Model description

Compartment model

A compartment model was used to describe the movement of herbicides in a river basin and to create herbicide pollutographs. In the model, a river basin was divided into a grid of 1 km × 1 km grid cells. Each grid cell was subdivided into 12 compartments: each compartment was defined as consisting mainly of a river-water compartment (R compartment), a river-bed compartment (S compartment), a paddy-field-water compartment (W compartment), 2 paddy-field-soil compartments (X and Y compartments), or others (Figure 1). Water and herbicides from all compartments except the C compartment move laterally to the R compartment of one of the immediately surrounding 8 grid cells, specifically, to the cell along the steepest downhill slope from the source cell. Lateral movement from the C compartment goes to the R compartment of the next grid cell via the S compartment of that grid cell. The irrigation water in the W compartment comes from the R compartment of the same grid cell or from the grid cell that contains the intake gate (R compartment) for the paddy field. Vertical flows from all compartments except the R and S compartments are downward.

A set of differential mass-balance equations describing the dynamics of a solute (herbicide) and water in each compartment was formulated, based on the law of conservation of mass for the herbicides and the water. In the hydrology (water flow) part of the model, the rates of lateral water flow into ($Q_{W,in}$) and out of ($Q_{W,out}$) the W compartment are described as functions of the water level (h_W) in the compartment:

$$Q_{W,in} = A\, a_{W,in} \max(0, h_{W,0} - h_W) + A\, q_W \tag{1}$$

$$Q_{W,out} = A\, a_{W,out} \max(0, h_W - h_{W,0}) \tag{2}$$

The water depth in the paddy field (h_W) is artificially controlled at various levels according to the growth stage of rice and the weather conditions. The desired water level in the rice paddy field ($h_{W,0}$) and the spill-over irrigation flow rate (q_W) are input variables, which are determined by the rice farming schedules.

Vertical flow from W compartments ($Q_{W,V}$) is described as a function of water level in the rice paddy field; this water goes into the X compartment beneath the W compartment

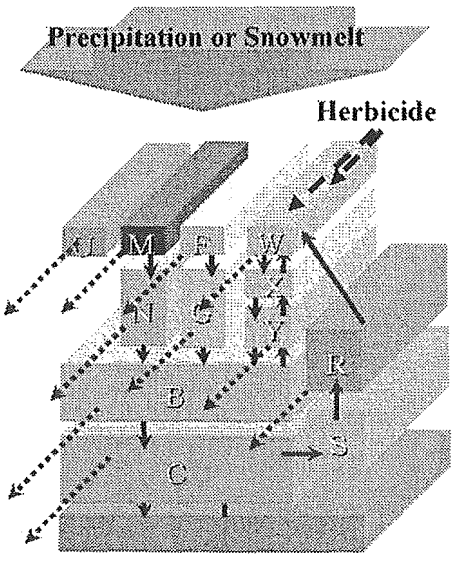

Figure 1 Compartments in a grid cell (R: river, S: river bed, W: paddy field, U: urban area, M: mountain, F: upland field)

in the same grid cell

$$Q_{W,V} = a_{W,V} A \left(\frac{h_W}{h_{W,0}} \right) \tag{3}$$

The rates of lateral flow (Q_H) from the M, F, and U compartments are described by the Manning equation:

$$Q_H = \frac{A}{B} h \frac{1}{n_M} h^{2/3} I^{1/2} \tag{4}$$

The rates of lateral interflow from X, Y, N, G, B, and C compartments are described as a function of the water level in the compartment and the slope of the compartment:

$$Q_H = a_H I(A/B)h \tag{5}$$

Vertical flows from the X, Y, M, N, F, G, B, and C compartments are described as percentages of each water content, which is equivalent to the water level relative to the compartment height:

$$Q_V = a_V A(h/h_0) \tag{6}$$

The Manning equation is also used to describe the flow rate in the R compartment:

$$Q_R = \frac{A}{L_R} h \frac{1}{n_M} h^{2/3} I^{1/2} \tag{7}$$

For solute movement between compartments, advection and diffusion are considered. Solute advections are given as the product of the concentration and water flow rate calculated from Eqs. (1)–(7). However, the maximum real concentration for each solute is limited by its solubility in water, so any amount of herbicide over the solubility limit must exist in the solid phase and is not subject to movement. The rate of solute movement by diffusion between compartments is given by the linear driving force model:

$$q_D = A \frac{D}{L} (C_1 - C_2) \tag{8}$$

Within a compartment, both the solute concentration and the water level are assumed to be uniform, each represented by a single variable. For example, rainfall is assumed to mix completely and uniformly with herbicides in the paddy-field-water compartment (W compartment). If a compartment consists of multiple subelements (soil–solid and soil–water), a dynamic equilibrium exists between the dissolved and sorbed fractions at the solid–water interface. These phases are assumed to be in equilibrium at all times; sorption processes are considered to be instantaneous and are described by a single constant (the solid–water partition coefficient) in the linear equilibrium relationship. Hence, once the concentration in one phase is known, the concentration in the other phase can be calculated. Degradation of herbicides in each compartment follows first-order kinetics. The processes of herbicide uptake by plants and herbicide evaporation into the atmosphere were not considered in this model. The flow rate coefficient in each type of compartment (W, X, etc.) is assumed to be a single value (for each compartment) throughout the entire set of grid cells in the basin. These assumptions were made to reduce the total number of hydrologic parameters, even though the target river basin was divided into numerous grid cells, which contributed to preventing too much uncertainty in determining the model parameter values.

Site description and model application

Two river basins were selected to test the model and to analyze the effects of uncertainties in agricultural work schedules on modeled predictions of herbicide concentrations: the Chikugo River basin (1,884 km^2; Figure 2) and the Oirase River basin (667 km^2, Matsui *et al.*, 2002). The Chikugo basin includes rice paddy fields (261 km^2) cultivated by 22,860 farmers, and the Oirase basin includes rice paddy fields (92 km^2) cultivated by 3,400 farmers. The Oirase River basin was divided into 667 grid cells (each 1 km^2), for a total of 8,004 compartments. A set of 16,008 equations was solved to describe the movements of water and herbicides in the river basin. The Chikugo River basin was divided into 1,884 grid cells. Because the Chikugo River basin includes several dams, where river flow rates are artificially controlled, model calculations were conducted for the catchment area of each dam. The model equations were solved as a system of ordinary differential equations by Gear's stiff method from the IMSL MATH/LIBRARY.

Application of the compartment model to the river basin requires geographic data: the altitude of each compartment was determined from Geographic Information System (GIS) data (The Geographical Survey Institute, Japan, 1999), and water flow directions between compartments were determined based on the direction of the steepest gradient. The GIS data (The Geographical Survey Institute, Japan, 1990) were also used to calculate the areas of the compartments (paddy field, river, forest, etc.) in each grid. However, the GIS data available were old and may not reflect current land utilization. The area of paddy fields, which is the most important geographical information in this research, was corrected with data published by the local governments (Census of Agriculture Japan, 1995 and 2000), which include data on the percent of rice paddy area removed from cultivation due to compulsory adjustments in production. The fallow paddy fields were regarded as upland field compartments.

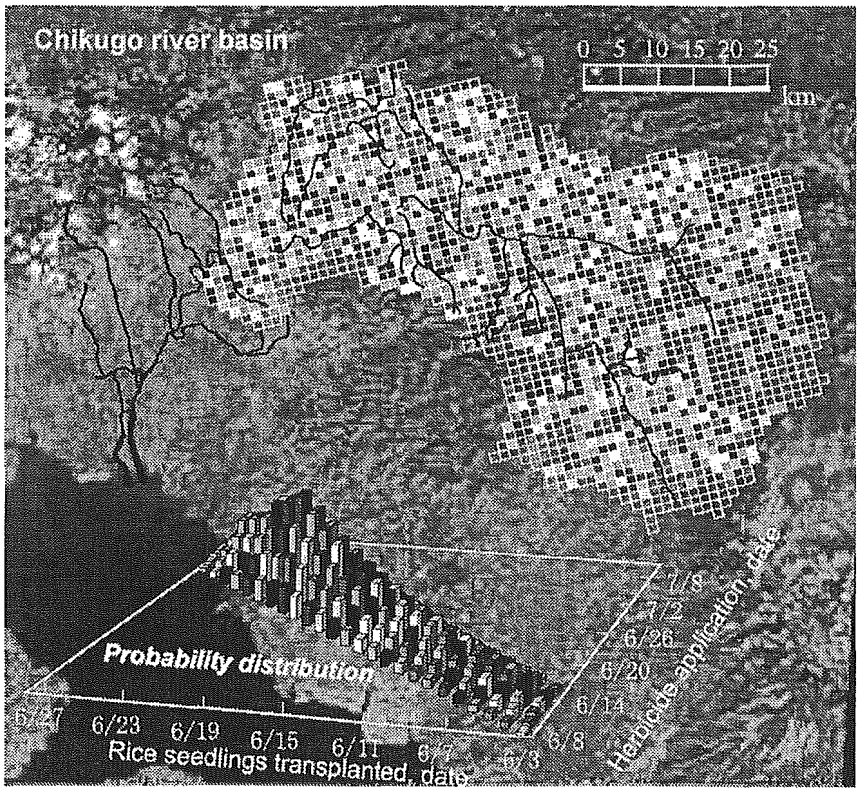

Figure 2 Probability distribution of farming schedules (histogram) and an allocation pattern of farming schedules in a target river basin (each farming schedule shown as a colored bar in the histogram was allocated to compartments randomly)

Hydrologic model inputs and system parameters

The amount of precipitation in each grid cell was estimated by interpolating the observed data from observation points in and around the target area and applying three-dimensional corrections for variations in altitude and location. Evapotranspiration was estimated by the method of Brutsaert and Stricker (1979) from data on air temperature, wind velocity, duration/intensity of sunshine, and celestial declination. For the Oirase river basin, the effects of snowfall and snowmelt were estimated by a temperature index method (Ikebuchi *et al.*, 1984, 1985).

The hydrologic (water flow) model requires 11 parameters optimized. The values of the vertical flow rate coefficients in the W and X compartments ($a_{W,V}$) were determined to be a typical value for the field percolation rate of water in paddy fields, 0.01 $m\,s^{1}$. The spill-over flow rate (q_W) was estimated to be 0.02 $m\,s^{1}$. Irrigation and drainage rate coefficients of rice paddy fields ($a_{W,in}$ and $a_{W,out}$) were inputs as 5 and 2 d^{-1}, respectively, after talking with a farmer, considering the structures and dimensions of outlets of several rice paddy fields, and also actually measuring the drainage flow rate of a paddy field. The values of the remaining parameters were seached by iteration to give the best fit to the observed river flow rate within the minimum error. In addition to this best-fit criterion, the parameters were set so as to give no annual long-term water loss in the C compartment. The hydrologic parameters of the model were successfully calibrated, with the result that they fit the observed stream flow data to the model simulation with reasonable accuracy.

Monte-Carlo generation of rice cultivation schedule

Figure 3 shows the irrigation schedule recommended by a local government, which was used to determine the input data for water depth and irrigation rate. The irrigation schedule is set according to the rice-transplanting date. To maintain the water depth in a field during various periods of rice growth, the water consumed due to evapotranspiration and percolation is replaced by irrigation. The irrigation and drainage schedule can be adjusted for herbicide dusting and ambient temperature. For example, after herbicide dusting, drainage is halted for 5 days. The water depth is also changed according to whether the ambient temperature is high (>20 °C) or not. Therefore, the input rice farming schedule can be based on the dates of rice transplanting and herbicide dusting. For a large paddy field cultivated by numerous farmers, however, the schedule of agricultural tasks in the entire paddy field is not homogeneous: rice transplanting and herbicide dusting are not each performed on a single date. The transplanting season continues for several weeks, and each herbicide is dusted once within a certain period after transplantation is finished.

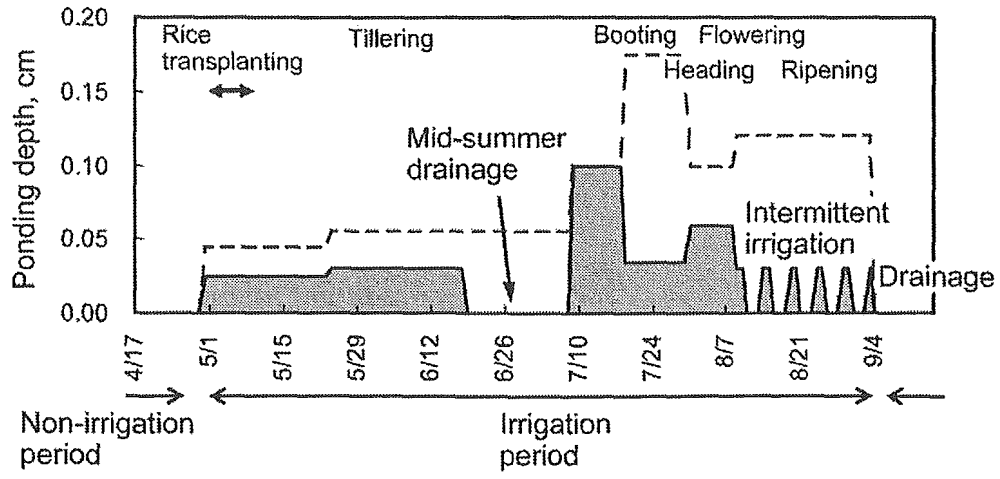

Figure 3 A pattern of irrigation (solid line for ordinary temperature and dashed line for high temperature)

For example, dusting with the herbicide mefenacet is done once, between 5 and 15 days after transplantation.

Modeling the agricultural schedules of each farmer after detailed data acquisition is ideal but too troublesome to be feasible. However, due to the large scale of the study area and its cultivation by many farmers, it should be possible to consider agricultural tasks as random events within defined periods of time, which can be estimated from data published by local governments. Two hundred farming work schedules (combinations of dates for rice transplanting and herbicide dusting) were created for each herbicide; the probability of occurrence of each work schedule is indicated by the histogram in Figure 2. For example, in about 5% of the rice paddy fields, rice seedlings were transplanted on June 23 and herbicide was dusted on July 2. Farming schedules were allocated to the paddy field compartments of the grid cells in the river basin randomly, within an expected occurrence probability (the histogram in Figure 2 shows an allocation pattern for the herbicide mefenacet). A total of 100 schedule-allocation patterns for each herbicide were created and used as input to the modeling. For comparison, model predictions with deterministic input were also conducted, for which a single farming schedule for rice transplanting and herbicide dusting was used throughout the entire river basin (the dates of the highest bar in the histogram in Figure 2). The amounts of herbicides consumed in the target river basin were estimated from marketing information on the sales of commercial herbicide products.

Monte-Carlo generation of degradation rates and soil–water partition coefficients of herbicides

Many factors (aerobic/anaerobic conditions, soil-sediment organic content, etc.) affect herbicide decomposition and its partition between soil and water. Because of a lack of information regarding the reaction environment in the field, however, it is impossible to quantify the specific decomposition rate and sorption equilibrium in each grid cell of the model, so these values are subject to various kinds of uncertainties. Although some reports include values for the decomposition rates or half-lives, the reported ranges of these rate coefficients are very wide, partly owing to variability in the reaction conditions. Therefore, a single reported value is not appropriate for representing decomposition rates in a whole area. It is more reasonable to assume that all rate parameter uncertainties are random. Using the Monte-Carlo method, a degradation rate coefficient for each herbicide in each compartment was randomly selected from values in the ranges of the reported values. The solid–water sorption coefficient of each herbicide was treated the same way. For comparison, we repeated the model calculation using the average of reported values as deterministic parameter value inputs.

Predicting herbicide concentrations

Herbicide concentrations were measured 5 days a week during 1999 and 2000 in the Chikugo River and once a week during 1995 and 1996 in the Oirase River. After the hydrological system parameters were calibrated, the hydrological and solute models were solved simultaneously by substituting solute input data, yielding predicted concentrations of herbicides in the river waters. Model-based predictions from the Monte-Carlo inputs were made, starting with each of the 100 schedule-allocation patterns. Figure 4 shows the probability distribution for the concentration of the herbicide pretilachlor on one day in 1999 in the Chikugo River. The predicted concentrations are distributed broadly, due to the Monte-Carlo generation of the farming schedules and herbicide parameters. The highest 95th percentile concentration was 7 times the lowest 5 percentile concentration. Figure 5 shows time variations in the pretilachlor concentration. About half the observed

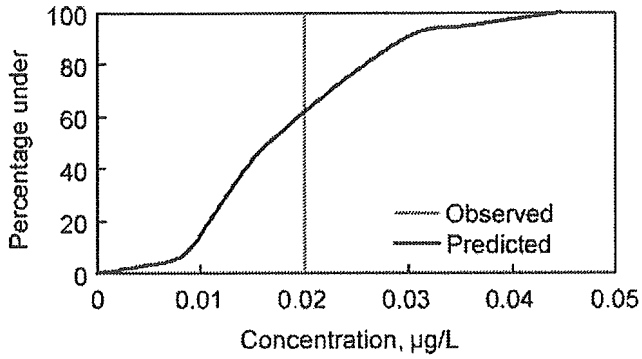

Figure 4 Probability distribution of predicted pretilachlor concentration on June 9, 1999

data points are within the 1%–99% probability range of the predicted concentrations. Although the herbicide dusting date and the amount applied are important factors in predicting the concentrations, our modeling did not consider the amount and date of herbicide dusting by individual farmers. Moreover, the simulations were conducted without optimizing the herbicide decomposition/sorption parameters. In light of these limitations, we consider the model prediction for pretilachlor to be reasonably successful. As shown in Figure 5, compared with the prediction using Monte-Carlo inputs, the prediction obtained with deterministic input yielded a rather discrete concentration variation with improper peaks.

Although prediction of pretilachlor concentration with the Monte Carlo method was good, the results for other herbicides were less successful. For example, the predicted concentration of dimethametryn was lower than that observed (Figure 6). These discrepancies could be due to poor estimates of herbicide consumption (from the sales volume) and/or of the herbicide decomposition parameter. A precise evaluation of local herbicide sales would improve the prediction. More than 60% of the predictions based on Monte-Carlo inputs were within 0.1 to 10 times the observed values, and the Monte-Carlo method reduced the percentage of 'bad' predictions, shown yellow in Figure 7.

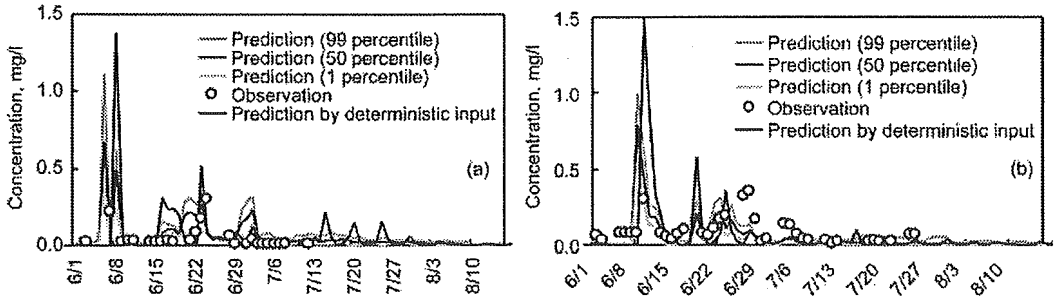

Figure 5 (a) Predicted and observed pretilachlor concentrations in Chikugo River in 1999. (b) Predicted and observed pretilachlor concentrations in Chikugo River in 1999

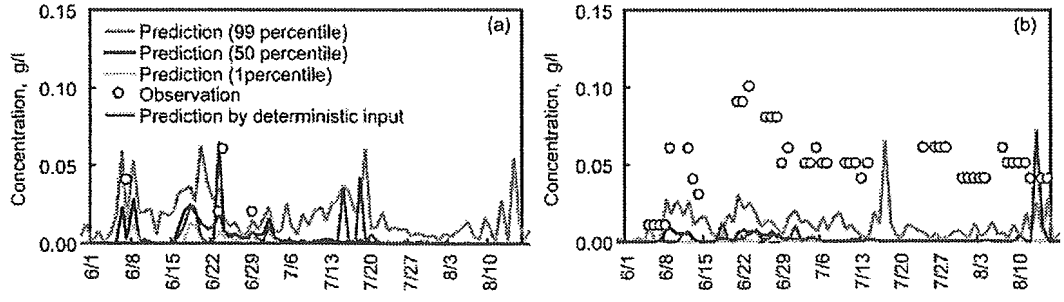

Figure 6 (a) Predicted and observed dimethametryn concentrations in Chikugo River in 1999. (b) Predicted and observed dimethametryn concentrations in Chikugo River in 2000

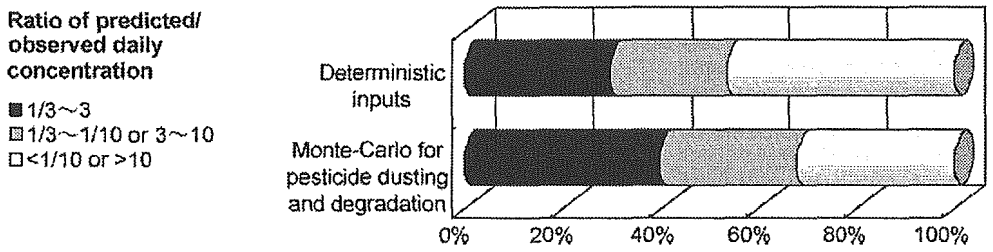

Figure 7 Comparison of model predictions with inputs generated by Monte-Carlo and deterministic methods (Chikugo River)

Conclusions

The effectiveness of the Monte-Carlo method for creating input data for agricultural work schedules and pesticide decomposition/sorption parameters was studied. The Monte-Carlo method was used to randomly allocate 200 patterns of farming work schedules to each paddy field in grid cells of a GIS-based basin-scale pesticide run-off model. The degradation rate and sorption coefficient for each herbicide in each compartment were also randomly selected from values in the ranges of reported values. Prediction of pesticide concentrations in river water by the runoff model was better with Monte-Carlo input than with deterministic input. The Monte-Carlo method alleviates the difficulty of obtaining precise data on individual farming schedules (including pesticide dusting dates) and on individual degradation rates and sorption coefficients in each soil. Once better values are available for the amounts of pesticides applied and for pesticide degradation rates and sorption parameters under actual field conditions, the GIS-based basin-scale runoff model should successfully predict river water pesticide concentrations.

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〈論 文—Original Paper〉

農薬の分子構造別の塩素分解性に関する研究

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浅見真理** 相澤貴子****

Degradation of Pesticides by Chlorination According to Their Basic Structures

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Abstract

One hundred and one pesticides listed as "Complementary Items for Setting the Targets for Water Quality Management in Japan" were divided into 16 groups on the basis of their structures. The degradation of 96 pesticides and 5 oxidation by-products in chlorination was studied to determine the potential of the purification process. Pesticides containing sulfur (S) were easily degraded; for 38 pesticides out of 46 examined pesticides, only 50 % or less of their original concentration remained after 4 hours. On the other hand, pesticides without S were hardly degraded; for 45 pesticides out of 50 examined pesticides, more than 50 % of their original concentration remained even after 24 hours. Thus, the potential for degradation of the pesticides by chlorination can be approximated by the presence or absence of S in their chemical formulae. Oxons (P=O), which are oxidation by-products of organophosphate pesticides containing P=S, were hardly degraded. Therefore, oxons may remain in chlorinated water for a long time.

Key Words: pesticide, chlorination, oxidation by-product, oxon, drinking water

1. はじめに

環境中で使用される可能性のある農薬は，農薬取締法に登録された約550種類であり，出荷量は約30万トンに及ぶ。水道においては，水源域で用いられた農薬が，浄水からもしばしば検出されている。

新しい「水質基準に関する省令（厚生労働省令第101号)」（平成15年5月30日公布，平成16年4月1日施行）の中で，農薬については，効率的な監視を行うため，約550種類を基本として，以下のように選定された。①国内推定出荷量とADI（許容一日摂取量）の比，②国内推定出荷量，③その他過去の経緯等及び測定方法から，水道原水で検出されるおそれのあるものとして，101種類がリストアップされ，水質管理目標設定項目と定められた。そして，下記の式で与えられる，検出指標値が1を超えないこととする総農薬方式により，浄水に対する水質管理を行うこととなった[1-3]。

$$DI=\Sigma(DVi \div GVi)$$

(DI：検出指標値，DVi：農薬 i の検出値，GVi：農薬 i の目標値)

測定を行う農薬については，各水道事業者が検出状況，使用量等，その地域の状況を勘案し，101種類から選定することとなった。この選定手法の一つの考え方として，出荷量，ADI, Log Kow（オクタノール/水分配係数），生分解性の情報を1～5のスコアとし，その合計値から選定する監視農薬プライオリティーリストが検討されている[4]。監視農薬プライオリティーリストを参考に測定すべき農薬を選定する場合，原水ではリストの上位順から測定することになるが，浄水では消毒効果維持のために給水栓水で遊離残留塩素を0.1mg・l^{-1}以上保持するよう定められているため[5]，浄

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水処理過程で塩素処理を行うことにより注入した塩素が農薬を分解し，それに伴い副生成物が生成する可能性があることを想定して，監視農薬プライオリティーリストを作成する必要がある。これまでにも農薬の塩素分解性についての研究が行われてきたが[6-9]，実験条件が異なり，農薬原体の分解性の比較に利用できる研究は少ない。また，農薬の分子構造から塩素との反応性が予測できれば有用であるが，そのような視点からの解析は限られている。

そこで，著者ら及び水道事業体等の複数の試験研究機関において，農薬101種類を対象に，初期農薬濃度，塩素濃度，反応時間をほぼ一定にした条件下で塩素分解実験を行った。その残存率を基に，監視農薬プライオリティーリストと同様に5段階に分類した。次に，官能基等の分子構造別に農薬を分類し，それぞれの農薬群が塩素により分解されやすいか否かを示し，分解されやすい場合は，どの部位が分解されやすいのか考察した。さらに，硫黄(S)を含む構造別にも分類し，塩素分解性の傾向を考察した。また，有機リン系農薬の酸化物である，オキソン体の塩素分解性も農薬残存率により5段階に分類するとともに，オキソン体の毒性に関する知見を示した。

2. 実験方法

2.1 塩素処理実験

浄水場における実際の塩素処理に近い条件で評価するため，処理に要する時間と塩素濃度を考慮に入れ，4時間後の塩素濃度が概ね1.0mg・l^{-1}になるように実験を行った。それぞれの実験条件において農薬の初期濃度は200μg・l^{-1}である。実際には，**Table 1**に示す混合農薬数及び塩素濃度で実験を行った。以下に示した条件では，当院で使用した試薬，分析機器の製造元を記したが，その他の試験研究機関で行った場合はこれに準拠した。

2.1.1 試薬

(1)1mM リン酸緩衝液：リン酸二水素ナトリウム・二水和物（和光純薬製）2.96g,リン酸水素二ナトリウム・十二水和物（和光純薬製）29.0g を精製水（日本ミリポア製,超純水装置Milli-Q Gradient）1lに定容し，100mMリン酸緩衝液を作製した。これを100倍希釈し，1w/v(%)水酸化ナトリウム（和光純薬製）溶液を用いpH7に調製した。(2)10mg・l^{-1}次亜塩素酸ナトリウム溶液：次亜塩素酸ナトリウム溶液（関東化学製）の塩素濃度を使用毎に残留塩素電流滴定器（磯村豊水機工業製，AT-Ⅱ型）で測定し，精製水で希釈し10mg・l^{-1}に調製した。(3)5w/v(%)亜硫酸ナトリウム溶液：亜硫酸ナトリウム（関東化学製）5gを精製水100mlに定容した。(4)1000mg・l^{-1}農薬標準液：農薬標準品10mgをメタノール（和光純薬製），アセトニトリル（和光純薬製）または精製水10mlに定容した。

2.1.2 実験手順

(1)密閉可能な10mlガラス製容器（ガラスに吸着しやすいジクワットはポリプロピレン製容器）に1mMリン酸緩衝液を1～9ml注入し，1000mg・l^{-1}農薬標準液を2μl添加した（農薬初期濃度200μg・l^{-1}）。(2)浄水場における実際の塩素処理に近くするため，あらかじめ4時間後の残留塩素濃度が概ね1.0mg・l^{-1}になるような初期添加濃度を求め，10mg・l^{-1}次亜塩素酸ナトリウム溶液を1～9ml添加した。1mMリン酸緩衝液，10mg・l^{-1}次亜塩素酸ナトリウム溶液の量は条件により適宜変更し，合計10mlにした。残留農薬測定用，残留塩素測定用としてサンプルはそれぞれ数本用意した。(3)容器を密閉後，十分に攪拌させ，暗所，20±1℃の条件で一定時間（15分，4時間，24時間）静置した。標準的な浄水場として，前塩素注入→凝集沈殿→砂ろ過→後塩素注入という浄水処理過程を想定し，塩素注入直後の15分，浄水場出口までを4時間，塩素注入より給水栓に到達するまでを24時間として，反応時間を設定した。(4)一定時間経過後，数本用意したサンプルのうち1本ずつ取り出し，残留農薬測定用サンプルは5w/v(%)亜硫酸ナトリウム溶液の添加により残留塩素を消去した。農薬の残留濃度は主に，酸アミド系，トリアジン系，アニリド系，ジニトロアニリン系，ジフェニルエーテル系，ピリジン系，有機塩素系農薬はGC/MS（Agilent 製，GC：G1098A，MS：5973MSD），ウレア系，スルホニルウレア系農薬はLC/MS（Agilent製，HPLC：Agilent1100，MS：1100LC/MSD SL），カーバメート系，チオカーバメート系，有機リン系，フェノキシ系農薬はGC/MS，LC/MSの両方を用いて測定した。残留塩素測定用サンプルはラピッドDPD試薬（関東化学製）100mg・10ml^{-1}を溶解させ，発色後に分光光度計（日立ハイテクノロジーズ製，U-2800）で残留塩素濃度を測定した。

2.2 対象農薬

101種類の農薬のうち，後に示す5種類を除いた96種類（うち3種類は参考値），及びP=S型有機リン系農薬の酸化物であるオキソン体（P=O型）5種類について検討を行った。101種類のうち，イプロジオン，オキシン銅及びプロシミドンについては，実験データが十分でなかったため，同様の条件で行われた文献値を参考値とすることとした。101種類のうち対象外とした5種類は，浄水における検出例がない，もしくは

Table 1 Experimental conditions for different data groups

Number	The Concentration of Chlorine (mg/L)		Number of Pesticide Mixtures	Number	The Concentration of Chlorine (mg/L)		Number of Pesticide Mixtures
	0	4hr			0	4hr	
(1)☆	3.9	<0.1	93	(14)	2.3	0.9	8
(2)	5.0	0.4	11	(15)	1.7	1.0	15
(3)	1.9	0.5	14	(16)	2.3	1.0	17
(4)	2.9	0.5	7	(17)	2.3	1.1	11
(5)	3.84	0.5	43	(18)	2.7	1.1	4
(6)	5.0	0.5	24	(19)	1.5	1.3	6
(7)	2.1	0.65	6	(20)	2.3	1.3	5
(8)	2.8	0.68	22	(21)	7.8	1.3	93
(9)	1.0	0.7	16	(22)	2.6	1.45	14
(10)	1.0	0.75	1	(23)☆	20	5.2	34
(11)	1.4	0.76	6	(24)☆	40	26.8	77
(12)	0.9～2.4	0.7～0.8	1	(25)	1.0	-	1
(13)	1.8	0.9	3	(26)☆	0.1	-	135

(25) is obtained from reference 7) and (26) from reference 6).
The initial concentration of pesticides is 200μg・l^{-1}.
☆The concentration of chlorine in 4 hours is beyond the appropriate range.

測定が困難であった1,3-ジクロロプロペン，CNP-アミノ体，ダラポン，イミノクタジン酢酸塩，ポリカーバメートであった。

2.3 塩素分解性の分類方法

2.1.2に記述した理由で，塩素注入より15分，4時間，24時間後に残留農薬濃度を測定したこと，従来の監視農薬プライオリティーリストにおいて各項目が5段階に分けられていることを踏まえ，農薬の塩素分解性を以下のように5段階に分類した。農薬の残存率が，15分で50%以下となる農薬をA，4時間で50%以下となる農薬をB，24時間で50%以下となる農薬をC，24時間で50%を超え90%以下残存となる農薬をD，24時間で90%を超え残存となる農薬をEと分類した。Aが最も塩素により分解されやすく，順にEに近づくにつれて塩素により分解されにくいことを表す。

3. 結果と考察

3.1 分子構造別の塩素分解性

代表的な官能基又は基本構造を中心とする分子構造別に農薬を分類し[10]，それぞれの農薬における15分後，4時間後，24時間後の残存率及び塩素分解性(A～E)を**Table 2**に示す。

3.1.1 ウレア系農薬

ウレア系農薬は尿素(H_2NCONH_2)に環状構造の基やアルキル基などの置換体が結合した構造を持つ薬剤である。除草剤として使用され，雑草の光合成阻害を主な作用とするものが多い[11]。ウレア系農薬5種類は全て分解されにくかった（D,E）。これを個別に見ると，ペンシクロン，シデュロンは24時間後もほぼ100%残存していたが（E），メチルダイムロン，ジウロン，ダイムロンは24時間後に約63～85%とやや分解していた（D）。

Mascoloら[12]によると，101種類の農薬以外のウレア系農薬であるイソプロツロンは，農薬40mg・l^{-1}，塩素160mg・l^{-1}の条件下で，塩素の加水分解や酸化作用により，側鎖の一部が-OHに置換され，ベンゼン環は最終的に開環すると報告している。しかし，ウレア構造は形を保っていると報告しており，本研究においてもウレア構造は残存していたと推測される。

3.1.2 スルホニルウレア系農薬

スルホニルウレア系農薬は尿素にスルホニル基(SO_2)が結合し，それに環状の置換体が結合した構造を持つ薬剤で，除草剤として使用される[11]。スルホニルウレア系農薬3種類は塩素により比較的分解されやすかった（A,B）。3.1.1で示したように，ウレア系農薬は全て分解されにくかったが，ウレア系農薬とスルホニルウレア系農薬の相違点は，SO_2を有するか否かである。したがって，スルホニルウレア系のSO_2の部分が分解に関与していたと考えられる。スルホニルウレア系以外でSO_2を有していた，アシュラムもAに分類された。ただし，SO_2を複素環中に有するプロベナゾール，ベンタゾンはそれぞれC,Dに分類されたため，分解性はSO_2が結合する位置によって異なると推測される。

3.1.3 カーバメート系農薬

カーバメート系農薬は-HN-COO-の構造を持つ薬剤である。除草剤としては根や茎葉から吸収され，植物体内全体を移行して作用し，殺虫剤としては害虫などの神経機能を阻害する[11]。カーバメート系農薬は11種類のうち5種類が分解されやすかった（A,B）。チオホスホリル基(P=S),チオメチル基(-SCH_3)等を含むチオアルキル基(-SR),ジチオラン環（五員環の1,3-位置換基にSを有する）等，一連の含イオウ農薬は分解されやすいことが既に報告されている[6,9]。5種類のうち4種類は-S-の構造を有していた。Milesら[13]によると，カーバメート系農薬であるメソミルは，加水分解と塩素化反応により，メソミルスルホキシドやN-クロロメソミル等を経てメタンスルホン酸に分解されると報告しており，その他の分解生成物は酢酸，ジクロロメチルアミン等であることを示した。

ベノミルは，水中では加水分解物であるMBC(methyl benzimidazol-2-yl-carbamate)として存在していると考えられ，本研究の塩素分解実験においてもMBCを測定した結果，非常に分解されやすかった（A）。MBCは-S-の構造を有していないが，電子過剰のベンゾイミダゾール[14]の構造を有しているため，これが塩素と反応したと推測される。

例外的に，チオファネートメチルはC=S構造を有していたが，分解されにくかった（E）。

3.1.4 チオカーバメート系農薬

ジチオカーバメート系を含むチオカーバメート系農薬は-HN-COS-,-HN-CSS-等の構造を持つ薬剤である[11]。チオカーバメート系農薬は6種類とも極めて分解されやすかった（A）。特にピリブチカルブ，モリネート，ジメピペレート，エスプロカルブは15分で10%以下に減少していた。ジチオカーバメート系農薬は-S-やC=Sの構造を有しているため，分解されたものと考えられる。高橋ら[15]はチオカーバメート系農薬であるチオベンカルブと塩素の反応は，主として酸化反応であり，その他に塩素付加，塩素置換反応などであるとし，塩素によりチオベンカルブはクロロトルエンまたは塩化クロロベンジルを経て，クロロベンジルアルコール，クロロベンジルアルデヒド，クロロ安息香酸の順に分解すると報告している。またMagaraら[16]は，チオベンカルブのようにベンゼン環を持つ農薬は，塩素により塩素化芳香族化合物を生成すると報告している。

3.1.5 酸アミド系農薬

酸アミド系農薬は-HNCO-構造に置換基が結合した構造を持つ薬剤である。除草剤として使用され，光合成阻害作用による脂質の合成阻害，あるいは生体内酵素のアルキル化などによる薬理作用を示す[11]。酸アミド系農薬8種類は全て分解されにくく（D,E），ナプロパミド以外は24時間後も80%以上残存していた。この結果より，酸アミド構造は塩素により分解されにくいと推測される。

3.1.6 トリアジン系農薬

トリアジン系農薬はトリアジン環（それぞれ3個の炭素と窒素からなる環）を母核とした薬剤で，1-位置換基に-Cl,-OCH_3,-SCH_3のいずれかの構造を有する。光合成を阻害することにより，除草剤として使用される[11]。トリアジン系農薬は4種類のうち2種類が分解されやすかった（A）。分解されたシメトリン，ジメタメトリンはどちらもチオメチル基(-SCH_3)の構造を有し

Table 2 101 pesticides and their degradation by chlorination

Group	Number*1	Pesticides	Category*2	Residual Pesticides*3 (Average, Minimum-Maximum, %) 15min	4hr	24hr	Using Data for Residual Pesticides*4
Urea	46	Methyldymron	D	94.4 (80.5 - 104)	92.1 (72.0 - 105)	85.7 (59.5 - 106)	(1)☆,(15),(24)☆
	68	Diuron (DCMU)	D	90.2 (76.5 - 104)	80.0 (76.5 - 83.5)	62.9 (52.0 - 73.9)	(10),(21)
	84	Dymron	D	88.6 (66.0 - 105)	82.1 (54.0 - 97.9)	82.7 (49.2 - 108)	(1)☆,(3),(4),(7) (11),(21),(23)☆
	33	Pencycuron	E	103 (83.4 - 117)	101 (80.7 - 115)	101 (81.4 - 114)	(1)☆,(21),(23)☆
	98	Siduron	E	97.8 (97.1 - 98.5)	97.7 (95.0 - 103)	95.2 (88.5 - 101)	(1)☆,(8),(21)
Sulfonylurea	86	Bensulfuron -methyl	A	40.2 (21.0 - 67.8)	2.7 (1.6 - 3.5)	0.1 (0 - 0.2)	(3),(8),(11) (21)
	95	Flazasulfuron	A	39.6 (5.1 - 78.0)	11.5 (1.3 - 18.0)	5.2 (0 - 19.0)	(1)☆,(3),(8) (21)
	94	Halosulfuron -methyl	B	65.8 (51.0 - 75.5)	14.8 (12.2 - 19.5)	4.1 (0.9 - 5.9)	(3),(8),(21)
Carbamate	36	Asulam	A	6.9 (1.3 - 12.1)	1.8 (0 - 4.9)	1.6 (0 - 4.4)	(1)☆,(3),(21)
	75	Benomyl	A	3.5 (0.9 - 7.7)	2.1 (0.1 - 5.9)	2.1 (0.1 - 6.1)	(1)☆,(11),(21)
	76	Benfuracarb	A	0 -	0 -	0 -	(6)
	74	Methomyl	B	68.2 (43.8 - 90.3)	1.4 (0 - 3.5)	0 (0 - 0)	(3),(8),(21)
	96	Thiodicarb	B	78.2 (77.5 - 78.8)	4.9 (3.8 - 6.0)	2.0 (0 - 4.0)	(3),(8)
	12	Fenobucarb (BPMC)	E	101 (93.7 - 109)	101 (96.9 - 107)	91.1 (82.3 - 99.0)	(5),(6),(9) (16),(21)
	18	Carbofuran	E	94.1 (92.0 - 96.5)	97.1 (91.0 - 110)	97.3 (92.7 - 100)	(1)☆,(5),(11) (21)
	38	Terbucarb (MBPMC)	E	93.8 (85.4 - 105)	102 (93.0 - 113)	94.7 (89.5 - 97.8)	(1)☆,(5),(21) (24)☆,(25)
	48	Carbaryl (NAC)	E	97.1 (90.2 - 104)	97.4 (88.9 - 106)	96.4 (79.7 - 104)	(1)☆,(3),(5) (8),(21)
	54	Isoprocarb (MIPC)	E	102 (96.7 - 108)	102 (98.1 - 104)	94.0 (80.0 - 100)	(1)☆,(9),(16) (21)
	55	Thiophanate -methyl	E	134 -	119 -	111 -	(7)
Thiocabamate	1	Thiram	A	31.6 (0 - 51.9)	27.6 (0 - 41.6)	15.0 (0 - 22.9)	(1)☆,(3),(21)
	3	Thiobencarb	A	28.1 (0 - 62.4)	4.2 (0 - 21.3)	1.7 (0 - 4.9)	(5),(6),(15) (16),(18),(23)☆
	40	Pyributicarb	A	1.5 (0 - 7.5)	0.9 (0 - 5.6)	1.0 (0 - 4.4)	(1)☆,(15),(18) (20),(21),(23)☆
	60	Molinate	A	5.9 (0 - 22.5)	3.1 (0 - 17.0)	1.1 (0 - 10.6)	(1)☆,(2),(5),(6) (9),(15),(17) (18),(21),(23)☆
	78	Dimepiperate	A	2.6 (0 - 5.2)	1.2 (0 - 2.3)	1.2 (0 - 2.3)	(1)☆,(21)
	83	Esprocarb	A	8.7 (0 - 41.8)	1.0 (0 - 3.1)	0.5 (0 - 2.8)	(1)☆,(4),(5) (22),(23)☆,(24)☆
	93	Polycarbamate	-	- -	- -	- -	
Acide amide	32	Flutolanil	D	98.0 (91.9 - 102)	96.2 (89.3 - 106)	82.0 (39.7 - 98.5)	(5),(6),(9) (15),(20),(23)☆
	35	Mepronil	D	99.9 (90.5 - 105)	101 (74.8 - 119)	84.9 (46.4 - 105)	(1)☆,(5),(15) (20)
	39	Napropamide	D	96.1 (91.8 - 100)	82.2 (48.6 - 102)	63.4 (1.6 - 94.5)	(1)☆,(5),(15)
	53	Pretilachlor	D	94.9 (84.0 - 101)	98.0 (91.7 - 106)	89.4 (77.5 - 104)	(1)☆,(2),(5) (6),(9),(17)
	10	Propyzamide	E	102 (85.0 - 115)	99.2 (78.5 - 114)	98.2 (87.5 - 107)	(1)☆,(21),(24)☆
	47	Alachlor	E	95.6 (84.5 - 103)	97.2 (88.5 - 103)	92.1 (83.0 - 103)	(1)☆,(6),(16) (24)☆
	58	Carpropamid	E	101 (95.9 - 105)	100 (93.9 - 104)	98.5 (91.8 - 103)	(1)☆,(7),(20) (21)
	59	Bromobutide	E	100 (92.0 - 107)	102 (75.3 - 115)	95.7 (75.3 - 106)	(1)☆,(2),(5) (9),(17)
Triazine	77	Simetryn	A	3.2 (0 - 8.2)	2.7 (0 - 5.7)	2.5 (0 - 6.4)	(1)☆,(2),(5) (6),(9),(21) (22),(24)☆
	89	Dimethametryn	A	2.0 (0 - 5.2)	1.6 (0 - 3.8)	1.6 (0 - 4.5)	(1)☆,(17),(21) (23)☆
	2	Simazine (CAT)	E	107 (92.0 - 118)	109 (87.0 - 134)	105 (85.5 - 120)	(1)☆,(5),(21) (23)☆,(24)☆
	63	Atrazine	E	96.9 (88.1 - 103)	97.6 (82.5 - 105)	93.2 (82.0 - 103)	(1)☆,(5),(16) (24)☆
Organophosphorous (P=S)	6	Diazinon	A	2.7 (0 - 6.9)	0 (0 - 0)	0 (0 - 0)	(5),(6),(12) (15),(23)☆
	7	Fenitrothion (MEP)	A	16.7 (0 - 53.2)	5.2 (0 - 35.1)	0.4 (0 - 1.7)	(2),(5),(6) (12),(14),(22) (23)☆
	16	EPN	A	0.5 (0 - 1.8)	0 (0 - 0)	0 (0 - 0)	(5),(12),(16) (21)
	22	Isofenphos	A	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	(1)☆,(5),(21) (24)☆
	23	Chlorpyrifos	A	16.0 (0 - 45.5)	12.4 (0 - 37.2)	11.9 (0 - 35.6)	(1)☆,(21),(24)☆
	25	Pyridaphenthion	A	7.8 (0 - 31.1)	0.2 (0 - 0.9)	0 (0 - 0)	(1)☆,(5),(23)☆ (24)☆
	31	Tolclofos -methyl	A	28.4 (8.0 - 64.8)	9.6 (0 - 28.9)	2.7 (0 - 8.0)	(1)☆,(5),(24)☆
	41	Butamifos	A	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	(1)☆,(21),(24)☆
	42	Bensulide (SAP)	A	8.4 (1.0 - 28.0)	1.4 (0 - 5.0)	1.1 (0 - 4.6)	(1)☆,(3),(8) (11),(21)
	57	Methidathion (DMTP)	A	5.0 (0 - 17.9)	2.2 (0 - 10.9)	2.0 (0 - 10.0)	(1)☆,(5),(16) (21),(23)☆
	62	Anilofos	A	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	(1),(12),(17) (21)
	66	Dimethoate	A	4.1 (0 - 12.2)	2.3 (0 - 7.0)	2.1 (0 - 6.4)	(1)☆,(14),(21)
	71	Fenthion (MPP)	A	0.3 (0 - 1.7)	0.3 (0 - 1.3)	0.3 (0 - 1.7)	(1)☆,(5),(6) (21),(23)☆
	73	Malathion	A	12.5 (0 - 42.7)	8.2 (0 - 33.6)	7.1 (0 - 28.5)	(1)☆,(6),(17) (21)
	79	Phenthoate (PAP)	A	8.4 (0 - 18.1)	4.0 (0 - 12.0)	3.7 (0 - 11.0)	(1)☆,(5),(16) (21)
	81	Ethylthiometon	A	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	(1)☆,4),17) (21)
	88	Piperophos	A	0.1 (0 - 0.5)	0 (0 - 0)	0 (0 - 0)	(1)☆,(5),(12) (16),(21)
	5	Isoxathion	B	51.0 (2.3 - 89.8)	1.5 (0 - 3.7)	0 (0 - 0)	(12),(14),(22)
Organophosphorous (P=O)	21	Acephate	A	0 -	0 -	0 -	(7)
	72	Glyphosate	A	0 -	0 -	0 -	(12)
	92	Fosetyl	C	70.0 -	72.0 -	0 -	(12)
	11	Dichlorvos (DDVP)	D	92.9 (84.0 - 100)	96.5 (89.6 - 100)	76.2 (49.9 - 96.0)	(5),(6),(14) (22)
	15	Iprobenfos (IBP)	D	95.9 (80.5 - 105)	78.3 (41.4 - 100)	53.7 (1.5 - 93.1)	(1)☆,(4),(5) (9),(16),(22)
	49	Edifenphos (EDDP)	D	102 (99.0 - 106)	91.4 (66.0 - 105)	78.6 (64.0 - 96.0)	(5),(6),(16) (22),(23)☆
	24	Trichlorfon (DEP)	E	96.5 -	94.5 -	91.5 -	(8)
Oxon	5'	Isoxathion oxon	D	75.3 -	54.9 -	54.6 -	(12)
	6'	Diazinon oxon	D	107 -	81.5 -	78.3 -	(12)
	16'	EPN oxon	D	101 -	87.5 -	85.2 -	(12)
	7'	Fenitrothion oxon (MEP oxon)	E	96.9 -	110 -	104 -	(12)
	-	Chlorpyrifos -methyl oxon	D	84.1 -	85.0 -	82.8 -	(12)

Group	Number*1	Pesticides	Category*2	Residual Pesticides*3 (Average, Minimum-Maximum, %) 15min	4hr	24hr	Using Data for Residual Pesticides*4
Anilide	34	Metalaxyl	E	102	103	92.3	(1)☆,(6)
				(99.2 - 104)	(99.2 - 106)	(78.5 - 106)	
	52	Mefenacet	E	99.8	99.0	91.7	(2),(5),(6)
				(91.7 - 104)	(82.6 - 108)	(76.0 - 104)	(8),(9),(17)
							(18),(23)☆
Dinitroaniline	43	Benfluralin	D	85.7	87.2	86.5	(1)☆,(21),(24)☆
				(68.5 - 108)	(60.0 - 105)	(70.0 - 100)	
	100	Trifluralin	D	96.5	83.4	87.1	(4),(9),(15)
				(87.0 - 108)	(70.0 - 96.0)	(67.0 - 100)	(16)
	44	Pendimethalin	E	98.4	105	94.8	(1)☆,(15)
				(93.8 - 103)	(102 - 107)	(81.6 - 108)	
Diphenyl ether	13	Chlornitrofen (CNP)	D	94.9	86.2	82.5	(14),(22)
				(93.7 - 96.0)	(78.3 - 94.0)	(71.0 - 94.0)	
	85	Bifenox	E	96.9	101	92.6	(5),(14),(15)
				(93.3 - 99.3)	(97.0 - 104)	(87.0 - 95.8)	
	14	CNP-amino	-	-	-	-	
				-	-	-	
Pyridine	37	Dithiopyr	D	95.2	94.6	87.5	(5),(21),(22)
				(85.6 - 108)	(87.0 - 105)	(82.2 - 92.2)	
	99	Pyriproxyfen	D	79.7	77.0	78.2	(1)☆,(24)☆
				(71.0 - 88.4)	(50.0 - 104)	(61.5 - 94.8)	
	20	Triclopyr	E	95.8	94.7	102	(3),(5),(13)
				(90.8 - 100)	(80.6 - 100)	(90.3 - 113)	(21)
Phenoxy	19	2,4-D	E	90.6	90.1	92.0	(2),(5),(13)
				(79.5 - 96.0)	(73.5 - 99.0)	(71.0 - 107)	(19)
	45	Mecoprop (MCPP)	E	96.7	98.8	102	(1)☆,(3),(21)
				(86.1 - 103)	(86.5 - 107)	(85.2 - 112)	
Organochlorine	26	Iprodione*5	D	99.6	94.2	69.8	(25)
				-	-	-	
	30	Chloroneb	D	92.3	88.5	81.4	(1)☆,(21)
				(86.9 - 97.7)	(85.2 - 91.7)	(70.0 - 92.7)	
	51	Phthalide	D	94.8	88.9	89.4	(2),(4),(6)
				(76.5 - 103)	(75.5 - 102)	(82.0 - 100)	(15),(16)
	69	Endosulfan	D	91.2	92.0	86.7	(1),(16),(21)
				(84.7 - 99.0)	(81.6 - 102)	(79.9 - 97.0)	
	9	Chlorothalonil (TPN)	E	99.2	94.6	92.5	(1)☆,(6),(21)
				(90.5 - 111)	(90.8 - 104)	(77.3 - 112)	(22)
	27	Etridiazole	E	100	105	97.4	(1)☆,(21),(24)☆
				(79.0 - 124)	(97.0 - 109)	(88.5 - 115)	
	61	Procymidone*5	E	100	100	100	(26)☆
				-	-	-	
	65	Dichlobenil (DBN)	E	92.1	97.9	94.4	(1)☆,(5),(6)
				(73.6 - 104)	(88.0 - 107)	(85.8 - 102)	(9),(17)

Group	Number*1	Pesticides	Category*2	Residual Pesticides*3 (Average, Minimum-Maximum, %) 15min	4hr	24hr	Using Data for Residual Pesticides*4
Others	28	Oxine-copper*5	A	3.7	0	0	(25)
				-	-	-	
	29	Captan	A	35.4	19.9	6.9	(5),(21),(22)
				(1.5 - 88.5)	(0 - 46.0)	(0 - 11.4)	
	56	Thenylchlor	A	37.7	16.3	0	(1)☆,(8),(9)
				(0 - 71.0)	(0 - 65.0)	(0 - 0)	(23)☆
	67	Diquat	A	3.0	0	0	(12)
				-	-	-	
	80	Buprofezin	A	10.7	0.9	1.4	(4),(15),(23)☆
				(0 - 20.0)	(0 - 2.3)	(0 - 4.2)	
	8	Isoprothiolane (IPT)	B	75.6	17.6	0	(12)
				-	-	-	
	82	Probenazole	C	94.4	77.5	39.9	(6),(8),(15)
				(87.5 - 101)	(68.5 - 90.0)	(34.5 - 45.8)	
	17	Bentazone	D	95.4	90.3	76.2	(2),(3),(5)
				(94.2 - 97.0)	(80.0 - 95.0)	(22.0 - 101)	(13),(19),(21)
	70	Etofenprox	D	90.9	79.0	65.2	(17),(21)
				(75.7 - 106)	(62.0 - 96.0)	(50.3 - 80.0)	
	50	Pyroquilon	E	103	103	95.6	(1)☆,(5),(6),(9)
				(94.9 - 114)	(96.5 - 112)	(89.7 - 102)	(15),(16),(21)
	87	Tricyclazole	E	95.5	96.8	92.6	(1)☆,(3),(5),(11)
				(83.0 - 105)	(81.0 - 105)	(86.0 - 100)	(20),(21),(23)☆
	90	Azoxystrobin	E	101	99.8	104	(1)☆,(8),(21)
				(98.6 - 102)	(97.5 - 101)	(98.5 - 109)	
	97	Propiconazole	E	96.5	94.8	99.3	(8),(17),(24)☆
				(92.5 - 102)	(86.0 - 101)	(97.0 - 101)	
	101	Cafenstrole	E	100	94.8	94.5	(8),(9),(23)☆
				(97.5 - 105)	(90.0 - 97.5)	(84.0 - 104)	
	4	1,3-Dichloropropene (D-D)	-	-	-	-	
				-	-	-	
	64	Dalapon	-	-	-	-	
				-	-	-	
	91	Iminoctadine -acetate	-	-	-	-	
				-	-	-	

*1 Number: The serial number of the pesticides within Complementary Items for Setting the Targets for Water Quality Management in Japan.

*2 Category: Category was divided as follows: A (residual≦50% in 15min), B (residual≦50% in 4hr), C (residual≦50% in 24hr), D (50%<residual≦90% in 24hr), E (90%< residual in 24hr),and A and B (decomposed by chlorine rapidly) are painted over with gray.

*3 Residual Pesticides: The upper column shows the average of residual pesticides in each time after adding chlorine. The lower column shows the minimum and maximum of residual pesticides in each time after adding chlorine.

*4 Using Data for Residual Pesticides: (1)～(26) refer to **Table1**.

*5 Reference Value

ており，塩素の酸化作用により-SCH_3は-$SOCH_3$に変化したためと考えられる。Fairhead ら[17]は，この反応はプロメトリンやテルブトリン等，-SCH_3の構造を有するトリアジン系農薬に共通であると報告している。Mascolo ら[18]によると，トリアジン環と結合している-SCH_3は酸化により-$SOCH_3$,-SO_2CH_3に変化し，その後分解により-OH に変化する。-$SOCH_3$への反応は速やかであるが，それ以外の反応は比較的ゆっくり進むと報告している。更に Mascolo ら[19]は，プロメトリンに関しては-SCH_3,-$SOCH_3$,-SO_2CH_3の順に反応が進み，直接あるいは-OSO_2CH_3を経て-OH に変化することを示している。

3.1.7 チオノ (P=S) 型有機リン系農薬

有機リン系農薬は炭素骨格にリンが結合した有機リン酸エステル化合物で，除草剤，殺虫剤，殺菌剤として使用される。特に殺虫剤においては，害虫などの神経機能を阻害する[11]。有機リン系農薬のうちチオノ(P=S)型を有する 18 種類は，塩素により速やかに変化し（A,B），イソフェンホス，ブタミホス，アニロホス及びエチルチオメトンは15分後には残存率が0%（検出下限値以下）であった。小野寺ら[20]は P=S 型有機リン農薬からオキソ(P=O)型を合成するために，P=S 型原体のメタノール溶液(5mg・ml^{-1})1m*l* と，これに対して5～10 倍モル量の次亜塩素酸ナトリウムを含むリン酸緩衝液(pH6)100m*l* を混合し，30 分反応させた。これに 1M 亜硫酸ナトリウム 1m*l* を加え，抽出操作後 GC により定量したところ，P=O への変換率が 60%以上になったと報告している。この結果より，本研究においても P=O に変化したと考えられる。

3.1.8 オキソ (P=O) 型有機リン系農薬

オキソ(P=O)型有機リン系農薬は，7 種類のうち 2 種類が分解されやすかった（A）。1 つは-SCH_3を有するアセフェートで，もう 1 つは陰イオン系農薬であるグリホサートであった。グリホサート(CO_2^--NH-PO_3^{2-})は塩素により容易にグリシン(CO_2^--NH_2)を生成するとされていることから[21]，アミンと塩素の反応により N-P 間の結合が切れたものと考えられる。グリホサートと同様に陰イオン系農薬であるホセチルは，4 時間

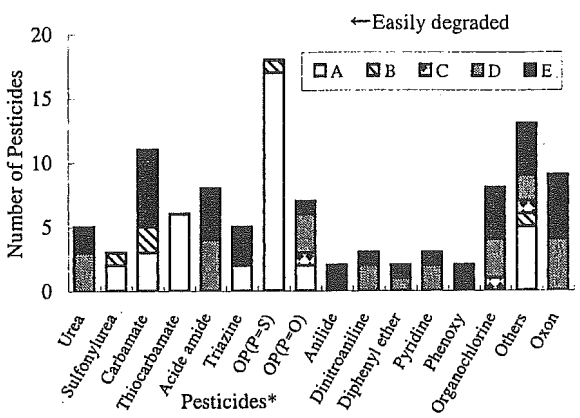

Fig.1 Degradation of pesticides by chlorination according to their basic structures: A (residual≦50% in 15min), B (residual≦50% in 4hr), C (residual≦50% in 24hr), D (50%<residual≦90% in 24hr), and E (90% <residual in 24hr). *Pesticides were divided into 16 groups.

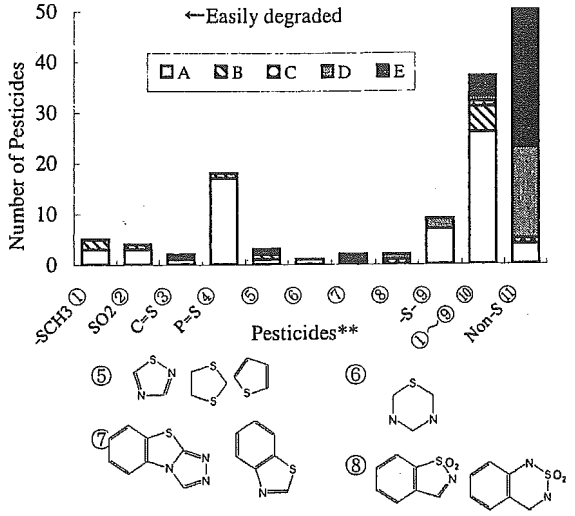

Fig.2 Degradation of pesticides by chlorination according to sulfur structures:A (residual≦50% in 15min), B (residual≦50% in 4hr), C (residual≦50% in 24hr), D (50%<residual≦90% in 24hr), and E (90%<residual in 24hr).**Pesticides were divided according to their structures into 11 groups as follows:① with SCH_3, ② with SO_2, ③ with C=S, ④ with P=S, ⑤ with S in five-membered ring, ⑥ with S in six-membered ring, ⑦ with S in heterocyclic ring, ⑧ with SO_2 in heterocyclic ring, ⑨ (except ①~⑧) with S, ⑩ with S (total of ①~⑨), and ⑪ without S.

後までは70%以上検出されたが，24時間後には検出下限以下となった（C）。

3.1.9 オキソン体

有機リン系農薬の酸化物であるオキソン体について，標準品が入手でき，測定が可能であった5種類において実験を行った。4種類は101種類の農薬，1種類は101種類の農薬以外のオキソン体である。オキソン体5種類は塩素により非常に分解されにくく（D,E），水中に長時間残存することが明らかになった。

3.1.10 その他の農薬

3.1.1～3.1.9以外に分類した，アニリド系，ジニトロアニリン系，ジフェニルエーテル系，ピリジン系，フェノキシ系，及び有機塩素系農薬は，塩素添加から24時間経過してもほとんどが残存しており，塩素により非常に分解されにくいことが明らかになった（D,E）。

上記の分類に入らなかった，その他の農薬14種類のうち，6種類がA,Bに分類された。そのうち，キャプタン，テニルクロール，ブプロフェジン及びイソプロチオランの4種類は，分子中にS（-S-あるいは複素環式における-S-）を含んでいた。Sを含まないオキシン銅は，8-ヒドロキシキノリン2分子が銅1原子とキレートした化合物であるが，小島ら[22]は，塩素により8-ヒドロキシキノリンの5位，7位，5及び7位に塩素原子が導入された3種の化合物を生成すると報告している。

3.2 101種類の農薬全体

分子構造別に分類した結果を**Fig.1**に示す。分子構造により速やかに分解される農薬，ほとんど分解されない農薬，もしくは両方を含む農薬に分かれており，構造から塩素分解性がある程度推定できると考えられる。奥村[6]によると，分子構造別に農薬を分類した結果，チオカーバメート系は塩素により分解されやすく，カーバメート系，トリアジン系，有機リン系は分解されやすい農薬と分解されにくい農薬があり，ジニトロアニリン系，ジフェニルエーテル系，有機塩素系は分解されにくいと報告しており，これらは本研究における結果と一致していた。

次にSの構造により分類した結果を**Fig.2**に示す。Sを含む農薬は46種類中38種類がAとBに分類され，分解されやすかった。特に-SCH_3,-SO_2-,P=Sを含む農薬は全てA,Bに分類された。一方，Sを含まない農薬は，50種類中45種類がDとEに分類され，分解されにくかった。そのうちAに分類された農薬は，4種類中2種類がイオン系農薬であった。これらのことから，分子中のSの有無により塩素の分解性はある程度推定でき，特に-SCH_3,-SO_2-,P=Sを含む農薬は分解されやすいことが明らかになった。

3.3 有機リンおよびオキソン体の毒性

有機リン系農薬の神経毒性は，急性毒性であるアセチルコリンエステラーゼ(AChE)活性阻害作用と遅発性毒性である神経毒エステラーゼ(NTE)活性阻害作用に大別される[23,24]。急性毒性は有機リンによるAChE阻害作用の結果，コリン作動性ニューロンのシナプス周辺に神経伝達物質であるアセチルコリン(ACh)が過剰に蓄積するために発現する[23]。さらに，遅発性毒性は，NTEの減少あるいは阻害が，多動性障害などの異常を引き起こすことが近年解明された[25]。その後の研究で，NTEはリゾレシチンを分解するリゾホスホリパーゼの一種の酵素であり，リゾレシチン分解が阻害されることによりリゾレシチンが局所的に蓄積し，神経に障害を与えることが明らかになった[26]。

有機リンは生体内でミクロソーム酸化酵素系によって酸化され，エポキシド型中間体を経てオキソン体(P=O型）に変換される[10]。小野寺ら[27]によると，オキソン体はもとの化合物に比べて強い阻害作用を持つことが知られているが，これはP=O型はP=S型に比較してP+σの求電子性が強く，コリンエステラーゼ(ChE)の活性中心と反応しやすいためであると報告している。一例として，Rompasら[28]は，クルマエビ幼生における成長の3段階で原体とオキソン体のAChEの50%阻害濃度(I_{50})を測定し，I_{50}の平均はフェニトロチオンで

195(μM)，フェニトロチオンオキソンで0.015(μM)であり，ダイアジノンで1194(μM)，ダイアジノンオキソンで1.27(μM)であることを報告している。原体に対するオキソン体のI_{50}はフェニトロチオンで1/13000,ダイアジノンで1/900であり，オキソン体のAChE活性阻害が高いことを確認している。またマラソンのI_{50}は2900(μM)，マラオキソンでは0.7(μM)であり[10]，原体に対するオキソン体のI_{50}は1/4000であった。

以上のことより，農薬は原体として速やかに分解されても，分解物や酸化物としてより毒性の高い物質に変化している可能性があることが示唆された。すなわち，塩素により分解されにくいD,Eは農薬原体に注意を要するが，塩素により速やかに分解されるA,Bにおいては，農薬原体よりも塩素の分解物や酸化物に注意する必要があると考えられる。

4. まとめ

本研究では，浄水を対象とした監視農薬の選定手法を検討する目的で,水質管理目標設定項目とされた101種類の農薬のうち，96種類及び5種類の酸化物について塩素分解実験を行い，一定時間ごとに各農薬の残存率を示した。この残存率を基に，塩素分解性が高い順にA～Eの5段階に分類し，また，農薬を共通となる分子構造別に分けた。その結果，$-SCH_3$,$-SO_2-$,P=S等，Sを含む農薬は分解されやすく，46種類のうち38種類がAとBに分類された。一方,Sを含まない農薬は，50種類のうち45種類がDとEに分類された。このことから，分子中のSの有無により，浄水過程における農薬の分解性が大部分推測でき，監視農薬を選定する指標の一つとして有効であると考えられた。

P=S型有機リン系農薬は，塩素により速やかに減少し，オキソン体(P=O型)に変化したと考えられるが，オキソン体は塩素により非常に分解されにくかった。すなわち，塩素処理によりP=S型有機リン系農薬は見かけ上消失するが，その酸化物であるオキソン体は水中に長時間残存すると推測された。

有機リン系農薬の中で，ダイアジノン等のAChE活性阻害は，原体（P=S型）よりオキソン体（P=O型）で高いと報告されている。このため，分解物や酸化物の毒性が高い農薬に関しては，原体のみならず，分解物や酸化物を監視することが不可欠であり，浄水に対する水質管理を行う上では，分解物の毒性も考慮に入れて，監視対象物質を決定することの重要性が示唆された。

今後，酸化物や酸化物の動態を把握し，毒性や塩素分解性の評価していくことが必要である。

謝　辞

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SEMI-QUANTITATIVE IMMUNOHISTOCHEMICAL ANALYSIS OF MALE RAT-SPECIFIC α_{2u}-GLOBULIN ACCUMULATION FOR CHEMICAL TOXICITY EVALUATION

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ABSTRACT — We purified male rat urinary α_{2u}-globulin, prepared the antibody in rabbits, and improved an immunohistochemical detection method using this antibody for male rat-specific α_{2u}-globulin accumulation appearing as hyaline droplets in the kidneys. Our prepared antibody reacted specifically with α_{2u}-globulin in both immunohistochemical and Western blotting analyses, furthermore, and the graded immuno-reactivities on the slide were well associated with computational image analyzing results. Using this method, we retrospectively analyzed the renal sections from the toxicity studies of 12 nephrotoxic chemicals, which had already been conducted under the Japanese Existing Chemicals Survey Program. We demonstrated that the hyaline droplets induced by treatment with 10 chemicals (1,4-dibromobenzene, dicyclopentadiene, 3,4-dimethylaniline, 1,4-dicyanobenzene, tetrahydrothiophene-1,1-dioxide, 1,3-dicyanobenzene, acenaphthene, 3,4-dichloro-1-butene, 3a,4,7,7a-tetrahydro-1H-indene and 3,5,5-trimethylhexan-1-ol) were directly associated with α_{2u}-globulin accumulation. This immunohistochemical method is convenient for applying, even retrospectively, paraffin sections from general toxicity studies and could be useful for qualifying male rat-specific hyaline droplets consisting of α_{2u}-globulin and renal risk in humans.

KEY WORDS: α_{2u}-globulin, Immunohistochemistry, Hyaline droplet, Nephrotoxicity

INTRODUCTION

For risk assessment of chemicals, the most critical data are derived from animal toxicity studies because of a general lack of information on humans. Although all available results from animal studies have been applied to human risk assessment, in principle, exclusion of some specific toxicities, which might not occur in humans, should be taken into account. Among laboratory animals, the rat has been commonly used for toxicity studies, especially sub-acute, long-term or carcinogenicity studies. Nephropathy with hyaline droplets and renal tubular neoplasia caused by chemicals inducing α_{2u}-globulin accumulation (CIGA) are considered to be a male rat-specific toxicity, not occurring in female rats or other animals, including primates. Although low molecular proteins homologous to α_{2u}-globulin can be detected in other species, including mice and humans, none of these proteins have been confirmed to bind to CIGA, followed by accumulation of the protein-CIGA complex as in the case of α_{2u}-globulin. It is therefore believed that renal toxicity induced by CIGA in male rats is unlikely to occur in humans (Hard *et al.*, 1993).

α_{2u}-Globulin was first identified in male rat urine (Roy and Neuhaus, 1966), and had been reported to be a male rat-specific protein with a molecular weight of 18 to 20 kDa. The major source of urinary α_{2u}-globulin

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is the liver, where α_{2u}-globulin mRNA constitutes approximately 1% of the total hepatic mRNA (Sippel *et al.*, 1976; Kurtz and Feigelson, 1977). Neither α_{2u}-globulin nor its mRNA is detectable in the female liver (Sippel *et al.*, 1975, 1976; MacInnes *et al.*, 1986). The blood α_{2u}-globulin secreted from the liver is freely filtered through the glomerulus, and in mature rats, about two-thirds of the filtered protein is reabsorbed by tubules and the remainder is excreted through the urine (Neuhaus *et al.*, 1981). CIGA binds noncovalently to α_{2u}-globulin, and the resulting complex shows less degradability with proteolytic enzymes, causing an accumulation of the complex that is detectable as hyaline droplets with a light microscope. Various chemicals have been suspected of being CIGA based on detection of the evidence for exacerbation of hyaline droplets in renal proximal tubules in male rats, though not in females. Direct evidence for increasing α_{2u}-globulin levels has been demonstrated for only a few of these chemicals, however, including 2,2,4-trimethylpentane (Stonard *et al.*, 1986; Charbonneau *et al.*, 1987; Lock *et al.*, 1987), decalin (Kanerva *et al.*, 1987), d-limonene (Lehman-McKeeman *et al.*, 1989; Webb *et al.*, 1989), 1,4-dichlorobenzene (Charbonneau *et al.*, 1989), isophorone (Strasser *et al.*, 1988), lindane (Dietrich and Swenberg, 1990), tri- or per-chloroethylene and pentachoroethane (Goldsworthy *et al.*, 1888).

A number of initial safety assessments has so far been conducted for industrial chemicals, including both new and existing chemicals by the Japanese government or the OECD high production volume chemicals programs. Certain chemicals among these industrial chemicals have been suspected of being CIGA. In some cases, however, renal changes in male rats have been assessed as the endpoint for extrapolation to human health risk owing to a lack of direct evidence caused by α_{2u}-globulin accumulation, because no antibody against α_{2u}-globulin is commercially available for general toxicity studies. Some immunohistochemical α_{2u}-globulin analysis methods had already been developed (Burnett *et al.*, 1989; Hashimoto and Takaya, 1992; Caldwell *et al.*, 1999). As these methods required glycolmethacrylate embedding or specific computational analysis, they would be inappropriate for confirming α_{2u}-globulin accumulation in routinely conducted guideline-based toxicity studies. We therefore improved an immunohistochemical α_{2u}-globulin detection system using paraffin sections, which are generally used for standard toxicity studies. We evaluated the several chemicals suspected of being CIGA, moreover, and indicated the direct evidence caused by α_{2u}-globulin accumulation.

MATERIALS AND METHODS

Preparation of anti α_{2u}-globulin antibody

α_{2u}-globulin as an antigen was obtained from the urine collected from aged male rats, pooled, and used to immunize rabbits. The immunization procedures, including the amount of antigen and immunizing intervals, were determined from the results of a preliminary test referring to the methods of Kurtz *et al.* (1976). The antigen was injected under the skin at a dose of 1 mg/animal (1st injection) or 0.5 mg/animal (2nd and subsequent injections) once at two weeks. Blood sampling was conducted periodically and the antibody titer measured. When the antibody titer level reached a plateau, whole blood was collected and antiserum was obtained from the blood. The antiserum was used for immunohistochemistry and immuno-electron microscopy. For measurement of the α_{2u}-globulin content in the urine and tissues, the antibody was purified from the antiserum using a DEAE ionic exchange column after ammonium sulfate precipitation. The singularity of the antibody was confirmed as a single diffuse band of approximately 19 kDa by Western blotting analysis. This study and the following study were carried out in accordance with the Law for the Humane Treatment and Management of Animals and the Standards Relating to the Care and Management, etc. of Experimental Animals in Japan.

Experiment 1 Confirmation of specific reactivity of the antibody to α_{2u}-globulin

1. Preparation of α_{2u}-globulin nephropathy rats

To confirm the specific reactivity of the anti-α_{2u}-globulin antibody, we prepared α_{2u}-globulin nephropathy rats as follows. Male and female Crj:CD(SD)IGS rats were obtained from Charles River Japan Inc. and used at the age of 11 weeks. d-Limonene (Nacalai Tesque Inc.), a well-known α_{2u}-globulin nephropathy inducer, was administered to the rats, consisting of 4 males and 4 females each, for 10 days at doses of 0, 150 and 300 mg/kg/day by gavage using corn oil as a vehicle. The rats were housed individually in stainless steel wire cages in an animal room with a controlled temperature of 24±2°C, humidity of 55±10% and a 12-hr light/dark cycle (lighting from 7:00 to 19:00) and allowed access to food and water ad libitum.

Pooled urine was collected for 24 hr on the day before the start of administration and on Day 9 of administration. After the 10-day administration period,

the rats were anesthetized with intraperitoneal injection of 30 mg/kg of sodium pentobarbital and perfused with physiological saline-added lactose (Lactec, Otsuka Pharmaceutical Factory Inc.) through the sinus aortae, after which the liver and kidneys were removed. The urine and a part of the liver and kidneys were used for measurement of their α_{2u}-globulin content and the remainder of the liver and kidneys for histopathology, immunohistochemistry and immuno-electron microscopy. The samples for histopathology and immunohistochemistry were embedded in paraffin following fixation with 10% neutral buffered formalin solution for about two weeks. The samples for immuno-electron microscopy were dehydrated with an ascending series of ethanol and embedded in spurr resin following pre- and post-fixation with 2.5% glutaraldehide and 1% osmium tetroxide solutions, respectively.

2. Histopathology and immunohistochemistry

The serial paraffin sections were prepared, deparaffinized and then stained with hematoxylin and eosin (HE) accompanied by Azan-Mallory staining and periodic acid shiff(PAS) reaction.

For immunohistochemistry, the paraffin sections were deparaffinized and incubated with 0.25% pronase E for 20 min at 37°C, after which they were washed 3 times in Tween-PBS (PBS containing 0.1% Tween 20, pH7.6). The specimens were incubated with 0.3% H_2O_2 in methanol at room temperature for 30 min to inactivate the endogenous peroxidase activity, and then washed 3 times in Tween-PBS. After blocking against nonspecific immuno-reactions with 10% FCA was conducted at room temperature for 20 min, the sections were incubated overnight with rabbit anti-α_{2u}-globulin antiserum at 4°C at a dilution of 1:80000 in PBS containing 1% BSA. Negative controls were incubated with an equivalent volume of diluent solution alone. The sections were washed 3 times in Tween-PBS and incubated with biotynilated secondary antibody (goat anti-rabbit and goat anti-mouse immunoglobulins, Dako, LSAB2 kit) at room temperature for 30 min. After they were washed 3 times inTween-PBS, the sections were incubated with horseradish peroxidase (HRP)-labelled streptavidin (Dako, LSAB2 kit) at room temperature for 30 min. The sections were then washed 3 times in PBS and reacted with 3,3-diaminobenzidine (DAB) for 5 min. The reactions were quenched by placement in running tap water, and the sections were then counterstained lightly with methylgreen, dehydrated in n-butanol, cleaned in xylene, and mounted.

3. Immuno-electron microscopy

Ultra-thin sections were prepared and reacted overnight with the anti-α_{2u}-globulin antiserum at a dilution of 1:5000 at 4°C. Protein A-colloidal Gold (10 nm, British Bio Cell International Inc.) was used at a dilution of 1:10, after which the sections were double stained with uranyl acetate and lead citrate.

4. Measurement of α_{2u}-globulin content in the liver, kidneys and urine

The α_{2u}-globulin content was measured in the liver and kidneys in all males in all the groups of α_{2u}-globulin nephropathy rats, and in the urine in two males each in the control and highest dose groups. The liver and kidneys were homogenized with phosphate buffer weighing 4 times their tissue weights and centrifuged at 105,000 g for one hour. The protein content of the supernatant thus obtained was measured for every molecular weight and the urine was measured similarly as is. Western blotting was then conducted using purified anti-α_{2u}-globulin antibody and the content of the protein showing a positive reaction was regarded as α_{2u}-globulin content.

Experiment 2 α_{2u}-globulin analysis for industrial chemicals

The selected chemicals are listed in Table 1. We selected 10 chemicals, which are suspected of being CIGA, among all the chemicals in the Japanese Existing Chemicals Survey Program (JECSP). In addition, two chemicals which caused renal toxicity without hyaline droplet accumulation were selected as negative controls. We used paraffin-embedded renal specimens originating from the JECSP toxicity studies conducted in several laboratories and stored for four to seven years in each. For each toxicity study, three groups (the control and low- and high-dose groups for 11 chemicals) or two groups (the control and high-dose groups for the other) were selected. The low-dose group has the dose showing the lowest effect for hyaline droplets in tubules or other renal changes, and the high-dose group has the highest dose administered in each toxicity study. The doses selected for each chemical are described in Table 1. Three male specimens were arbitrarily selected for each dose group based on the results obtained from HE-stained sections in the original studies.

The serial paraffin sections were prepared, deparaffinized and then stained with HE accompanied by Azan-Mallory staining and PAS reaction. The sections were also stained immunohistochemically using anti-

Table 1. Chemical name and effect dose derived from the general toxicity studies.

Chemical	Test type	Original study doses (mg/kg/day)	Effect doses (mg/kg/day) [a]			Original reported NOEL (mg/kg/day) [a]	The selected doses for analyzing (contr./low/high) (mg/kg/day)
			Histopathological findings		Non histopathological observations		
			AN	Other			
1,4-Dibromobenzene	RD	0/ 4/ 20/100/500	20≤ / –	100≤	100≤ / 20≤	4	0/ 20/500
Dicyclopentadiene	RT	0/ 4/ 20/100	4≤ / –	20≤ / 100	20≤ / 100	<4 / 20	0/ 4/100
3,4-Dimethylaniline	RD	0/10/ 50/250	50≤ / –	250	250 / 50≤	10	0/ 50/250
1,4-Dicyanobenzene	RD	0/ 1.25/ 5/ 20/ 80	5≤ / –	20≤ / –	20≤	1.25 / 5	0/ 5/ 80
Tetrahydrothiophene-1,1-dioxide	RD	0/60/ 200/700	200≤ / –	–	700	60 / 200	0/200/700
1,3-Dicyanobenzene	RD	0/ 8/ 40/200	8≤ / –	40≤ / 200	40≤	<8 / 8	0/ 8/200
Acenaphthene	RD	0/12/ 60/300	60≤ / –	300	300 / 60≤	12	0/ 60/300
3,4-Dichloro-1-butene	RT	0/ 0.4/ 2/ 10/ 50	10≤ / –	50	10≤ / 50	2 / 10	0/ 10/ 50
3a,4,7,7a-Tetrahydro-1*H*-indene	RT	0/ 67/200/600	67≤ / –	600	67≤ / 200≤	<67 / 67	0/ 67/600
3,5,5-Trimethylhexan-1-ol	RT	0/ 12/ 60/300	12≤ / –	60≤	60≤	12	0/ 12/300
2,4-di-*tert*-butylphenol	RD	0/ 5/ 20/ 75/300	– / –	300	300 / 75≤	75 / 20	0/ – /300
4-aminophenol	RD	0/ 4/ 20/100/500	– / –	100≤	100≤	20	0/100/500

[a] The data were described in a pattern of male/female when the data were different between the male and female.
RD, 28-day Repeat Dose Toxicity Test; RT, Combined Repeat Dose and Reproductive/Developmental Toxicity Test.
AN, α_{2u}-globulin nephropathy including hyaline droplets and subsequent tubular alteration.