000 mg/kg body weight, mainly due to the variation in the individual isomer composition.[2] The acute oral LD_{50} value of HCH obtained for the rats supplemented with vitamin A at the recommended dietary level is comparable to the earlier report of Krishnakumari.[31] The significant lowering of the LD_{50} value in the vitamin A-deficient rats suggests the enhanced susceptibility of these rats to the acute oral toxicity of HCH. Also, the fact that more of the insecticide was required to achieve the same effect in the rats supplemented with vitamin A at 10^5 I.U./kg diet compared with those supplemented with the recommended dietary level illustrated the additional protective effect offered by the excess, but not hypervitaminotic, level of vitamin A against HCH toxicity. Similarly, though the signs of toxicity were exhibited by all three groups of HCH-intubated rats, the earlier onset, longer duration, and higher intensities noted in the vitamin A-deficient rats compared with the supplemented ones are evidences for the greater vulnerability of the deficient rats to HCH toxicity.

Assay of the activities of the soluble enzymes GOT, GPT, ALP, and LDH that are released into the blood during conditions of hepatic injury is an important diagnostic indicator of xenobiotic toxicity in animals. Alterations in the serum and liver activities of these enzymes have been reported during biochemical lesions of liver caused by insecticides.[3,32,33] Also, a dose-dependent increase in the serum and liver levels of β-GLR, an important enzyme involved in the detoxification of xenobiotics, has been reported in HCH-intoxicated rats.[3] Therefore, the higher alterations of the serum and liver enzymes found in the vitamin A-deficient rats following HCH administration, compared with their vitamin A-supplemented counterparts, confirm the enhanced susceptibility of these rats to the toxicity of HCH, as well as the protective action of vitamin A supplementation against the toxicity.

In animals HCH is first oxidized by cyt. P-450–dependent monooxygenases to relatively non-toxic metabolites that undergo further conjugation reactions including glucuronidation before they are excreted.[34–37] In this study we found that the activities of the hepatic XMEs in rats are greatly influenced by their dietary vitamin A status. Reduced activities of cyt. P-450, DM, and GT have been reported earlier in vitamin A-deficient rats.[8,9,38] With regard to the normal activity of AH in the vitamin A-deficient rats, our data is supported by Hauswirth and Brizuela[38] and Siddik *et al.*[39] Similarly, GST, an important phase II enzyme, is known to show higher activity in the vitamin A-deficient rats.[38,40] However, to the best of our knowledge, the activity of β-GLR has not been investigated so far in vitamin A-deficient rats and we found that like the other microsomal enzymes, β-GLR, an important phase II enzyme of xenobiotic metabolism, also showed reduced activity in the deficient rats.

In experiments on albino rats, Pentuik et al.[41] showed that vitamin A deficiency delays, and its excess supplementation enhances, biotransformation of aminopyrine and benzoic acid. The same authors also reported that vitamin A in excess exerted a protective effect in acute intoxication with bromobenzene, cyclophosphamide, and dimethylnitrosamine. Similar observations have been noticed with other nutrients such as proteins, lipids, minerals, and other vitamins.[7,42–44] Thus, the results of the present study suggest that the reduced activities of the hepatic XMEs, with the exception of GST, noted in the vitamin A-deficient rats could be one of the reasons for an impairment of HCH detoxification, which possibly resulted in accumulation of the more toxic parent compound leading to the greater susceptibility of these rats to HCH toxicity. On the other hand, the higher activities of the XMEs in the

vitamin A-supplemented rats resulted in a faster detoxification and clearance of HCH and the consequent protection against its toxicity in the rats. Though this study has provided evidences that the effect of vitamin A on HCH toxicity in rats was due to the modification of the detoxification capacity of the animals, other possibilities such as interaction between vitamin A and HCH at the sites of absorption and excretion are not excluded. In short, limited data presented in this paper warn of the possible potentiation of chemical toxicity in people whose dietary as well as body vitamin A contents are lower than the normal values. Also, it may be beneficial to incorporate more vitamin A resources in the daily diet of those who are at an occupational risk of exposure to chemicals.

References

1 Anonymous. (1987). Production of technical grade pesticides in India during 1986–87. *Pesticides Information.* **13,** 22

2 Ulman. (1972). Monograph of an insecticide, Verlag, K. Schillinger-Freiburg, Breisgau.

3 Shivanandappa, T. and Krishnakumari, M.K. (1981). Histochemical and biochemical changes in rats fed dietary benzene hexachloride. *Indian J. Exp. Biol.* **19,** 1163–1168

4 Shivanandappa, T. and Krishnakumari, M.K. (1983). Hexachlorocyclohexane induced testicular dysfunction in rats. *Acta Pharmacol. et Toxicol.* **52,** 12–17

5 Shivanandappa, T., Krishnakumari, M.K., and Majumder, S.K. (1982). Inhibition of steroidogenic activity in the adrenal cortex of rats fed benzene hexachloride (hexachlorocyclohexane). *Experientia* **38,** 1251–1253

6 van Velsen, F.L., Danse, L.H.J.C., van Leeuwen, F.X.R., Dormans, J.A.M.A., and van Logten, M.J. (1986). The subchronic oral toxicity of the β-isomer of hexachlorocyclohexane in rats. *Fund. Appl. Toxicol.* **6,** 697–712

7 Parke, D.V. and Ioannides, C. (1981). The role of nutrition in toxicology. *Ann. Rev. Nutr.* **1,** 207–234

8 Becking, G.C. (1973). Vitamin A status and hepatic drug metabolism in the rat. *Can. J. Physiol. Pharmacol.* **51,** 6–11

9 Colby, H.D., Cramer, R.E., Greiner, J.W., Robinson, D.A., Krause, R.F., and Canady, W.J. (1975). Hepatic drug metabolism in retinol-deficient rats. *Biochem. Pharmacol.* **24,** 1644–1646

10 Phillips, W.E.J. and Hatiana, G. (1972). Effect of dietary organochlorine pesticides on the liver vitamin A content of the weanling rat. *Nutr. Rep. Int.* **5,** 357–362

11 Pius, J., Shivanandappa, T., and Krishnakumari, M.K. (1990). Protective role of vitamin A on the male reproductive toxicity of Hexachlorocyclohexane (HCH) in the rat. *Repro. Toxicol.* **4,** 325–330

12 Lakshmanan, M.R., Junganwala, F.B., and Cama, H.R. (1965). Metabolism and biological potency of 5,6-monoepoxyvitamin A aldehyde in the rat. *Biochem. J.* **95,** 27–34

13 Chapman, D.G., Castillo, R., and J.A. Campbell. (1959). A new salt mixture for use in experimental diets. *J. Nutr.* **14,** 273–285

14 Hubbell, R.B., Mendel, L.B., and Wakeman, A.J. (1937). Evaluation of protein in foods 1. A method for the determination of protein efficiency ratios. *Can. J. Biochem. Physiol.* **37,** 679–686

15 Olson, J.A. (1979). A simple dual assay for vitamin A and carotene in human liver. *Nutr. Rep. Int.* **19,** 807–813

16 Lowry, O.H., Rosebrough, N.G., Farr, A.L., and Randall, R.J. (1951). Protein measurement with folin-phenol reagent. *J. Biol. Chem.* **193,** 265–275

17 Alphons, A.J.L.R., Falke, H.E., Catsburg, J.F., Topp, R., Blaauboer, B.J., Holsteijn, I.V., Doron, L., and Leewwen, F.H.R.V. (1987). Interlaboratory comparison of total cytochrome P-450 and protein determinations in rat liver microsomes. *Arch. Toxicol.* **61,** 27–33

18 Omura, T. and Sato, R. (1964). The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem* **239,** 2370–2378

19 Shimazu, T. (1965). Response of 20-methylcholanthrene of hepatic aniline and acetanilide 4-hydroxylase of rats with hypothalmic lesions. *Biochim. Biophys. Acta* **105,** 377–380

20 Pettit, F.H. and Zeigler, D.M. (1963). The catalytic demethylation of N,N′-dimethylaniline-N-oxide by liver microsomes. *Biochem. Biophys. Res. Commun.* **13,** 193–197

21 Fishman, W.H. (1974). β-glucuronidase. In *Methods of enzymatic analysis, Vol. 2,* (H.U. Bergmeyer, ed.) p. 929–943, Academic Press, New York, NY, USA

22 Isselbacher, K.J., Chrabas, M.F. and Quinn, R.C. (1962). The solubilization and partial purification of a glucuronyl transferase from rabbit liver microsomes. *J. Biol. Chem.* **237,** 3033–3036

23 Booth, J., Boyland, E., and Sims, P. (1961). An enzyme from rat liver catalyzing conjugation with glutathione. *Biochem. J.* **79,** 516–524

24 Moron, M.S., Dupierre, J.W., and Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim. Biophys. Acta* **582,** 67–78

25 Finney, D.J. (1977). *Probit analysis,* Cambridge University Press, Cambridge, UK

26 Snedecor, G.W. and Cochran, W.G. (1967). *Statistical methods,* Iowa State University Press, Ames, Iowa, USA

27 Anonymous. (1982). Nutrient requirements of laboratory animals. In *National Academy of Sciences-National Research Council, Publication 990.* Washington, DC, USA

28 Rozman, K., Gorski, J.R., Dutton, D., and Parkinson, A. (1987). Effects of vitamin A and or thyroidectomy on liver microsomal enzymes and their induction in 2,3,7,8-tetrachlorodibenzo p-dioxin treated rats. *Toxicology* **46,** 107–122

29 Melin, A.M., Carbonneau, M.A., Maviel, M.J., Perromat, A., and Clere, M. (1990). Free radical inhibitor effect of retinol after carbon tetrachloride intoxication in the rat. *Food Additives and Contaminants* **7 Supp.,** S182–S187

30 Zbinden, G. and Roversi-Fluri. (1981). Significance of the LD50 test for the toxicological evaluation of chemical substances. *Arch. Toxicol.* **47,** 77–99

31 Krishnakumari, M.K. (1973). Acute oral toxicity of Hexachlorocyclohexane to albino rats. In *Annual Report of Central Food Technological Research Institute,* Mysore, India.

32 Srinivasan, K. and Radhakrishnamurthy, R. (1988). Biochemical changes produced by α- and γ-hexachlorocyclohexane isomers in albino rats. *J. Environ. Sci. Health* **B19,** 367–373

33 Rahman, M.F., Siddiqui, M.K., Mahboob, M., and Mustafa, M. (1990). Hematological and hepatotoxic effects of isoprocarb in chicken. *J. Appl. Toxicol.* **10,** 187–192

34 Engst, R., Macholz, R.M., and Kujawa, M. (1979). Recent state of lindane metabolism, Part III. *Residue Rev.* **72,** 71–95

35 Tanaka, K., Kurihara, N., and Nakajima, M. (1979). Oxidative metabolism of tetrachlorocyclohexanes, pentachlorocyclohexanes and hexachlorocyclohexanes with microsomes from rat liver and housefly abdomen. *Pest. Biochem. Physiol.* **10,** 79–95

36 Tanaka, K., Kurihara, N., and Nakajima, M. (1979). Oxidative metabolism of lindane and its isomers with microsomes from rat liver and housefly abdomen. *Pest. Biochem Physiol.* **10,** 96–103

37 Ronis, M.J., Walker, C.H., and Peakall, D. (1987). Hepatic metabolism of cyclodiene insecticides by constitutive forms of cytochrome P-450 from lower vertebrates. *Comp. Biochem. Physiology - C: Comp. Pharmacol. Toxicol.* **87,** 375–388

38 Hauswirth, J.W. and Brizuela, B.S. (1976). The differential effects of chemical carcinogens on vitamin A status and on microsomal drug metabolism in normal and vitamin A-deficient rats. *Can. Res.* **36,** 1941–1946

39 Siddik, Z. H., Drew, R., Litterst, C.L., Minnaugh, E.G., Sikie, B.I., and Gram, T.E. (1980). Hepatic cytochrome P-

450 dependent metabolism and enzymatic conjugation of foreign compounds in vitamin A-deficient rats. *Pharmacol.* **21,** 383–390

40 Dogra, S.C., Khanduja, K.L., and Sharma, R.R. (1982). Effect of vitamin A-deficiency on the levels of glutathione and glutathione S-transferase activity in rat lung and rat liver. *Experientia* **38,** 903–904

41 Pentuik, A.A., Gutsol, V.I., Lutsiuk, N.B., and Bogdanov, N.G. (1987). Body vitamin A allowance of rats as a factor affecting the metabolism and toxicity of xenobiotics. *Farmakologiia I Toksikologiia* **50,** 51–54

42 Meydani, M. (1987). Dietary effects on detoxification processes. In *Nutritional toxicology, Vol. 2* (J.N. Hathcock, ed.) p. 1–39, Academic Press, New York, NY, USA

43 Boyd, J.N. and Campbell, T.C. (1983). Impact of nutrition on detoxication. In *Biological basis of detoxication,* (J. Caldwell and W.B. Jacoby, eds,) p. 287–306 Academic Press, New York, NY, USA

44 Campbell, T.C. and Hayes, J.R. (1974). Role of nutrition in the drug metabolizing enzyme system. *Pharmacol. Rev.* **25,** 171–197