福岡医誌 66 (10): 547—548, 1975

序　　言

油症治療研究班長，九州大学医学部教授
尾　前　照　雄

Foreward

Teruo OMAE

Chief of the Study Group for the Therapy of "Yusho", Professor of Medicine, Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

In succession to the 4th report (Fukuoka Acta Medica, Vol. 65, No. 1, January, 1974), the outline of the recent studies made by the Study Group for the Therapy of "Yusho" was described. The total number of the patients reached 1291 on April, 30th, 1975, among which 29 died.

Elevation of blood PCB concentration and abnormality in gas chromatographic pattern of blood PCB, acneiform eruptions, pigmentation (skin, conjunctiva, gingiva or nail) and hypertrophy or hypersecretion of Meibomi' glands have been considered as most important for the diagnosis of "Yusho". Some changes in clinical profiles were noticed as the time elapsed.

Studies concerning the pathogenesis or pathophysiology of "Yusho" were also described.

既に，油症と PCB に関する研究報告は，第4集までが本誌に刊行されている（福岡医学雑誌60巻6号，昭和44年；62巻1号，昭和46年；63巻10号，昭和47年；65巻1号，昭和49年）．今回はその後，昭和50年6月までの研究成績を第5集として論文にまとめたものである．

油症治療研究班は，昭和50年4月現在，九州大学医学部を中心に，同薬学部，同歯学部，久留米

表 1. 油症治療研究班員（昭和50年5月15日現在）

九大医	第二内科	教授	尾前照雄（班長）	〃	眼科	助手	大西克尚
〃	第三内科	助教授	平山千里（副班長）	〃	耳鼻咽喉科	〃	林田邦彦
〃	皮膚科	教授	占部治邦	九大歯	歯科保存	教授	岩野正男
〃	公衆衛生	〃	倉恒匡徳	九大薬	衛生裁判化学	〃	吉村英敏
〃	ウィルス学	〃	森　良一	〃	生理化学	〃	加藤敬太郎
〃	呼吸器科	助教授	置松信昭	〃	薬化学	助教授	佐伯清太郎
〃	第二内科	助手	山中正義	福岡大医	第一内科	教授	奥村　恂
〃	皮膚科	助教授	幸田　弘	〃	皮膚科	〃	利谷昭治
〃	〃	講師	旭　正一	〃	病理	〃	菊池昌弘
〃	産婦人科	助教授	楠田雅彦	久留米大医	第一内科	〃	倉田　誠
〃	整形外科	助手	喜多正臣	〃	小児科	〃	山下文雄
〃	神経内科	〃	岩下　宏	〃	眼科	〃	吉岡久春
〃	中検	部長	大河内一雄	〃	環境衛生	〃	高松　誠
〃	小児科	教授	合屋長英	〃	〃	助手	井上馨人
〃	〃	講師	本田　憲	第一薬科大	分析	教授	増田義人
				国立栄養研究所	栄養改善室	室長	印南　敏

DSW 033172

STLCOPCB4017134

福岡医誌 66 (10): 549–554, 1975

PCBの代謝に関する研究(第6報)

2,4,3',4'-テトラクロロビフェニルの代謝と急性毒性との関連性について

九州大学薬学部衛生裁判化学教室
吉村英敏・山本弘明
木下春喜

Metabolic Studies on Polychlorinated Biphenyls. VI.

Contribution of the Hydroxylated Metabolites to the Acute Toxicity of 2,4,3',4'-Tetrachlorobiphenyl

Hidetoshi YOSHIMURA, Hiro-aki YAMAMOTO and Haruki KINOSHITA

Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University

In our previous study, the acute toxicity of 5-hydroxy-2,4,3',4'-tetrachlorobiphenyl (TCB), the major metabolite of 2,4,3',4'-TCB, was found to be about 5 times as high as that of 2,4,3',4'-TCB in mice. In order to learn the contribution of this metabolite to the toxicity of the parent compound, it was studied whether 2,4,3',4'-TCB would exert more toxic effect or not after pretreatment of mice with phenobarbital (PB) or 3-methylcholanthrene (3-MC), the typical inducers of liver microsomal drug metabolizing enzymes system. Effects of the same pretreatment of rats on the biliary excretion rate of 5-hydroxy-2,4,3',4'-TCB after iv injection of 2,4,3',4'-TCB and on the *in vitro* metabolic rate of 2,4,3',4'-TCB using liver 9000 *g* supernatant fraction were also investigated.

By these studies the following findings were obtained.

1. Pretreatment with 3-MC increased the acute toxicity of 2,4,3',4'-TCB, but on the contrary PB pretreatment decreased that toxicity in mice.

2. Liver 9000 *g* supernatant fraction obtained from the rat pretreated with 3-MC showed much higher activity to hydroxylate 2,4,3',4'-TCB than that of the control rat, but PB pretreatment did not increase the activity at all.

3. By pretreatment with PB or 3-MC, rats increased their biliary excretion of 5-hydroxy-metabolite about 2 times or more than 10 times, respectively, comparing with that of untreated rats, during 24 hr after iv injection of 2,4,3',4'-TCB. It was suggested that the increase of excretion of 5-hydroxy-metabolite in 3-MC pretreated rats should be due to marked elevation of the metabolic rate, whereas that in PB pretreatment could be explained by increase of biliary flow rate.

4. From these results, it was concluded that at least a part of the acute toxicity of 2,4,3',4'-TCB was attributable to the more toxic metabolite, 5-hydroxy-2,4,3',4'-TCB, which was produced in the body.

いわゆる油症の原因物質がカネクロール-400に基ずくことが明らかにされ[1]，著来筆者らはその治療法の確立および中毒機序の解明に手掛りを得る目的で，今日までカネクロール-400中の主成分 2,4,3',4'-テトラクロロビフェニル (2,4,3',4'-TCB)[2][3] および 3,4,3',4'-テトラクロロビフェニル(3,4,3',4'-TCB)[4][5]

DSW 033173

STLCOPCB4017135

福岡医誌 66 (10): 555—562, 1975

Iochem. J.

Biochem.

.: J. Phar-

aith, R. L.
nacol. 19:

PCB の代謝に関する研究（第7報）

2, 5, 2′ 5′-テトラクロロビフェニルのラットにおける代謝

九州大学薬学部衛生裁判化学教室
吉村英敏・山本弘明
米沢和明

Metabolic Studies on Polychlorinated Biphenyls. VII.

Metabolic Fate of 2, 5, 2′, 5′-Tetrachlorobiphenyl in Rats

Hidetoshi YOSHIMURA, Hiro-aki YAMAMOTO
and Kazuaki YONEZAWA

Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University

In the previous studies from this laboratory, it was found that both of 2, 4, 3′, 4′- and 3, 4, 3′, 4′-tetrachlorobiphenyl (TCB) were metabolized to the monohydroxylated derivatives in rats and the metabolites were excreted almost exclusively into their feces. Hutzinger *et al.*, on the other hand, reported that when 2, 5, 2′, 5′-TCB was injected intraperitoneally into rats, large quantities of the unchanged material could be extracted from the feces and only a monohydroxy derivative was identified in the urine. In order to confirm this discrepancy for excretion route of the metabolite, we re-investigated the metabolic fate of 2, 5, 2′, 5′-TCB orally given to rats and obtained the following results.

1. In the case of 2, 5, 2′, 5′-TCB, the major metabolite was excreted mostly in the feces. By GLC analysis, this metabolite excreted during 7 days accounted for about 45 % of the dose, whereas that excreted in the urine during the same days accounted for only 0.4 % of the dose. This metabolic rate was much higher than those of 2, 4, 3′, 4′- and 3, 4, 3′, 4′-TCB (about 10 % and 3.3 % of the dose, respectively, during 12 days). The structure of this major metabolite was elucidated to be 3-hydroxy-2, 5, 2′, 5′-TCB by various spectrometries and finally by comparison with the authentic sample. A trace amount of 4-hydroxy-metabolite was also identified in the urine.

2. In above experiments, about 7 % of the dose was excreted unchanged into 7 days feces, most of which was done in the first day. A trace amount of unchanged material could be detected in the urine. This result also suggested that the absorption rate of 2, 5, 2′, 5′-TCB from the gastrointestinal tract was quite higher than those of 2, 4, 3′, 4′-and 3, 4, 3′, 4′-TCB (the absorption rate of the latter two TCB was calculated to be about 60 % and 40 %, respectively).

3. 2, 5, 2′, 5′-TCB was injected intraperitoneally into rats with cannulated bile duct and excretion of the metabolite in the bile was examined by GLC. The result indicated clearly the excretion of 3-hydroxy-2, 5, 2′, 5′-TCB as the major metabolite and also of 4-hydroxy-metabolite in a trace amount, but no unchanged material could be detected in the bile. Since 4-hydroxy-metabolite was not excreted in the faces, above result suggested that a part of the metabolites excreted through the biliary system to the small intestine might be re-absorbed from the intestine.

DSW 033174

STLCOPCB4017136

福岡医誌 66 (10): 563—567, 1975

ラット肝ミクロゾーム膜のリン脂質に及ぼす塩化ビフェニール (PCB) の影響

九州大学薬学部生理化学教室
前田康夫・高橋慶吉
加藤敬太郎

Effect of Biphenyl Chloride on Rat Liver Microsomal Phospholipids

Yasuo MAEDA, Keikichi TAKAHASHI and Keitaro KATO
Faculty of Pharmaceutical Sciences, Kyushu University Fukuoka, Japan

The proportion of different phospholipids and fatty acid component of phosphatidyl choline, phosphatidylethanolamine and phosphatidylinositol were studied in rat liver microsomal membranes, after a long period administration (60 days; total dose, 100 mg) of Kanechlor-400 (KC-400). The proportion of different phospholipids did not change, but KC-400 caused the proportion of stearic acid in phosphatidylcholine to increase to 125 % of the control and the proportion of palmitic acid to decrease to 70 %. The effect of a long period administration of KC-400 on drug metabolizing emzymes was also studied, and the relationship between elevation of the emzymes and change of phospholipids was discussed.

緒言

動物に PCB を投与すると，肝細胞の滑面小胞体が増殖し[1)2)]，薬物代謝酵素系の活性が上昇することが知られている[1)3)]．Phenobarbital を投与した場合には Cytochrome P-450 (Cyt. P-450) が，Methylcholanthrene を投与した場合には Cyt. P-448 が，それぞれ肝ミクロゾームに増加するが[3)4)5)6)7)]，PCB を投与した場合には Cyt. P-450 と Cyt. P-448 の両者が，肝ミクロゾームに増加することが知られている[1)3)]．

Phenobarbital と Metylcholanthrene では，さらに，肝ミクロゾーム膜を構成しているリン脂質におよぼす影響が詳細に研究されている．これらの薬物を投与すると，ミクロゾーム膜を構成している各リン脂質の組成には変化が起らないが，Phosphatidylcholine と Phosphatidylethanolamine では，構成脂肪酸組成の変化，特に不飽和脂肪酸組成の変化が起こることが報告されている[8)]．このうちPhosphatidylcholine の脂肪酸組成の変化は，Phenobarbital による薬物代謝酵素系の誘導と密接な関連を持つことが報告されている[4)7)9)]．

Phenobarbital や Metylcholanthrene に比べると，PCB の肝ミクロゾーム膜リン脂質に及ぼす影響についての研究は少ない[10)]．ラットに投与された PCB が，特に肝ミクロゾーム膜に濃縮されることから考えると[6)]，この点についての詳細な研究が必要であろうと思われる．本報告では，ラットに PCB を長期投与した場合に，肝ミクロゾーム膜にリン脂質の変動が起こるや否やを分析し，さらに肝ミクロゾーム薬物代謝酵素系の変動についても測定を行い，肝ミクロゾーム膜に起こる薬物代謝酵素系の増加と膜リン脂質の構成脂肪酸組成の関連について考察を加えた．

実験方法

1．投与方法

動物は Sprague Dawley ラット雄（約 300 g）を使用した．Kanechlor-400 (KC-400) を Corn oil

DSW 033175

STLCOPCB4017137

福岡医誌 66(10): 568—573, 1975

ポリ塩化ビフェニール (PCB) の培養 HeLa 細胞に及ぼす影響

九州大学医学部微生物学教室
森　良一・藤田晃一
山口みどり
九州大学薬学部衛生裁判化学教室
吉村英敏・山本弘明

Effect of Polychlorinated Biphenyls (PCB) on HeLa Cells.

Ryoichi MORI, Koichi FUJITA,
and Midori YAMAGUCHI
Department of Microbiology, Faculty of Medicine, Kyushu University, Fukuoka
Hidetoshi YOSHIMURA and Hiroaki YAMAMOTO
Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka

Suspension of Kanechlor 400 (KC 400), a commercial brand of polychlorinated biphenyls (PCB), was found to have cytotoxic and cytolytic activity against HeLa cells. The minimum effective dose was 3~6 μg/ml in media without serum protein. Time-lapse cinematography revealed that HeLa cells treated with KC 400 25 μg/ml undergo retraction of processes, bleb formations, balloon formations followed by burst of the cells.

ポリ塩化ビフェニール（以下 PCB と略す）の市販品の一つであるカネクロール 400（以下 KC 400 と略す）は，いわゆる油症の原因物質であり[1][2][3][4]，また長期間ラットに投与すると肝に腫瘍性の変化を生ずる[5]ことが報告されている点でも注目すべき物質である．

従来，この物質の作用については動物実験レベルの研究が主に行われており，試験管内培養細胞を用いての研究報告は見当らない．筆者らはヒト子宮癌由来の株化細胞である HeLa 細胞を用い，これに及ぼす KC 400 の影響について検討を行ったので報告する．

材料と方法

薬剤：PCB は主として KC 400 を使用した．KC 400 をジメチルスルフォキサイド (DMSO) に 10 mg/ml の濃度に溶解したものを原液とし，この状態で 121 °C, 15分のオートクレイブにより滅菌を行った．これを pH 7.2 の燐酸緩衝食塩水 (PBS) または Eagle の minimum essential medium (MEM) に加えて必要な濃度の懸濁液とした．

培養細胞：当教室で角瓶を用い，仔牛血清 10% 加 MEM を培地として継代されている HeLa 細胞 S 3 株を用いた．顕微鏡的観察のための容器としてはプラスチックミクロプレイト (Falcon 3040) および 35×10 mm のプラスチックペトリディッシュ (Falcon 3001) を使用した．

色素排除試験：細胞の生死を判定するための色素排除試験は，細胞浮遊液に等量の 0.5% トリパンブルー液を加えて血球計算盤に入れ，染色される細胞を死細胞と判定した．

映画による観察：Falcon 3001 に培養した HeLa 細胞を MEM で 5 回洗ったのち，必要な濃度の KC 400 を含む MEM 2 ml を加え，ビニールテープで容器を封じ，20倍の DLL 位相差対物レンズをつけ，

DSW 033176

STLCOPCB4017138

福岡医誌 66(10): 574—578, 1975

ポリ塩化ビフェニール (PCB) の溶血作用および血球凝集様作用

九州大学医学部微生物学教室
藤田晃一・森　良一
山口みどり
九州大学薬学部衛生裁判化学教室
吉村英敏・山本弘明

Hemolytic and "Hemagglutinating" Activity of Polychlorinated Biphenyls (PCB).

Koichi FUJITA, Ryoichi MORI and Midori YAMAGUCHI
Department of Microbiology, Faculty of Medicine, Kyushu University, Fukuoka
Hidetoshi YOSHIMURA and Hiroaki YAMAMOTO
Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka

Kanechlor 400 (KC 400) dissolved in dimethylsulfoxide and emulsified in PBS was found to have "hemagglunating" activity at 4°C and hemolytic acitivity at 37°C. The "hemagglutinating" phenomenon was not due to the dimer formation or aggregate formation. The phenomenon was blocked by serum protein and cardiolipin. Most of the isomers of tetrachlorobiphenyls, chief components of KC 400, were shown to have hemolytic activity at the same concentration as KC 400.

前報[1]でポリ塩化ビフェニール (PCB) の市販品の一つであるカネクロール 400 (KC 400) が，培養 HeLa 細胞に対して細胞障害作用があることについて報告した．その形態学的観察から細胞膜の変化が著しいことが明らかとなったので，系を簡易化し，PCB と赤血球との反応について検討を行った．その結果，ある種の動物の赤血球浮遊液は PCB 懸濁液と混合し 4°C に放置すると，赤血球凝集反応にみられると同様な凝集パターンをとることを見出し，また 37°C では溶血反応が観察されたので報告する．なお KC 400 による血球凝集反応の一部についてはすでに報告した[2]．

材料と方法

(1) 使用薬物

PCB としては主として KC 400 を使用した．KC 400 はジメチルスルフォキサイド (DMSO) に 10 mg/ml に溶解したものから必要な濃度の懸濁液を作製した．

ビフェニールおよび 1 塩素化ビフェニール (4—クロロビフェニール) は，それぞれ和光純薬および半井化学薬品の製品を購入し，2 塩素化ビフェニール (4,4'—ジクロロビフェニール) はベンチジンのジアゾニウムクロリドを銅粉で処理して合成したものを，また 4 塩素化ビフェニール，5 塩素化ビフェニール (Analabs 社製) は九大医学部倉恒匡徳教授より分与を受けたものを使用した．上記単品の溶血作用の定量には，温エタノールに 5 mg/ml に溶解させたのち，所定の濃度の懸濁液を作製した．

(2) 血球凝集試験

ハムスター，モルモット，マウス，ニワトリより採血し，Alsever 液に保存した血球を燐酸緩衝生理食塩水 (PBS) で 3 回洗浄したのち，最後の遠沈を 1500 rpm, 10 分行い PBS を用いて 0.4% (v/v) の血球

DSW 033177

STLCOPCB4017139

福岡医誌 66 (10): 579—584, 1975

(PCB) の結
i 66: 568-573

i—: PCB (ポ
反応. 医学のあ

カネクロールの
ついて. 福岡医

Polychlorinated Biphenyls[1] Toxicity and Nutrition IV

PCBs Toxicity and Vitamin A (3)

Satoshi INNAMI, Atsuko NAKAMURA, Katsushige KATO and Motoyoshi MIYAZAKI

The National Institute of Nutrition, Tokyo 162, Japan

Sumiko NAGAYAMA

Teikyo College, Tokyo 151, Japan

Eiichi NISHIDE

Department of Fisheries, College of Agriculture and Veterinary Medicine, Nihon University, Tokyo 154, Japan

The investigations have been made to elucidate the interaction between PCBs toxicity and vitamin A. The authors (Innami, Tojo *et al.*) have already reported that rats fed for 6 weeks a 0.1% PCBs diet supplemented with 3,400 IU of vitamin A showed better growth than those fed a 0.1% PCBs diet without supplementation. However, rats given a vitamin A deficient diet with 0.1% PCBs showed more significant growth retardation than those given a 0.1% PCBs diet without supplementation (Innami, Nakamura *et al.*). All rats which were fed a vitamin A deficient diet with PCBs had a bad coat of fur and closed their eyes 50% or completely at the 3rd week of feeding, and some of them died in the last period of the experiment. But such symptoms were not at all seen in the rats given a 0.1% PCBs diet without supplementation or a vitamin A deficient diet. It was also observed that the vitamin A content in the liver decreased significantly with 0.1% PCBs administration (Innami, Nakamura *et al.*).

This result was not inconsistent with that of Villeneuve *et. al.* who demonstrated the decrease of vitamin A content in the livers of rabbits and fetuses receiving Aroclor 1221 and 1254 for 28 days. Therefore, it seemed that the nature of the symptoms which appeared in the rats fed a vitamin A deficient diet with 0.1% PCBs was strongly possible to be of a vitamin A deficiency itself. However, Tanaka *et al.* previously described that almost similar symptoms appeared when rats fed a stock diet were dosed 0.1 g of PCBs per kg per day orally for 4 weeks.

For such reason, the authors have mentioned in the previous paper (Innami, Nakamura *et al.*) that further investigations would be required to clarify the cause of this phenomenon.

In the present study, the experiments have been conducted to determine whether these symtoms would be a characteristic phenomenon of PCBs poisoning or based on vitamin A deficiency.

Methods

Weanling male rats of the Sprague Dawley strain were housed individually in wire cages maintained in a laboratory with 12 hours of light and 12 hours of darkness. The temperature in the animal laboratory was controlled near to 22°C. The composition of the basal diet was (%): milk casein 20.0, sucrose 63.0, soybean oil 9.0, mineral mixture

1) The following abbreviation is used: PCBs, polychlorinated biphenyls.

DSW 033178

STLCOPCB4017140

(Harper, A. E.) 4.0, cellulose flour 2.0, vitamin mixture (Harper, A. E.) 0.85, choline·HCl 0.15 and vitamin oil 1.0 (one gram of this oil contains 300 IU of vitamin A-acetate, 30 IU of vitamin D_3 and 10 mg of α-tocopheryl E-acetate).

In the vitamin A deficient group, vitamin A was removed from vitamin oil and vitamin free casein (Wako Pure Chemical Inc.) was directly used without further purification. One hundred mg of PCBs was added to 100 g of the basal diet in substituting for an equal part of sucrose. PCBs used in this experiment were purchased from Wako Pure Chemical Inc., and were a mixture of the isomers, of which tetrachloride was dominant.

The diets were prepared every two weeks and stored in the refrigerator except feeding time. Six to seven rats of each group were fed the experimental diets. Food and water were given ad libitum throughout the experiment. Food consumption and body weight were recorded 3 times per week.

In the first experiment, rats were fed on the PCBs diets with and without vitamin A for 8 weeks. On the way of feeding, half of the rats receiving a vitamin A deficient diet with PCBs was switched to the diet containing 3,000 IU of vitamin A-acetate per 100 g of the PCBs diet after the characteristic symptoms appeared.

In the second experiment, after the characteristic symptoms appeared each 100 IU of vitamin A-acetate and β-carotene was directly ingested every two days into the stomach of the rats fed a vitamin A deficient diet with PCBs by using a syringe with a round-top needle (9.5 cm in length). The purity of crystal β-carotene purchased from Merck was found to be 80% by the alumina chromatographic analysis (Tabata *et al.*). Vitamin A-acetate and β-carotene were dissolved in the soybean oil (the Japanese Pharmacopoeia) and the potency was determined every two days.

At the end of experiment the food cups were removed at 7 : 00 a.m. After fasting for 7~8 hours, the rats were sacrificed by decapitation. The liver was quickly removed, weighed and stored in a freezer maintained under −20°C. As early as possible after sacrifice, the liver vitamin A was determined by a colorimetric method using a mixture ofantimony trichloride and glycerol-1, 3-dichlorohydrin following saponification in KOH (Fujita and Kimura).

Results and Discussion

The first experiment was carried out to further clarify whether the characteristic symptoms which appeared in the rats given a vitamin A deficient diet with PCBs in a comparatively early period of the experiment, as previously reported, would be based upon a vitamin A deficiency or not. Rats were fed on the PCBs diets with and without vitamin A for 8 weeks.

As shown in Fig. 1, rats fed a vitamin A deficient diet with PCBs showed more prominent growth retardation than the PCBs group at the 4th week after feeding started. At this time, all rats of this group had a bad coat of fur and eye mucus, and closed their eyes completely as shown in Fig. 2 (A). The results were completely the same as previously reported (Innami, Nakamura *et al.*).

On the other hand, the growth of the vitamin A deficient group was almost equal to that of the control group until the 4th week after feeding started, and since then the growth of this group tended to slow down slightly as compared with the control group. However, not any eye symptoms in this group have been detected by the end of experiment. Even in the PCBs-fed group, the same was true, but the only difference from the vitamin A deficient group was that two out of 6 rats died at the last week of the experiment. They had a bad coat of fur and a part of hair was lost. In the group on the vitamin A deficient diet with PCBs one out of 7 rats died at the 4th

DSW 033179

STLCOPCB4017141

week of feeding, and after that the remaining rats of this group died successively until the 6th week of feeding.

While, it was found that the rats which were switched from a vitamin A deficient diet with PCBs to the diet containing 3,000 IU of vitamin A-acetate per 100 g of the PCBs diet at the 26th day after feeding started, as shown in Fig. 1, recovered from their body weight loss rapidly and gained significantly over the PCBs group at the end of experiment. They regained a fine coat of fur, and remarkably, their closed eyes opened and the eye mucus disappeared, as seen in Fig. 2(B).

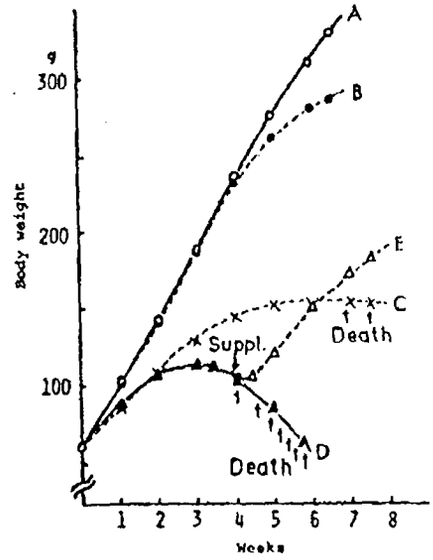

Fig. 1. Effect of vitamin A supplementation on recovery of growth retardation in rats fed a vitamin A deficient diet with PCBs
A: Control, B: Vitamin A deficient, C: 0.1% PCBs, D: V.A def.+0.1% PCBs, E: 3,000 IU of V.A-acetate was added to 100g of a 0.1% PCBs diet.

It was also observed in this experiment that the vitamin A content in the liver of rats fed a PCBs diet or a vitamin A deficient diet with PCBs decreased significantly, as shown in Table 1. The vitamin A content in the liver of rats fed 3,000 IU of vitamin A-acetate with 0.1% PCBs was significantly higher than that of rats fed a vitamin A deficient diet with PCBs.

However, the liver vitamin A content of these rats, despite that a large amount of vitamin A was administered, was much less than that in the previous experiment (Innami, Nakamura *et al.*). The reason may be due to the difference of experimental conditions between the present experiment and the previous one. At any rate, this means that if animals receiving PCBs would once



(A) (B)

Fig. 2. Appearance of characteristic symptoms in rats received a vitamin A deficient diet with 0.1% PCBs and recovery of these symptoms by vitamin A administration.
(A) A rat received a vitamin A deficient diet with 0.1% PCBs for 4 weeks.
(B) A rat received a diet containing 3,000 IU of vitamin A per 100 g of a 0.1% PCBs diet for 3 weeks after the characteristic symptoms appeared.

DSW 033180

STLCOPCB4017142

Table 1. Effects of vitamin A and β-carotene on weight gain and vitamin A content in liver of rats fed a vitamin A deficient diet with 0.1% PCBs

Experiment number	Group	Body weight gain	Liver weight		Vitamin A content
		g	g	%[1]	IU/g liver
Exp. 1	PCBs (7)	93±15*	14.39±1.66	9.47±0.61	2.57±0.51
	V.A def. + PCBs (7)	17± 4	8.10±0.38	10.51±0.26	1.67±0.22
	V.A def. + PCBs→ V.A suppl.[2] + PCBs (7)	137±21	17.80±2.10	9.03±0.53	12.35±2.70
Exp. 2	V.A def. + PCBs (6)	24± 3	8.03±0.61	9.60±0.57	1.17±0.31
	V.A def. + PCBs→ V.A dose[3] + PCBs (6)	75±20	12.42±1.67	9.35±0.48	5.55±2.09
	V.A def. + PCBs→ β-carotene dose[3]+PCBs (6)	91±23	14.14±1.88	10.32±1.75	0

* Mean ± S.E.
1) (Liver weight)÷(Body weight)×100
2) 3,000 IU of vitamin A-acetate was supplemented to 100 g of a 0.1% PCBs diet on and after the 26th day of feeding.
3) Each 100 IU of vitamin A-acetate and β-carotene was ingested every two days into the stomach of rats fed a vitamin A deficient diet with 0.1% PCBs on and after the 33rd day of feeding.
Figures in parentheses are the number of rats used.

become deficient in vitamin A, much time may be needed to restore vitamin A in the liver even though a large amount of vitamin A is supplied.

In the second experiment, the effectiveness of β-carotene on the PCBs toxicity in the vitamin A deficient animals was compared with that of vitamin A.

Each 100 IU of vitamin A-acetate and β-carotene was directly ingested every two days into the stomach of rats fed a vitamin A deficient diet with PCBs after the characteristic symptoms appeared. The results are shown in Fig. 3 and Table 1. All rats fed a vitamin A deficient diet with PCBs died until the 6th week of feeding. However, in the groups which ingested either vitamin A or β-carotene orally since the 33rd day of feeding, only one out of 6 rats in each group died within 10 days after the vitamin administration.

There were no differences between vitamin A and β-carotene in the recovery of reduced body weight and the characteristic symptoms caused by a vitamin A deficient diet with PCBs. However, the recovery of growth by vitamin A or β-carotene administration was not remarkable as compared with the first experiment.

The reasons for this or for the appearance of one dead rat in each case of this experiment might be due to the delay of time for the ingestion of vitamins and the low level of vitamin administration as compared with the first experiment.

Vitamin A content in the liver of rats ingested 100 IU of vitamin A every two days was not so high, but higher than that of rats fed a vitamin A deficient diet with PCBs. On the other hand, vitamin A was not detectable in the liver of rats ingested β-carotene every two days.

This seems to indicate that, as far as the present experimental conditions concern, the ingestion of 100 IU β-carotene every two days is inadequate to store vitamin A in the liver of rats fed a vitamin A deficient diet with PCBs for few weeks.

DSW 033181

STLCOPCB4017143

At any rate, the above results mean that a large part of the symptoms which appeared in the rats fed a vitamin A deficient diet with PCBs is based on a vitamin A deficiency. In other words, it means that PCBs act to accelerate vitamin A deficiency in the animals or make animals deficient in vitamin A.

Thus, the possible explanation on the PCBs toxicity is that the toxic effect, at least a part of it, appears through the occurrence of vitamin A deficiency.

However, it is not yet clear whether the symptoms described by Tanaka *et al.* would also be based upon a vitamin A deficiency caused by subacute toxicity of PCBs or not. Further investigations will be needed about it.

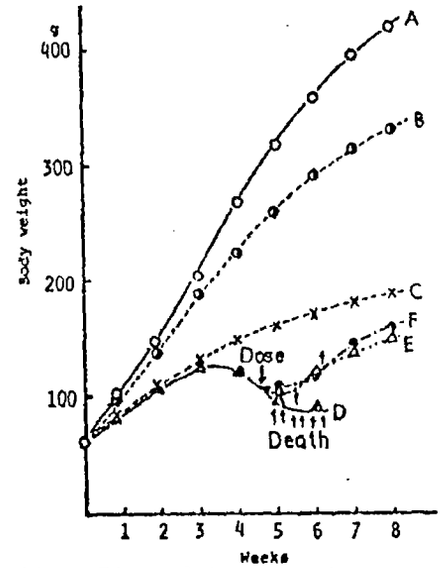

Fig. 3. Effects of vitamin A and β-carotene ingestion on recovery of growth retardation in rats fed a vitamin A deficient diet with PCBs
A: Control, B: Vitamin A deficient, C: 0.1% PCBs, D: V.A def.+0.1% PCBs, E: 100 IU of V. A was ingested, F: 100 IU of β-carotene was ingested.

Summary

The experiments have been conducted to determine whether the symptoms which appeared in the rats fed a vitamin A deficient diet with PCBs at an earlier time than expected would be a characteristic phenomenon of PCBs poisoning or based on a vitamin A deficiency.

Rats fed a vitamin A deficient diet with PCBs showed the characteristic symptoms until the 4th week after feeding started, and all rats in this group died until the 6th week of the experiment. While, the rats whose diet was switched from a vitamin A deficient diet with PCBs to the diet containing 3,000 IU of vitamin A-acetate per 100 g of the PCBs diet at the 26th day of feeding after the characteristic symptoms appeared, restored their body weight rapidly and gained significantly over the PCBs diet group without supplementation at the end of experiment. They regained a fine coat of fur and remarkably, their closed-eyes opened clearly and the eye mucus disappeared.

It was also found from another experiment that there were no differences between vitamin A and β-carotene in the recovery of reduced body weight and the characteristic symptoms caused by a vitamin A deficient diet with PCBs. However, a significant difference was seen in the vitamin A content of the liver between the rats dosed 100 IU of vitamin A and the rats dosed 100 IU of β-carotene.

These results indicate that a large part of the symptoms which appeared in the rats fed a vitamin A deficient diet with PCBs is based on a vitamin A deficiency. Thus, it is concluded that PCBs act to accelerate vitamin A deficiency in the animals or make animals deficient in vitamin A. Therefore, animals received PCBs require more vitamin A than usual.

References

1) Fujita, A. and Kimura, K.: An improved micromethod for fractional determination of vitamin A alcohol and ester and the changes in both types of vitamin A in blood plasma,

DSW 033182

STLCOPCB4017144

milk and liver after loading with both types of the vitamin A and β-carotene. J. Vitaminol. 6: 6-15, 1960.

2) Harper, A. E.: Amino acid balance and imbalance I. Dietary level of protein and amino acid imbalance. J. Nutr. 68: 405-418, 1959.

3) Innami, S., Nakamura, A., and Nagayama, S.: Polychlorobiphenyl toxicity and nutrition II. PCB toxicity and vitamin A (2). J. Nutr. Sci. Vitaminol., 20: 363-370, 1974.

4) Innami, S., Tojo, H., Utsugi, Y., Nakamura, A. and Nagayama, S.: PCB toxicity and nutrition I. PCB toxicity and vitamin A (1). Jap. J. Nutr. 32: 58-66, 1974 (in Japanese).

5) Tabata, R., Baba, H., Fukuba, H., Takai, Y., and Iwao, H.: The determination methods of vitamins in foods (2) The determination method of β-carotene. Jap. J. Nutr. 21: 54-56, 1956 (in Japanese).

6) Tanaka, K., Fujita, S., Komatsu, F. and Tamura, N.: Experimental subacute poisoning of chlorobiphenyls, particularly the influence on the serum lipids in rats. Fukuoka Acta Med. 60: 544-547, 1969 (in Japanese).

7) Villeneuve, D. C., Grant, D. L., Phillips, W. E. J., Clark, M. L., and Clegg, D. J.: Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Bull. Envir. Contam. Toxicol. 6: 120-128, 1971.

PCB の毒性とビタミン A (3)

国立栄養研究所栄養改善部
印南　敏・中村敦子
加藤勝重・宮崎基嘉
帝京短期大学
永山スミ子
日本大学農獣医学部水産学科
西出英一

シロネズミを PCB を含むビタミン A (以下 V. A) 不足飼料で飼育すると，比較的早期に特有の症状が発現するが，この症状が V. A 欠乏によるものなのか，あるいは PCB に特有の毒性症状であるのかを明らかにしたいと考え実験を行った．

Sprague Dawley 系の雄の幼シロネズミに 0.1% PCB (四塩化物主成分) を含む V. A 不足飼料を与えると，前報同様に飼育開始後 3 週目頃より体重が低下し始め，毛並みの乱れや眼やにが現れ，その後眼瞼がよさがるといった特有の症状が現れた．

そこで飼育後 26 日目にこれらの症状を呈したネズミを 2 群に分け，その 1 群の飼料を 0.1% PCB 飼料 100 g 当たり 3,000 IU の V. A を含む飼料に切換えると，成長は速やかに回復し，投与 3 週後では特有の症状の消失することを認めた．一方，V. A を添加しない他の 1 群では 6 週目末までに 7 匹全部が死亡した．0.1% PCB 群では 7～8 週目にかけて 6 匹中 2 匹死んだが，この 2 匹は毛並みが悪くなっただけで眼瞼症状には異常が認められなかった．

次に，これらの特有症状に対する β-カロチンの回復効果を V. A のそれと比較した．この実験でも，V. A 不足 0.1% PCB 群は 3～4 週目に特有症状を呈し，6 週目末までに全部死亡した．しかしながら，飼育 33 日目に V. A と β-カロチンをそれぞれ 100 IU ずつ 2 日置きに胃に注入したものでは，投与後 3 週目までに成長と症状の回復がみられた．

上記の 2 実験において，各群の肝臓 V. A 濃度を測定したところ，PCB 群，V. A 不足 PCB 群共に V. A の著しい減少がみられたが，V. A 投与群では V. A の貯留が認められた．しかし，β-カロチン投与群では肝臓に V. A を認めることができなかった．

以上のことから，0.1% PCB を含む V. A 不足飼料で飼育した場合に現れる特有症状は，V. A 欠乏によるものであることを認めた．すなわち，PCB は動物に対して V. A 欠乏を促進させるか，あるいは V. A 欠乏を起こさせるように作用すると推定されるので，PCB 投与動物では V. A の要求量が一応増大するといえる．

DSW 033183

STLCOPCB4017145

福岡医誌 66(10): 585—588, 1975

PCB の酵素誘導作用に及ぼす他物質併用の影響

九州大学医学部薬理学教室
荒木泰典*・日高賢志
田中　潔

Influences of Some Agents on the Enzyme-inducing Activity of PCB

Yasunori ARAKI, Tadashi HIDAKA and Kiyoshi TANAKA

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

The enzyme-inducing activity of PCB was measured pharmacologically with the method of testing hexobarbital sleeping time in young female mice (Araki & Tanaka: Fukuoka Acta Med. 65: 47, 1974). Combination of PCB with a small dose of methyl mercury or clofibrate enhanced the inducing activity, whereas alkylbenzene sulfonate counteracted the activity of PCB.

Cholestyramine strongly inhibited the shortening of hexobarbital sleeping time induced by a mixture of PCB, DDT and BHC, indicating that this anion exchange resin suppressed the intestinal absorption of the mixture.

PCB に対する生体の反応として最も鋭敏なものは，肝の薬物代謝酵素誘導作用であり，これを薬理学的に間接測定する簡便な方法が考案された (Araki & Tanaka)．この方法で調ると，PCB は DDT または BHC と協力し，小量ずつの併用で著しい誘導作用を示すことが知られている（荒木・田中）．現在のような複合汚染の時代においては，汚染農薬と PCB との協力は注目すべき現象であるから，他の汚染物質との複合作用も検査する必要がある．

今回は有機水銀と中性洗剤という普遍的汚染物質を取り上げ，PCB と併用したときの誘導作用をみることにした．また誘導性医薬の一つに挙げられる有機塩素系脱コレステロール薬 clofibrate（荒木・天ケ瀬）と PCB との併用も試みた．一方 PCB の腸内吸収を抑制する薬として cholestyramine が発見されたが (Tanaka & Araki)，これが PCB+DDT+BHC の3者複合汚染に対しても有効な防止薬になるかどうかを試験することにした．

実験方法

Araki & Tanaka の方法に従い，体重 15～20 g の若い雌の CF 1 系マウスを使用し，その 10 匹を 1 群として，hexobarbital-Na 100 mg/kg 腹腔内注射による麻酔時間を測定した．PCB としてはカネクロール 400 を CMC で水性懸濁液に作り，胃ゾンデで経口的に応用した．2 日，7 日，14 日（必要あれば 21 日にも）後に麻酔実験をして，そのときの対照群の麻酔時間に対する百分率で表示し，麻酔短縮がみられた場合は回復までの経過を曲線に描いた．また平均値±標準誤差 (S.E.) から 2 群間の有意差を検定した．

有機水銀としては塩化メチル水銀の水溶液，中性洗剤としては sodium dodecylbenzenesulfonate (ABS) の水溶液，clofibrate, DDT, BHC 等，水に難溶のものは CMC で水性懸濁液とし，いずれも胃ゾンデで経口投与した．

* 現所属：岡山大学医学部附属病院薬剤部

DSW 033184

STLCOPCB4017146

福岡医誌 66(10): 589–592, 1975

PCB の脳内カテコルアミンに及ぼす影響

九州大学医学部薬理学教室（主任 田中潔教授）
末永典子・山田勝士
日高賢志・福田健夫

Influences of PCB on the Brain Catecholamine Levels in Rats

Noriko SUENAGA, Katsushi YAMADA,
Tadashi HIDAKA and Takeo FUKUDA
Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Norepinephrine (NE) levels in the cerebral cortex, brain stem and cerebellum, and dopamine (DA) level in the brain stem were determined fluorometrically in rats treated with PCB (Kaneclor 400). When 100 mg/kg/day of PCB was given to rats for 7 days, NE in the cortex increased and NE in the cerebellum decreased. Methyl mercury chloride in the daily dose of 2～5 mg/kg did not change the NE levels. Combination of PCB with methyl mercury did not potentiate the NE decrease in the cerebellum. In the subacute experiment, in which 20～50 mg/kg of PCB was given twice a week for 7 weeks, a definite and dose-dependent decrease in the cerebellar NE and a slight decrease in the cortical NE were evidenced.

PCB の中枢作用の有無については，現在なお決定的結論を得ていない．油症患者には通常誘発異常を認めないし（永松），明らかな中枢症状を思わせるものはないが，動物実験では電撃けいれん後の昏睡期が延長し（福田ら），坐骨神経刺激による大脳皮質誘発電位の潜時延長が認められる（福田）．しかし動物実験でも脳波に変化はなく，電撃けいれんの経過も変らないし（福田ら），脳内セロトニン量も変化しない（古賀）．

今回はラットに PCB を投与して脳内カテコルアミンを定量し，同時に筋弛緩作用の有無と断頭けいれんへの影響を検して，PCB の中枢作用の有無を判定する新しい資料を提供したいと思う．また先に PCB とメチル水銀の併用が，酵素誘導作用において協力する事実をみたので（荒木ら），今回の実験でも両者の併用を試みることにした．

実験方法

動物は Wistar King A 系ラットの雄を使用した．PCB はカネクロール 400 を CMC で水性懸濁液に作り，メチル水銀は methyl mercury chloride に等モルの cysteine を加えて水溶液とし，いずれも胃ゾンデで経口的に応用した．急性実験は PCB を連続7日間与え，亜急性実験は週2回7週間（計14回）投与した．

筋弛緩作用の試験としては，傾斜板テスト（inclined screen test）を行った．これは板にラットをのせ，次第に傾けて滑り落ちる最小の角度を測定するものである．

断頭けいれん（decapitation convulsion）は，断頭したあとで一定の潜時をもって発現する脊髄性の間代性けいれんで，その潜時とけいれん持続時間（秒）を計測した．

急性実験では薬物応用最終日の午後筋弛緩試験を行い翌日断頭，亜急性実験では最終投与の2日後に筋弛緩試験を行い3日後に断頭した．断頭後素をすばやく取出し，大脳皮質，脳幹，小脳の3部分につき norepinephrine (NE) を，脳幹では dopamine (DA) をも，螢光分光光度計を用いて Shellenberger らの方法により測定した．

DSW 033185

STLCOPCB4017147

Chlorinated Dibenzofurans in Kanechlors and Rice Oils Used by Patients with Yusho

Junya NAGAYAMA[1], Yoshito MASUDA[2] and Masanori KURATSUNE[1]

Department of Public Health, Faculty of Medicine, Kyushu University, Fukuoka, Japan[1] and Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan[2]

It was reported that the causal agent of the epidemic of Yusho which occurred in the Western Japan in 1968 was polychlorinated biphenyls (PCBs)[1]. According to the animal experiments by Vos and his associates marked differences in toxicity were seen among foreign commercial brands of PCBs[2]. They reported that the more toxic brands made in France and West Germany contained 20 and 5 ppm of polychlorinated dibenzofurans (PCDF), respectively, while the less toxic one made in the United States no such contaminants[2]. It seems quite possible that the toxicity of commercial PCBs is greatly affected by their relatively small amount of contaminants such as PCDF[3][4][5]. On the other hand, it has been well known that "chick edema factor", the causal agent of an extensive poisoning of chickens which killed more than a million chicks in the United States in 1957, is polychlorinated dibenzo-p-dioxins (PCDD)[6][7], and that these compounds are extremely toxic and acnegenic to man, too[8][9].

All these facts raise a question whether or not PCDF or PCDD are involved in Yusho in any sense. Thus, it is without doubt needed to investigate the possible contamination by these compounds of Kanechlor-400, a commercial brand of PCBs of Japanese make which is known to have caused Yusho epidemic. In view of the fact that very few studies have been made in this respect, we analyzed various types of Kanechlor and samples of the toxic rice oil used by patients with Yusho for PCDD and PCDF. This paper describes the main results of such analysis.

Materials and Methods

1. Materials analyzed

1) Kanechlor-300, 400, 500, and 600: Kanechlor-300 and -500 were those donated Kanegafuchi Chemical Industry Co., Ltd. in 1968 and Kanechlor-400 and -600 were supplied by the Ministry of Health and Welfare in 1972.

2) Rice oil used by patients with Yusho ("Yusho oil"): 3 samples of rice oil used by 3 independent families with Yusho in 1968. These samples had been kept in glass bottles with glass stoppers at room temperature in our laboratory until 1974 when the analysis was made.

2. Chemicals

1) n-Hexane, ethanol, water, potassium hydroxide, anhydrous sodium sulphate, and silica gel: Purified as described previously[10].

2) Carbon tetrachloride and methylen chloride: Reagent grade, distilled with a fractional distillation column.

3) Activated alumina: Alumina for column-chromatography (about 300 mesh), Wako Pure Chemical Industry, Ltd., activated by heating at 150°C for 12 hours before every use.

4) PCDF and PCDD: Synthesized by chlorination of dibenzofurans or by conden-

DSW 033186

STLCOPCB4017148

sation of 2, 4, 5-trichlorophenol[19]. PCDF consisted mainly of tetra- and penta-chloro dibenzofurans, while PCDD mainly of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin.

5) Octachlorodibenzofuran and octachlorodibenzo-p-dioxin: Purchased from Analabs Inc., U.S.A.

3. Apparatus

1) Gas chromatograph (ECD-GC): Beckman GC 72-5 with a detecter of electron capture type, using a glass column (4 mm×2 m) containing chromosorb W AW DMCS (100-120 mesh) coated with 2% SE-30. Column temperature was 200°C and helium was used as carrier gas, flow rate 30 ml/min.

2) Gas chromatograph-mass spectrometer (GC-MS): Type D-100, GC-MS, Japan Electron Optic Laboratory. Column used was similar to the one used for gas chromatography. Ionization energy was 25eV.

4. Analytical methods

The alkaline decomposition method described in the official standard analytical methods for PCBs established by the Ministry of Health and Welfare was applied for the isolation of PCBs fraction from samples of Yusho oil (5-10g). Isolation of PCDF and PCDD from the PCBs fraction mentioned above or from Kanechlor samples (0.1-0.5 g) was made in the following ways. Each material was placed with 20 ml of n-hexane onto a column (1×7.5 cm) of activated alumina and then eluted with 120 ml of n-hexane containing 20% carbon tetrachloride, 10 ml of n-hexane, and then with 30 ml of n-hexane containing 20% methylenchloride. PCDF and PCDD were expected to be contained in the final eluate. If their separation from PCBs was not adequate, the above chromatographic fractionation was repeated or another similar column chromatography was tried using a shorter column of activated alumina (1×2.5 cm) and a half volume of each of the eluents mentioned above. The eluate consisting of n-hexane containing 20% methylen chloride thus collected was evaporated by a rotary evaporator, then dissolved in n-hexane and subjected to ECD-GC and GC-MS analysis.

Determination of PCDF and PCDD was made by two methods, namely by measuring the gas chromatographic peak heights and by measuring the gas chromatographic peak area after perchlorination of these compounds. The former consists of comparison of each of the peaks of gas chromatogram confirmed as those of PCDF or PCDD by GC-MS with the corresponding one of the synthesized PCDF or PCDD used as standard, assuming that all the PCDF or PCDD have the same peak height sensitivity regardless of the number of chlorine and the site of substitution by chlorine. The latter consisted of chlorination by the BMC reagent[19] of the evaporation residue of the fra tion containing PCDF and PCDD to yield octachlorodibenzofuran and octachlorodibenzo-p-dioxin, followed by the gas chromatographic determination of such octachloro-derivatives.

For determination of PCBs, the peak height calculation method and the perchlorination method, described in the official analytical methods for PCBs set by the Ministry of Health and Welfare, were used.

Results

The gas chromatograms of PCDF fractions separated from Kanechlor-400 and -500 are shown in Fig. 1, while the gas chromatogram of the corresponding fraction from Yusho oil in Fig. 2. Fig. 3 and 4 show the mass-spectra of tetra- and penta-chlorodibenzofurans isolated from these PCDF fractions, together with those of the standard PCDF.

Table 1 summerizes the results of analysis by ECD-GC and GC-MS, indicating that from dichloro to heptachlorodibenzofurans were contained in the samples analyzed. The Kanechlors designated larger numbers, starting from 300 to 600, contained the

DSW 033187

STLCOPCB4017149

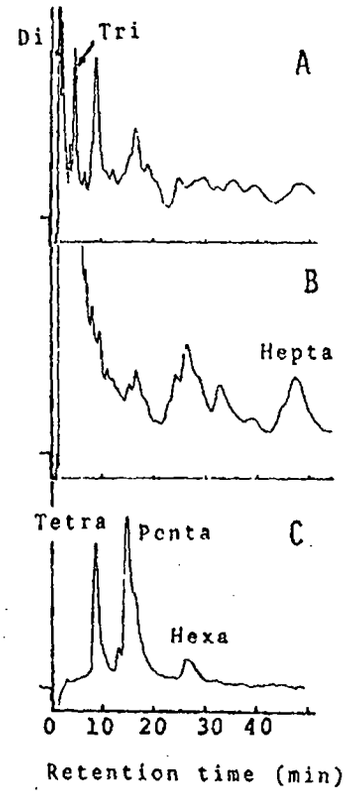

Fig. 1. Gas chromatogram of PCDF fraction
A, Kanechlor-400,
B, Kanechlor-500,
C, Authentic specimen

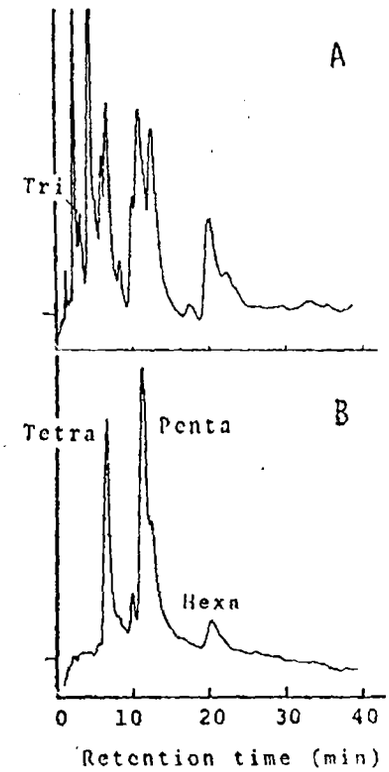

Fig. 2. Gas chromatogram of PCDF fraction,
A, Rice oil ("Yusho oil"),
B, Authentic specimen

Table 1. Concentration of chlorinated dibenzofurans in Kanechlors and "Yusho oil"

Samples		Chlorodibenzofurans						Concentration (ppm)	
		Di	Tri	Tetra	Penta	Hexa	Hepta	a	b
Kanechlors	300			+	+			1	1.5
	400																	18	17
	500				+	|||	+	4	2.5
	600			+	+	||	||	5	3
"Yusho oil"	A		+	||	|||	+		5	4.5
	B		+	||	||	+		4	5
	C		+	||	|||	+		5	5

a: calculated from peak heights, b: calculated by perchlorination method.

higher chlorinated isomers of dibenzofuran. The concentrations of PCDF in Kanechlor-300, -400, -500, and -600 were 1, 18, 4, and 5 ppm by the peak-height calculation method and 1.5, 17, 2.5, and 3 ppm by the perchlorination method, respectively. Thus,

DSW 033188

STLCOPCB4017150

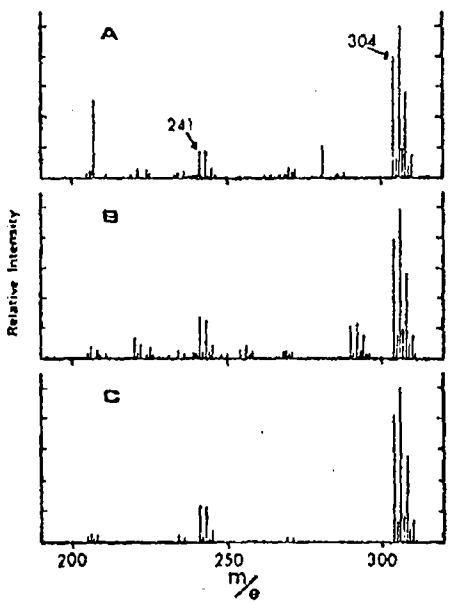

Fig. 3. GC-MS of tetrachlorodibenzofuran.
A. Kanechlor-400,
B. Rice oil ("Yusho oil"),
C. Authentic specimen

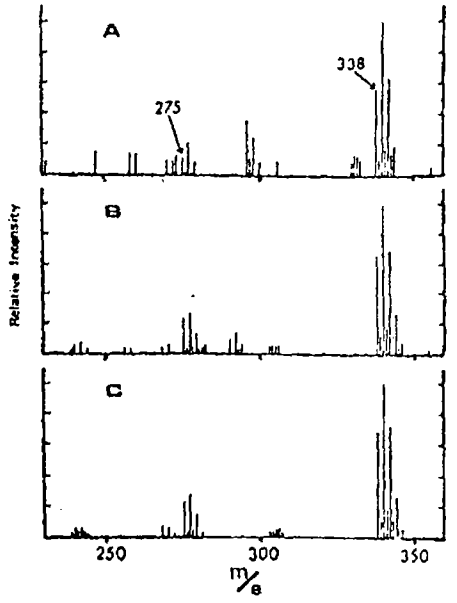

Fig. 4. GC-MS of pentachlorodibenzofuran.
A. Kanechlor-400,
B. Rice oil ("Yusho oil"),
C. Authentic specimen

Table 2. Concentration of PCBs in "Yusho oil"

"Yusho oil"	Concentration (ppm)	
	a	b
A	830	870
B	900	920
C	1,030	980

a: calculated from peak heights
b: calculated by perchlorination method

two methods gave similar results, demonstrating that Kanechlor-400 contains a considerably higher concentration of PCDF than the other types.

Three samples of Yusho oil showed similar gas chromatographic patterns, indicating that the PCDF contained in the oil consisted mainly of tetra- and penta-chlorodibenzofurans together with a small amount of tri- and hexa-chlorodibenzofurans. Both the peak height calculation method and the perchlorination method gave the same figure, 5 ppm of PCDF.

Contrary to the above findings in regard to PCDF, PCDD was not detected in any of the materials examined.

As shown in Table 2, the samples of Yusho oil contained 800-1,000 ppm of PCBs, and both calculations gave very close figures.

In additional experiments, 10 g of a vegetable oil containing 10 mg of Kanechlor-400 was subjected to a series of analytical procedures, the same as those employed in the above analysis, including the alkaline decomposition process maintained for 6 hours.

DSW 033189

STLCOPCB4017151

PCDF found from the oil thus treated was about 0.2 μg, just as much as expected from the PCDF content of 10 mg of Kanechlor-400. Similar blank experiments were repeated 3 times, yielding practically the same results. Therefore, any possible transformation of PCBs to PCDF during the analytical procedures could be denied.

The recovery ratio of 1 μg of PCDF added to 10 g of a vegetable oil was determined to be approximately 90%.

Discussion

Analysis of a trace amount of PCDF and PCDD is generally known to be fairly difficult. As mentioned, it is not conceivable that the PCDF found in our analysis were the artificial products due to the analytical procedures employed. In addition, the recovery ratio was also fairly high. Therefore, we can say with confidence that Kanechlors and "Yusho oil" contain PCDF.

It seems to be noteworthy that the concentrations of PCDF in Kanechlor-400 and in Yusho oil, as determined by the present analysis, are considerably higher than the figures so far reported. Roach *et al.*, found about 1 ppm of PCDF in Kanechlor-400[14]. According to Vos, Bowes, Space Science Laboratory, University of California, could demonstrate the presence of PCDF in Kanechlor samples and in a sample of "Yusho oil". The figures for the formers have not yet been supplied but the figure for the latter, approximately 1 ppm, have been provided us[10]. Thus, the informations so far accumulated do not very precisely coincide with each other, but they agree at least in that both Kanechlors and "Yusho oil" contain PCDF.

Since "Yusho oil" which is known to have been contaminated with Kanechlor-400 contained 5 ppm of PCDF and 1,000 ppm of PCBs, the ratio of the concentration of PCDF to that of PCBs is 5 : 1,000, much larger than the expected ratio 0.018 : 1,000 which can be calculated from our figure concerning PCDF concentration in Kanechlor-400. The reasons for this great discrepancy are not well understood, but it should be noted that the sample of Kanechlor-400 we analyzed was an "unused" one, while the Kanechlor-400 present in "Yusho oil" must be "used" one as heat transfer medium.

Very few studies have been made in which the toxicity of PCDF was directly compared with that of PCBs. Araki observed that PCDF was about 170 times stronger than Kanechlor-400 in the activity of hepatic enzyme induction[15]. Nishizumi and Masuda, however, could not observe any marked difference in acnegenicity between PCBs and PCDF when applied to the skin of the ears of rabbits. The ratio of the concentration of PCBs needed to exert a certain degree of acnegenicity to the corresponding concentration of PCDF needed to exert the same degree of acnegenicity was shown to be 1 : 0.3[15].

There are several studies, however, in which "Yusho oil" and Kanechlor-400 were compared in toxicity. Ikeda reported that "Yusho oil" was twice or more toxic, when fed to broilers, than expected by the concentration of Kanechlor-400 contained in the oil[16][17]. In feeding experiment with CF#1 mice, it was shown that both a diet added "Yusho oil" to attain about 110 ppm of PCBs in the diet and a diet containing 500 ppm of Kanechlor-400 exerted an approximately similar toxicity. The former diet, however, seemed to affect more the skin and liver than the latter and the authors suggested a possibility that "Yusho oil" might contain some chlorinated compounds which were more toxic than Kanechlor-400[18]. In a feeding experiment with chicken, Goto *et al.* observed that a diet containing 5% of "Yusho oil" showed similar toxic effects to those induced by feeding a diet containing 400 ppm of Kanechlor-400. If the concentration of PCBs in the "Yusho oil" used could be assumed to be about 2,000 ppm, the concentration of PCBs in the diet added "Yusho oil" would be about 100 ppm, considerably

DSW 033190

STLCOPCB4017152

lower than that of the diet added Kanechlor-400[19].

Furthermore, there seems to be a great difference in acute toxicity between PCDF and Kanechlors. LD_{50} of Kanechlor-400, when fed to mice and rats, was 1.1-1.9 g/kg and that of Kanechlor-300 0.6 g/kg when fed to rabbits[19]. PCDF, on the contrary, killed rabbits due to severe liver necrosis after a single oral administration of as little as about 1 mg/kg[5,20].

Toxicity of PCBs and possibly of PCDF must greatly vary from isomer to isomer. Rational comparison of toxicities of PCBs and PCDF does not seem to be easy. However, in view of all these past findings described above, PCDF seems to be considerably more toxic than PCBs. The possible role played by PCDF in Yusho should not be dismissed.

Summary

In order to answer the question whether or not polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) which are more toxic than PCBs are involved in causation of Yusho, various types of Kanechlor, PCBs of Japanese make, and 3 samples of "Yusho oil" used by Yusho families were analyzed for these compounds.

Kanechlor-300, -400, -500, and -600 were shown to contain 1, 18, 4, and 5 ppm of PCDF, respectively, as calculated by gas chromatographic peak heights and 1.5, 17, 2.5, and 3 ppm of PCDF, as calculated by the perchlorination method. Thus, Kanechlor -400 was most contaminated with PCDF. "Yusho oil" samples also contained about 5 ppm of PCDF, much higher than expected. PCDD, however, was not detected in any materials analyzed. The possible role of PCDF in causation of Yusho was suggested.

References

1) Tsukamoto, H. et al.: The chemical studies on detection of toxic compounds in the rice bran oils used by the patients of Yusho. Fukuoka acta Med. 60: 496-512, 1969 (In Japanese).

2) Vos, J. G. and Koeman, J. H.: Comparative toxicologic study with PCBs in chickens with special reference to porphyria, edema formation, liver necrosis and tissue residues. Toxicol. Appl. Pharmacol. 17: 656-668, 1970.

3) Vos, J. G., Koeman, J. H., Van der Mass, H. L., Ten Noever de Brauw, M. C. and de Vos, R. H.: Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial PCBs. Food cosmet. Toxicol. 8: 625-633, 1970.

4) Vos, J. G. and Beems, R. B.: Dermal toxicity studies of technical PCBs and fractions thereof in rabbits. Toxicol. Appl. Pharmacol. 19: 617-633, 1971.

5) Vos, J. G. and Notenboom-Ram, E.: Comparative toxicity study of 2,4,5,2',4',5'-hexachlorobiphenyl and PCB mixture in rabbits. Toxicol. Appl. Pharmacol. 23: 563-578, 1972.

6) Cantrell, J. S., Webb, N. C. and Mabis, A. J.: Identification and crystal structure of a hydropericardium producing factor. Chem. Eng. News. 45: 10, 1967.

7) Firestone, D., Flick, D. F., Ress, J. and Higginbotham, G. R.: Distribution of chick edema factor in chick tissues. J. Ass. offic. Anal. Chem. 54: 1293-1298, 1971.

8) Bauer, H., Schulz, K. H. und Spiegelberg, U.: Berufliche Vergiftungen bei der Herstellung von Chlorphenol-Verbindungen. Arch. Gewerbepath. Gewerbehyg. 18: 538-555, 1961.

9) Oliver, R. M.: Toxic effects of 2,3,7,8 tetrachlorodibenzo 1,4 dioxin in laboratory workers. Br. J. Ind. Med. 32: 49-53, 1975.

10) Roach, J. A. G. and Pomerantz, I. H.: The finding of chlorinated dibenzofurans in a Japanese PCB sample. Bull. Environ. Contam. Toxicol. 12: 338-342, 1974.

11) Masuda, Y., Kagawa, R. and Kuratsuno, M.: Polychlorinated biphenyls in Yusho patients and ordinary persons. Fukuoka Acta Med. 65: 17-24, 1974 (In Japanese).

12) Nishizumi, M., Masuda, Y. and Kuratsuno, M.: Comparison of hyperkeratosis induced by PCBs, PCDF and PCDD application. Fukuoka Acta Med. 66: 600-604, 1965 (In Japanese).

DSW 033191

STLCOPCB4017153

13) Hutzinger, O., Safe, S. and Zitko, V.: Analysis of chlorinated aromatic hydrocarbons by exhaustive chlorination: Qualitative and structural aspects of the perchloroderivatives of biphenyl, naphthalene, terphenyl, dibenzofuran, dibenzodioxin and DDE. Intern. J. Environ. Anal. Chem. 2: 95-106, 1972.
14) Vos, J. G.: From private communication, 1974.
15) Araki, Y.: Comparison of enzyme-inducing activities of chlorinated dibenzofuran and chlorinated dibenzodioxin. Fukuoka Acta Med. 65: 61-64, 1974 (In Japanese).
16) National Hygienic Laboratory, Ministry of Health and Welfare: Studies on toxic substances in rice-bran oil, with special reference to organochlorine compounds. (5) Studies on toxicity of noxious substances. Special studies on prevention, diagnosis and treatment of Yusho.: 74-124, 1969 (In Japanese).
17) Ikeda, Y.: Toxicity of PCB. J. Food Hyg. Soc. Japan. 13: 359-367, 1972 (In Japanese).
18) Livestock Hygiene Experiment Station, Ministry of Agriculture and Forestry: Studies on toxic substances in rice-bran oil, with special reference to organochlorine compounds. (5) Studies on toxicity of noxious substances, Special studies on prevention, diagnosis and treatment of Yusho.: 74-124, 1969 (In Japanese).
19) Goto, M., Sakaguchi, K. and Ogawa, K.: Hydropericardium assay of rice oil which caused Yusho and of Kanechlor 400 in chickens. Fukuoka Acta Med. 60: 533-538, 1969 (In Japanese).
20) Hofmann, H. Th.: Neuere Erfahrungen mit hochtoxischen Chlorkohlenwasserstoffen Arch. exp. Pathol. Pharmakol. 232: 228-230, 1958.

(和文抄録)

カネクロールおよび油症患者使用ライスオイル中の塩化ジベンゾフラン

九州大学医学部公衆衛生学教室[1]
第一薬科大学物理分析学教室[2]
長山淳哉[1]・増田錡人[2]
倉恒匡徳[1]

油症の原因に，PCB より毒性の強い塩化ジベンゾフラン（PCDF）あるいは塩化ジベンゾジオキシン（PCDD）が関与しているのではないかという疑問に答えるため，カネクロールおよび油症患者使用ライスオイルから PCDF および PCDD の分離，定量を行つた．

カネクロール 300，400，500 および 600 からピーク高法により，それぞれ 1，18，4 および 5 ppm の，また全クロル化法により，それぞれ 1.5，17，2.5 および 3 ppm の PCDF を検出した．カネクロール 400 の PCDF 濃度が最も高かつた．また油症患者使用ライスオイルからは 5 ppm の PCDF が検出された．しかし PCDD はいずれの検体からも検出されなかつた．

今回分析したライスオイル中の PCB 濃度は 500～1,000 ppm であり，今までいわれていた 2,000～3,000 ppm にくらべるとかなり低い濃度であつた．

PCDF の毒性の評価は，はつきりしていないけれども，油症患者使用ライスオイル中の PCB がカネクロール 400 よりかなり毒性が強いという報告があり，この PCDF が油症発症にかなり関与している可能性がある．

DSW 033192

STLCOPCB4017154

福岡医誌 66(10): 600–604, 1975

Polychlorinated Biphenyls, Polychlorinated Dibenzofuran および Polychlorinated Dibenzodioxin のウサギ耳塗布による過角化作用の比較

九州大学医学部公衆衛生学講座
西住昌裕・倉恒匡徳
第一薬科大学物理分析学教室
増田義人

Comparison of Hyperkeratosis Induced by PCBs, PCDF and PCDD Application

Masahiro NISHIZUMI and Masanori KURATSUNE
Department of Public Health, Faculty of Medicine, Kyushu University, Fukuoka
Yoshito MASUDA
Daiichi College of Pharmaceutical Sciences, Fukuoka

Polychlorinated biphenyls (Kanechlor 400, a commercial preparation), polychlorinated dibenzofuran (a mixture of tetra- and penta-chloro-dibenzofuran) or polychlorinated dibenzodioxin (2, 3, 7, 8-tetrachloro-dibenzodioxin) was dissolved into acetone in different concentrations, and was applied on the inside of depilated rabbit's ear for consecutive 3 days. The redness on the local skin was followed by thickening of the skin within 1 to 2 weeks.

Judging from the extent of hyperkeratosis induced by these three compounds, PCDD is approximately 1,000 times stronger than PCBs or PCDF as an acnegen.

Polychlorinated Dibenzofuran (PCDF) および Polychlorinated Dibenzodioxin (PCDD) の皮膚に対する毒性として，毛漏性角化や座瘡様皮疹の出現はすでに10数年前に報告されているが[1][2]，これらの化合物が化学構造上 Polychlorinated Biphenyls (PCBs) に類似しており，PCBs 製剤中にも不純物として PCDF の存在を確認した報告もある[3][4] ことから，油症の場合 PCBs による皮膚症状発現にこれらの化合物が関与している可能性を指摘する人もある．ただ，油症の原因物質とされている Kanechlor 400 中には微量の PCDF の存在は確認されている[4]が，PCDD の存在は現在までのところ報告されていない．

著者らは，PCDF および PCDD を合成し，PCBs と共にこれら化合物の皮膚に対する作用強度を比較し，これらが混在した場合の皮膚症状発現への影響の程度を推定する1つの手がかりとするため，この実験を行った．

材料と方法

(1) PCDF の合成

Dibenzofuran (東京化成) 5g，塩化第2鉄 0.5g ヨウ素 0.5g および Chloroform 100 ml の混合溶液に，濃塩酸および過マンガン酸カリウムより発生させ濃硫酸で乾燥させた塩素ガスを通じた．塩素化は室温で2.5時間，水浴で加熱しながら5時間行った．反応終了後すべての固型物を Chloroform により集め，溶媒を留去すれば 11g あった．風乾し，ヨウ素を揮発させた後，1部を Florisil column で n-Hexane および Benzene を用いて精製することにより無色の結晶が得られた (mp 180～195°)．この結晶を GC-MS (日立 PMU-6MG) で測定すると，Fig. 1 (左)

DSW 033193

STLCOPCB4017155

PCB 汚染油を摂取した母親から生まれた油症児の血漿中 PCB について

久留米大学医学部環境衛生学教室
阿部純子・井上義人
高松誠

Polychlorinated Biphenyl Residues in Plasma of Yusho Children Born to Mothers Who Had Consumed Oil Contaminated by PCB

Sumiko ABE, Yoshito INOUE and Makoto TAKAMATSU

Department of Environmental Health, Kurume University School of Medicine, Kurume, Japan

Medical examination and analysis of PCB in plasma were performed on 30 children who were born in the period of February 1967 to December 1973, and on 18 mothers who had taken oil contaminated by PCB in Tamanoura-cho of Nagasaki-ken. Plasma samples were also obtained from 11 ordinary children in Miiraku-cho.

The average PCB concentrations in plasma of Yusho children, mothers and ordinary children were 6.0 ppb, 10.8 ppb and 3.7 ppb, respectively. The average PCB concentration in plasma of Yusho children who had been fed on mother's milk in infancy was higher than that of children fed on none of mother's milk.

Gaschromatograms of PCB in plasma of Yusho children and mothers had a peculiar pattern in most cases.

Though the skin symptoms of the Yusho children had subsided, they still complained of atypical symptoms such as easily getting cold, diarrhea, physical dullness and abdominal pain.

吉村[1]は昭和47年5月に長崎県玉之浦町の油症患者家族108世帯のなかから，PCB に汚染したカネミ油を摂取した母親から生まれた児22名についての疫学的調査を行っている．そして彼は乳児の油症患者は経母乳油症児と経胎盤油症児に分類されること，その可能性のある小児については充分な臨床観察とともに，今後の患者の健康管理を中心とする継続的観察が望まれるといっている．

玉之浦町にはカトリックを信仰する地区があり，宗教の戒律によって堕胎が禁じられているので，特に玉之浦町では油症児の出生が多かったものと思われ，疫学的の注目をあびることになった．筆者らも昭和49年度中3回にわたり玉之浦町に赴き，町民の協力をえて，油症患者の精密検査を行うとともに，血漿中 PCB 値を分析することができた．

本論文ではその成績のなかから，カネミ油症の母親と，その母親から生まれた油症児と推定される小児について分析した血漿 PCB 値を中心にして，疫学的問題点をみるとともに検査時の所見とを併せて報告したい．

調査方法

玉之浦町のカネミ油症患者については，この町の患者の会と患者を守る会とで精密な調査に基づく患者名簿ができている．この名簿によって昭和49年1月と7月とにカネミ油症患者約500名の精密検査を行い，血液の提供をうけて血中 PCB 値を分析することができた．そしてこの名簿によって PCB に汚染したカネミ油を摂取した母から生まれた児を選びだし，その家庭を訪問して諸々の情報を聞きだし油症母子家系の実

DSW 033194

STLCOPCB4017156

福岡医誌 65(10): 610—616, 1975

血漿中 PCB ならびに DDT, BHC とそれらの組織内残留との関係

久留米大学医学部環境衛生学教室
井上義人・阿部純子・高松　誠
京都市堀川病院
青木信雄・三木昭作
京都市衛生研究所
藤原邦達

PCB, DDT and BHC Levels in Human Plasma as a Measurement of Tissue Residue

Yoshito INOUE, Sumiko ABE and Makoto TAKAMATSU
Department of Environmental Health, School of Medicine, Kurume University, Kurume
Nobuo AOKI and Shosaku MIKI
Horikawa Hospital, Kyoto
Kunisato FUJIWARA
Kyoto City Institute of Public Health, Kyoto

Polychlorinated biphenyls (PCB) in plasma has been used for diagnosis of Yusho (PCB poisoning) since 1974. But it was not obvious that PCB in plasma reflected the total residue in human body. This study was performed to this relation.

Pair samples of plasma and adipose tissue were obtained from 33 hospital patients at the time of operation. And the other samples—blood samples (21), subcutaneous fat tissue (2), liver tissue (1), and kidney tissue (1) were obtained from 21 persons. The PCB concentrations in plasma and adipose tissue, and the patterns of gaschromatograms were examined. At the same time β-BHC, pp'-DDE, pp'-DDT were measured.

(1) It was observed that PCB, β-BHC, pp'-DDE in plasma reflected the residual condition of these compounds in adipose tissue respectively.

(2) The concetrations of these compounds of the emaciated group were higher than those of non-emaciated group.

(3) The PCB gaschromatograms patterns varied with the type of PCB exposure.

(4) It was found that two cases had very high PCB concentrations in plasma (230 ppb, 92 ppb).

血中 PCB の量と質との分析は，油症患者の診断や追跡調査に極めて有用な示唆となることが認められて，昭和49年3月の油症診断要項にも取り上げられ，広く使用されることになつた．しかし血中 PCB による把握は，血液についての現象を見ているのであつて，組織内に残留している PCB の蓄積を考える場合，血中濃度によつてそれが適確に把握できるという確信は得られていなかつた．

すでに有機塩素系殺菌については，血中濃度と組織中濃度との間には相関があることが認められているので[7][10][13][19]，PCB についても同じような関係があるかどうかをみて，血液値によつて，PCB の体脂肪中

DSW 033195

STLCOPCB4017157

油症患者における血清酵素化学的異常とくに RNA 分解酵素を中心に

九州大学医学部第二内科学教室（主任 尾前照雄教授）
山中正義・赤木公博
平尾修恭・村井宏一郎

Abnormality of Serum Enzyme in PCB Poisoning Patients with Special Reference to Ribonuclease

Masayoshi YAMANAKA, Kimihiro AKAGI,
Nobuyuki HIRAO and Koichiro MURAI
Second Department of Internal Medicine, Faculty of Medicine, Kyushu University Fukuoka 812, Japan

Serological enzyme analysis was performed on 101 sera of patients with PCB poisoning, including RNase, GOT, LDH and alkaline phosphatase. The means±SD of RNase activity in young (under 40 yr) and old (above 41 yr) patients were 194±51 and 220±50, whereas those in each healthy control group were 142±26 and 162±39, respectively. The enzyme levels in both groups of PCB poisoning were highly significant statistically compared with those of normal subjects ($p<0.005$). The respective abnormal elevation of GOT, LDH and alkaline phosphatase was observed in 6.4, 7.3 and 9.0% of the cases examined. The mean values of abnormal GOT, LDH and alkaline phosphatase activities were 64.8±41.3 (mU/ml), 254±41.5 (mU/ml) and 169±27.9 (mU/ml), respectively. Electrophoretic analysis of alkaline phosphatase on agar gel indicated a single active band of hepatic origin.

油症患者の血清酵素化学的異常として，GOT, LDH, アルカリ性フォスファターゼの上昇が報告されているが[1]，その上昇はいずれも軽度で，大部分の患者では正常範囲にとどまっている．肝臓の光顕的組織所見でも，特別な異常はみられていない[7]．奥村らは[13]，油症重症患者に血清鉄の低下と銅の上昇を観察し，本症の消耗性疾患としての可能性を示唆している．

そこでわれわれは，白血病[8,9,10]，癌[3,6,19]，腎不全[4,8,10]などの消耗性疾患で血清 RNA 分解酵素（以下 RNase）が著明に上昇する知見に基づき，油症患者で本酵素活性を測定し，さらに血清 GOT, LDH およびアルカリ性フォスファターゼ活性についても検討を加えた．

対象ならびに方法

対象は昭和49年1月に行われた油症追跡調査受診者のうち，血清 RNase を測定した101症例（福岡地区：61人，北九州地区：17人，久留米地区：11人，大牟田地区：9人，田川地区：3人）である．対照として，九州大学職員およびその家族から採血を行った．

血清 RNase の測定は，Anfinsen ら[9]の方法を改良して使用した．基質としてイースト RNA (Sigma 社) を，50 mM トリス塩酸緩衝液 (pH 8.5) で3日間氷冷中で透析し，0.5%溶液として用いた．反応は RNA 溶液 1 ml に血清 50 μl を加え，37°C, 30分間インキュベートしたあと，0.5%酢酸ウラニルを含む3%過塩素酸を 0.2 ml を加えて反応をとめた．氷冷中に10分間放置してから，2,000×g で5分間遠心後，その上清 0.2 ml を 3 ml の蒸溜水で稀釈し，石英セルで 260 nm の吸光度を測定した．この条件下で血清 RNase は，A_{260nm} が 0.45 まで直線的に RNA を水解する．

DSW 033196

STLCOPCB4017158

福岡医誌 66(10): 620—623, 1975

油症(PCB中毒)患者における血清トリグリセライドの6年間の推移

福岡大学医学部第一内科学教室(主任 奥村 恂教授)
奥 村 恂
九州大学医学部第二内科学教室(主任 鬼頭昭進教授)
山 中 正 義・中牟田 澄 子
熊本大学体質医学研究所成人体質学研究部(主任 鵜沢春生教授)
鵜 沢 春 生

Consecutive Six Year Follow-up Study on Serum Triglyceride Levels in Patients with PCB Poisoning

Makoto OKUMURA
First Department of Internal Medicine, Fukuoka University Medical School
Masayoshi YAMANAKA, Sumiko NAKAMUTA
Second Depattment of Internal Medicine, Faculty of Medicine, Kyushu University
Haruo UZAWA
Department of Geriatrics, Institute of Constitutional Medicine, Kumamoto University, Japan.

Serum triglyceride (TG) concentrations of 40 subjects (14 males and 26 females) with PCB poisoning were determined at least once a year for 6 years successively from the onset of poisoning in 1969. The determination of TG was carried out under the supervision of cooperative triglyceride standardization program, Georgia, U. S. A. Total number of determinations of serum TG was 219 in 1969, 166 in 1970, 206 in 1972, 75 in 1973, and 46 in 1974, respectively.

In male subjects, the mean values and standard deviations of the results(mg/100 ml) were 159±57 in 1969, 166±55 in 1970, 169±60 in 1971, 174±69 in 1972, 164±68 in 1973, and 160±118 in 1974. No significant change was obesrved in these values for 6 year follow-up study. Contrarily, in female subjects, values obtained in these 6 years were 155±75, 161±70, 155±80, 153±63, 129±50, and 111±56, respectively. The differences of mean vaiues between 1973 or 1974 and those of 1969 to 1972 were found to be statistically significant. It was suggested that serum TG levels of female subjects with PCB poisoning are decreasing gradually, though those of males still remain in elevated levels through out the term of this study. The mechanism underlying these sex differences is yet to be elucidated.

昭和43年に九州北部を中心に集団発生をみた，いわゆる油症(塩化ビフェニールあるいはPCB中毒)も発生以来すでに7年を経過した．本中毒患者は定型的な皮膚症状を伴って発症し，多彩な症状がある．その後の経過とともに皮膚症状は軽快しつつある印象を受けるが，なおいくつかの愁訴は持続しているものも多い．検査所見では当初から血清中の高グリセライドが注目され[1][2][3]，3年間の観察ではほとんど低下傾向がみられないことを先に報告した[4]．

今回は，油症患者のうち発生以来6年にわたり，少

DSW 033197

STLCOPCB4017159

福岡医誌 66(10): 624—628, 1975

九州大学附属病院油症外来患者の血中 PCB と臨床症状との関係

九州大学医学部皮膚科学教室（主任：占部治邦教授）
幸田弘
第一薬科大学物理分析学教室（主任教授）
増田義人

Relation between PCB Level in the Blood and Clinical Symptoms of Yusho Patients

Hiromu KŌDA
Department of Dermatology (Director: Prof. H. Urabe), Faculty of Medicine, Kyushu University, Fukuoka, Japan
Yoshio MASUDA
Dailchi College of Pharmaceutical Sciences, Fukuoka

Blood PCB levels and clinical symptoms were studied in 72 patients with Yusho who had visited at the outpatient department of Kyushu University during April 1973 to March 1974. Blood PCB was analysed with gas chromatography.

Patterns of PCB on gas chromatogram were classified in three types, type A and B which were peculiar to Yusho patients and type C which showed similar pattern to controls. The mean value of blood PCB levels of 72 patients was 5.9±4.5 S. D. ppb. The mean ± S. D. of PCB levels of type A group composed of 43 patients (59.7%) was 7.2±4.9 ppb, that of type B group composed of 26 patients (36.1%) was 4.3±3.1 ppb, whereas that of type C group composed of 3 patients (4.2%) was 1.7±02 ppb.

Clinically, dermatological symptoms were mostly observed in type A group. Type C group hardly showed such symptoms. General signs such as fatigue and headache were noticed on patients with any types of PCB pattern. Same type of PCB pattern in all of the family members was observed in 15 families out of 21 families.

われわれは，昭和48年4月より九大附属病院油症外来患者の血中 PCB の測定を行っているが，その中の油症認定患者について，血中 PCB 濃度と臨床症状とくに皮膚症状との関連を調査し，一応まとめることができたので報告する．

血中 PCB 濃度測定

（1）対　象

昭和48年4月より1年間に九州大学附属病院油症外来を受診した油症認定患者の72名である．なお油症を心配して来院した9名（非油症患者）についても測定したので，その結果も附記する．

（2）材　料

PCB 分析用にとくに洗浄したガラス製注射器および瓶を用いて採血し，その血液 10 g を使用した．

（3）分析方法

分析方法の詳細については増田ら[1]がすでに報告しているので，それにゆずり，ここでは大略を記するにとどめる．

採血した全血 10 g に 10 ml のエタノールおよび水酸化ナトリウム 2 g を加え，1時間加熱する．冷却後ヘキサン 10 ml で3回抽出し，ヘキサン層を集め，芒硝で乾燥後，シリカゲル 1 g のカラムを通す．カラムはさらにヘキサン 50 ml で溶出，さきのヘキサンと合して濃縮し，ガスクロマトグラフ（電子捕獲型検出器付）で分析する．カラムは5% SE-30 を使用した．PCB はカネクロール 500+600（1：1）を標準物質とし，全ピーク高の和により定量した．

DSW 033198

STLCOPCB4017160

福岡医誌 66(10): 629–634, 1975

昭和48年度，49年度一斉検診における油症皮膚重症度の変動と新しい皮膚重症度評価試案

九州大学医学部皮膚科教室（主任：占部治邦教授）
旭　正一・幸田　弘
福岡大学医学部皮膚科教室（主任：利谷昭治教授）
利谷　昭治

Alteration in Skin Severity Grading of Yusho in the General Examination in 1973 and 1974, and Presentation of a New Standard for the Skin Severity of Yusho by Point Count System

Masakazu ASAHI, Hiromu KŌDA
Department of Dermatology (Director: Prof. H. Urabe) Faculty of Medicine, Kyushu University, Fukuoka, Japan
Shoji TOSHITANI
Department of Dermatology (Director: Prof. S. Toshitani) Faculty of Medicine, Fukuoka University, Fukuoka, Japan

Alteration in skin severity grading index of Yusho was investigated by analysing the results of the general examination of Yusho in 1973 and 1974. In the 1973 examination, almost no change was found in the skin severity index and in the 1974 examination, this time about half number of the whole patient showed improvement more or less.

Some problems in the standard for skin severity grading of Yusho was discussed and the new tentative standard for the skin severity was presented. This new standard was characterized by its quantitative nature (point count system).

油症が発生してすでに7年近くが経過した．この間に油症の皮疹がどのように経過してきたかについては，すでに4回にわたり本誌に報告してきたが[1)~4)]，今回はこれに続いて昭和48年度，49年度の油症追跡調査の結果をまとめ，皮膚重症度の推移について考察するとともに，従来の皮膚重症度の評価基準の問題点をとりあげ，一つの改良案として，定量的表現の意味をもたせるように考慮した新しい点数方式の評価試案をのべる．

はじめに油症追跡調査（一斉検診）について，その施行年次などに分りにくい点があるので，これまでの実施状況を簡単に記すと，表1にみられるように，油症発生の昭和43年の翌年から始まり，年1回の割合ですでに6回行なわれているが，47年度以降は施行が半年遅れて，翌年の1月に行ったかたちとなっている．このため実施時期と表示年度に1年のずれが生じていて誤解しやすいが，ここでは県衛生部の通達に従い，公式の表示年度で示すことにした．

本来この検診は，油症発生後間もないころに，急死した症例が続いたりしたため[2)]，患者全員を検診して危険の予測される症例には至急に対策を講じようという目的で開始されたものであるが[3)]，その後急性中毒期をすぎて慢性中毒期に移行するにつれて，そのような緊急対策の意味は薄らぎ，患者の方の受診意欲が低下したこと，患者の一部に検診に対する強い不満が生じ受診拒否の動きが出てきたため，表1にみるように受診者数が47年度まで次第に減少してきた．48，49年度は再び増加する傾向にあるが，このように患

DSW 033199

STLCOPCB4017161

福岡医誌 66(10): 635—639, 1975

油症患者の下垂体前葉機能

九州大学医学部産婦人科学教室（主任：滝一郎教授）
楠田雅彦・永田行博
中村正彦

Anterior Pituitary Function of "YUSHO" Patients (Polychrolinated Biphenyls Poisoning)

Masahiko KUSUDA, Yukihiro NAGATA
and Masahiko NAKAMURA

Department of Obstetrics and Gynecology (Director: Prof. I. Taki)
Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Twelve patients, consisting of 9 women and 3 men who had been suffering from "YUSHO" (polychlorinated biphenyls poisoning), were subjected to this study.

Anterior pituitary function was examined with regard to basal levels of gonadotropins and its response to synthetic LH-Releasing Hormone in total number of 16 times. One hundred mcg. of LH-RH was administered intravenously, blood was drawn just before the injection and 15, 30, 60, and 120 minutes after that. Serum specimens were kept at −20°C until assayed. All samples from the same patient were assayed with the same radioimmunoassay system.

Criterion on pituitary response to LH-RH was settled as indicated in Table 1. Results obtained were listed in Table 2-B and Table 3-B and these were compared with the control groups (Table 2-A, 3-A). In 10 cases out of 11 patients normal basal levels of LH and FSH were noted, and pituitary response to LH-RH also revealed "good response" in each phases of menstrual cycles, postmenopausal women, a castrated woman, and men, respectively. One patient in which the test was performed in preovulatory phase showed rather high basal level and strong response. However, in midfollicular phase it showed normal "good response". Although pituitary response of "YUSHO" patients seems high in normal range, any decisions could not be drawn, because criteria on "abnormally strong response" have not been established as yet.

From these results it can be mentiond: anterior pituitary lobe function concerning with gonadotroph is not deteriorated at least. Consequently, the cause of abnormality of sexual function might be pursued in hypothalamus or peripheral steroid metabolism.

著者はさきに，油症婦人の性機能について検索し，月経周期の乱れ，月経血の量的，質的変化，持続や間隔の異常等が高率に認められること，また一部婦人においては尿中諸ホルモンの濃度や排泄量にも異常がみられることを報告し，油症婦人では何らかの機序で性機能が障害されていることを推定した[1]．その後，油症患者の下垂体前葉機能をゴナドトロピン産生・放出能の面から検索する機会を得たので，これまでの成績について報告する．

研究対象ならびに方法

九大病院油症外来に通院する患者の中，あらゆる面から診断が確定している女子9名，男子2名について，のべ16回の合成 LH-RH (LRF または Gn-RH) 負荷による下垂体前葉刺激試験を施行し，正常男女の対照群と比較検討した．なお女子の2例は閉経後婦人，1例は子宮頸癌根治手術後3年目の婦人である．6名の性周期を有する婦人に対しては基礎体温の測定を依頼し，その体温表から，測定していない婦人

DSW 033200

STLCOPCB4017162

福岡医誌 66(10): 640—641, 1975

油症患者の眼症状(第3報)

九州大学医学部眼科学教室
大西克尚・生井浩
北九州市立小倉病院眼科
栗本晋二
久留米大学医学部眼科学教室
川島謙一郎

Further Ophthalmic Studies on Patients of Chronic Chlorobiphenyls Poisoning ("Yusho")

Yoshitaka OHNISHI, Hiroshi IKUI, Shinzi KURIMOTO and Kenichiro KAWASHIMA

Department of Ophthalmology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Since 1968 many patients have been registered as chronic chlorobiphenyls poisoning in the western part of Japan. One hundred and forty-seven registered patients (male 67 and female 80) of the disease were re-examined for follow-up study on January, 1975. Hypersecretion of the Meibomian gland and abnormal pigmentation of the conjunctiva, which are main ocular signs of the disease, have been improved.

昭和43年6月以来，西日本一帯に発生した油症患者の眼症状についてはさきに報告[1][2]したが，その後も，随時あるいは一斉検診において，症状の把握と治療に努めてきた．

昭和50年1月27日から5日間にわたり，厚生省委託による昭和49年度油症患者の一斉検診（追跡調査）を，九州大学附属病院，久留米大学附属病院および北九州市立休日急患診療センターで行つた．その際受診した患者175名中，未認定者を除外した147名についての眼所見について報告する．

1．対　象

集計の対象とした147名の油症患者の年令および性別分布は表1に示した通りである．

2．油症患者の自覚症状

検診の際，受診者に問診票を配り，自覚症状を記入させ，また診察の際にその訴えを聞き，自覚症状の把握に努めた．眼科的な自覚症状がないものは皆無に等しく，例えば眼脂を例にとつてみると表2のようになる．眼脂のほかに「眼が痛くなる」，「時々涙が出る」などの愁訴が多かつた．また特徴的なのは，「眼やにが出て，眼に膜を張つたようになり，新聞や本が読みにくくなる」という訴えであつた．

表 1. 年令別・性別患者数

年　令	0～10	11～20	21～30	31～40	41～50	51～60	61～	計
男	8	8	7	7	19	10	8	67
女	4	10	12	14	19	19	2	80
計	12	18	19	21	38	29	10	147

DSW 033201

STLCOPCB4017163

福岡医誌 66(10): 642—645, 1975

油症患者の眼所見およびPCB投与ラットの眼瞼結膜の組織所見

久留米大学医学部眼科学教室（主任：吉岡久春教授）
青木昭彦

Ocular Findings of Chlorobiphenyls Intoxication and Histological Changes of the Palpebral Conjunctiva in Rats Fed with Kaneclor 500

Akihiko Aoki
Department of Ophthalmology, Kurume University School of Medicine

Light and electron microscopic observations of the palpebral conjunctiva including the fornix in rats, fed with Kaneclor 500 in the dose of 50 mg/kg 4 times a week, were carried out. Light microscopically, marked lymphocytic and plasmacytic infiltrations were recognized in the submucosal area, and slightly in the Meibomian gland which contained condensed secretion.

Electron microscopically, no abnormal findings could be seen in the pigment epithelium and in the visual cells of the retina. In the palpebral conjunctiva, an increase of melanin-like pigment was recognized.

昭和43年西日本を中心として，家族的に集団発生した油症は，ライスオイルの製造中に塩化ビフェニール混入による中毒と判名してから，やがて7年を経過している．今回久留米大学眼科教室も福岡県衛生部の依頼で油症患者の追跡調査のため検診を行つた．本症の臨床的特徴は，関係各科より報告されているが，九大油症治療研究班の診断基準にもとづいて，眼所見についてのみ検討を行つた．また一方，実験的にラットにカネクロール400より毒性の強いといわれているカネクロール500を投与し，円蓋部を含めた眼瞼結膜を試料として，光顕および電顕観察を行つたので報告する．

油症患者の眼症状

昭和49年8月および昭和50年1月に，油症の未認定，認定患者の検診を行つたが，今回は久留米，大牟田地区に在住する油症認定患者で，昭和50年1月に検診を行つた患者25名についてのみ報告する．25名のうちわけは，男12名，女13名で，年令は，6才より58才までであつた．

眼検査所見としては，①眼瞼結膜色素沈着，②マイボーム腺肥大，③眼脂過多，④眼瞼浮腫について検討し，その程度が高度なもの（++），強度なもの（+），かすかにある（±），なし（−）と分類した．

検診結果をまとめると表1となるが，高度なもの（++）の患者は1名も見られなかつた．

ラットにおける実験

体重85～105 g のラット7匹に，カネクロール500を50 mg/kg の割合で1週中に4回，強制的に経口投与し，合成固形飼料で4週間飼育後に断頭した．円蓋部を含む眼瞼結膜を採取し，光顕用試料を作成しHE染色を行つた．また1部はLufft法に従つて電顕用試料を作成し，日本電子T7S電顕で観察した．

光顕的には，眼瞼結膜の粘膜下にびまん性のリンパ球とプラズマ細胞を主体とする細胞浸潤が高度に見られる．また近傍のマイボーム腺にも軽度のリンパ球浸潤を認め，1部腺腔内にも褐色色素の沈着を認めた（図1，2）．

電顕による観察では，眼瞼結膜の表皮下に多数のメラニン顆粒が細胞質中にびまん性に存在していた．また散在性に直径2～3 μ程度の不定形な dense body

DSW 033202

STLCOPCB4017164

福岡医誌 66(10): 646—648, 1975

絶食療法を実施した油症患者の追跡調査

兵庫県津名郡（淡路島）五色町都志診療所
今村基雄

A Follow-up Study on Fasting Therapy of Yusho Patients

Motoo IMAMURA
Tsushi Clinic, Goshikicho, Tsunagun, Hyogoken

Thirty three patients with Yusho, who had sumitted to a fasting therapy more than once, were followed up. Beneficial effects of the therapy were still confirmed in 79 percent of the patients 0.3～4.6 years after leaving hospital. The mechanism responsible for the symptomatic relief resulted from this this therapy was discussed.

私は1970年10月から1975年7月までに34名の油症患者に絶食療法を実施した．その成績はすでに2回発表したが[1][2]，その効果が認められて1972年10月には厚生省の「油症治療指針」に正式採用された．

今回は1975年2月までに退院した33名について，退院時の成績と退院後4カ月～4年8か月経過した時点における成績を追跡調査し，両者を比較してその推移を観察した．

1．対　象

（1）患者の年令，性別，地域

年令　16～67才

性別　男性　14名，女性　19名

地域　福岡県　25名
　　長崎県　3名
　　広島県　3名
　　岡山県　2名

（2）絶食日数

8日　1名
10日　16名
12日　10名
14日　6名

（3）絶食の回数

1回　24名
2回　8名
3回　1名

2．絶食の方法と心身の変化

私の方法を簡単に述べる．

1）準備期　絶食前1～2日間はカユ食．

2）絶食期の第1日目に下剤を与え，その後も2～3日目ごとに下剤または完腸で排便をはかる．

3）絶食期の初め2日間は水だけ1日量1500～2000 ml与える．

4）絶食期の3日目よりジュース（数種の生野菜と果物の絞り汁）を200 mlずつ午前，午後2回与える（緩法）．

5）年令，体力，併発症等を考慮して絶食5～6日目より更に牛乳を朝，夕200 mlずつ与えることもある（緩法）．

6）緩法20名，緩法13名であつた．

7）復食期になるとオモユ，三分ガユ，五分ガユ，全ガユ，常食と2日ごとに上がつていく．常食は1日量約1800～2000 Calくらいである．

8）全期を通じて体力に応じた軽い運動（散歩，釣，その他）を励行させる．

9）絶食者の80%くらいは，絶食初日の夜から2日目にかけて脱力感，軽い吐き気，頭重を訴えるが，3～4日目になれば気分良好となる．

10）油症患者は，絶食3，4日ごろまでは激烈な頭痛発作を訴える者があるので，その都度，堪えられぬほどの激痛の場合はパピナールアトロピン，ノブロンA等の鎮痛剤の注射を行つた（33名中3名）．

11）水分補給，栄養補給のための注射による補液はやらない．

3．症状好転の時期と程度

1）全身症状（全身倦怠感，頭痛，頭重，不定の腹

DSW 033203

STLCOPCB4017165