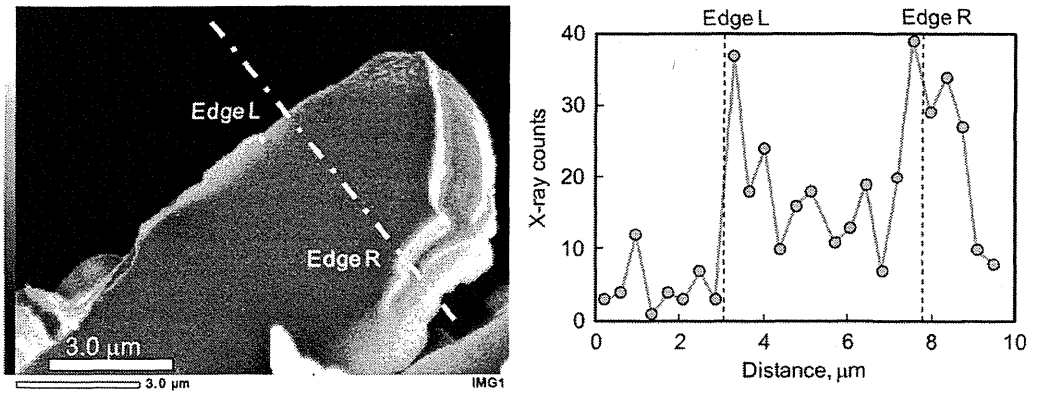

Fig. 3 – Left panel: SEM image of the cut face of a PSS-loaded PAC-T particle. EDXS line-scan analysis was conducted along the broken line in 0.047-μm increments. Right panel: Profile of X-ray counts from S obtained by EDXS line-scan analysis. The dashed lines indicate the particle boundaries.

estimate the penetration depth of 1.2 μm using 10-kV acceleration voltage for bulk carbon. Therefore, the EDXS would be regarded as center-weighted average metering scheme in the circle of around 0.6 μm radius if the penetration depth of 1.2 μm and the spherical penetration region were assumed and the diameter of the spherical penetration region was assumed to be equal to the penetration depth.

3. Results and discussion

3.1. *Adsorption isotherms and solid-phase concentration profile*

Our goal was to verify the hypothesis that organic macromolecules are adsorbed mainly on the shell region close to the external surface of powdered activated carbon particles and not in the inner region. Because the specific outer surface area (surface area per unit mass) available for adsorption is greater for smaller particles than for larger particles, this hypothesis would explain why the adsorption capacity of SPAC is larger than that of PAC. To verify the hypothesis, we used PSS as a model adsorbate and directly observed the cross-sectional profile of the PSS concentration in PAC-T particles by FE-SEM/EDXS. In previous work (Ando et al., 2010; Matsui et al., submitted for publication), in which we observed that the adsorption capacity of SPAC was larger than that of PAC, we used a PSS solution with a natural ion composition (including sulfate-ion). In contrast, in this study, we observed the PSS concentration profiles of PAC-T particles with adsorbed PSS in sulfate-ion-free water. Therefore, we first confirmed that in sulfate-ion-free water, the amount of PSS adsorbed on the SPAC was higher than that adsorbed on PAC-T (Fig. 2). Fig. 2 also suggests that a higher initial liquid-phase concentration lead to a lower solid-phase concentration. The reason for this phenomenon is not known, but this might be due to the slight heterogeneity of the PSS (Matsui et al., submitted for publication): for heterogeneous adsorbate, the isotherm results depend on the initial concentration (Sontheimer et al., 1988).

Because the sulfur (S) in PSS is in a sulfonic acid group and activated carbon contains little S (0.28 mg-S/g), the characteristic X-ray emissions from S (Kα, 2.307 keV; He et al., 1999) were counted as a measure of PSS concentration. The X-ray emission counts were scanned in 0.047 μm increments along the FIB-cut surface of a PSS-loaded PAC-T particle (Fig. 3, left

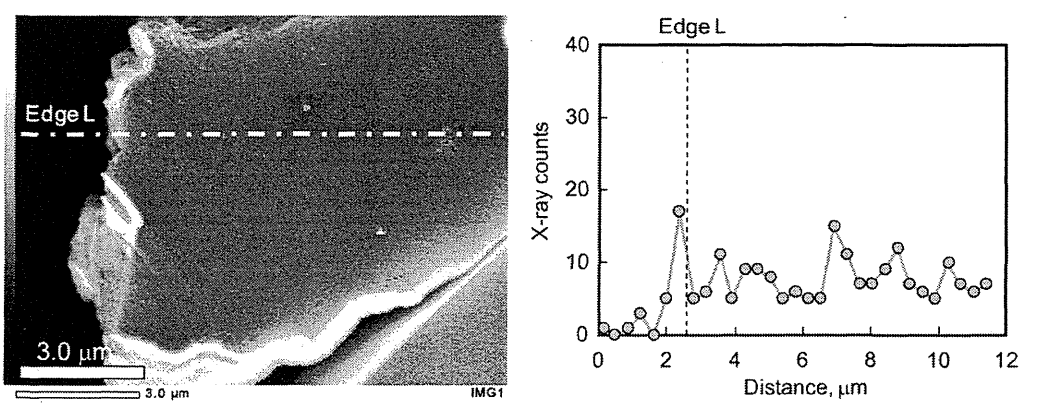

Fig. 4 – Left panel: SEM image of the cut face of a blank PAC-T particle. EDXS line-scan analysis was conducted along the broken line crossing. Right panel: Profile of X-ray counts from S obtained by EDXS line-scan analysis. The dashed line indicates the particle boundary.

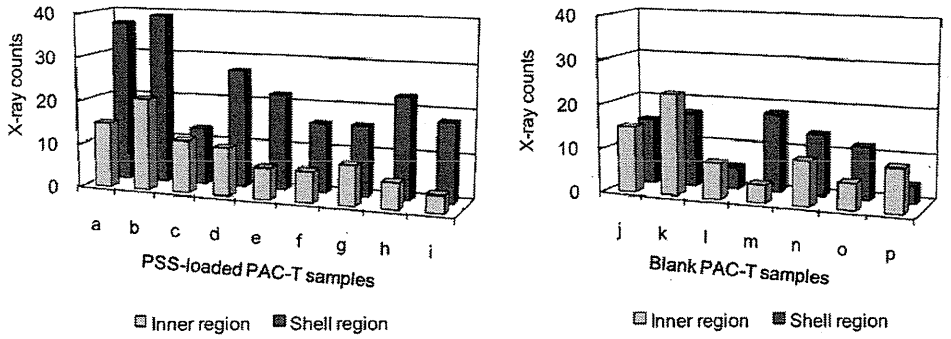

Fig. 5 – Comparison of X-ray counts in the shell and inner regions of PAC-T particles. Left panel: X-ray counts for PSS-loaded PAC-T particles a–i. Right panel: X-ray counts for blank PAC-T particles j–p. X-ray counts obtained from 0.024 to 0.400 μm (0.376-μm range) from the outer surface were summed as the counts for the shell region, and the counts obtained from 2.024 to 2.400 μm (0.376-μm range) from the interface were summed as the counts for the inner region.

panel, broken line). The characteristic X-ray emission from S was weak because X-ray production is inherently low for light elements such as S and because the amount of PSS adsorbed on the PAC-T was small (63 mg-S/g) relative to the amount of carbon. Therefore, the X-ray emission counts obtained for each 0.047-μm increment were small, and the counts were summed for each 0.376-μm interval and are plotted in Fig. 3 (right panel). The data showed some scatter, but the X-ray counts for S were clearly higher in the region close to the outer surface of the particle than in the inner region. High X-ray counts were also observed beyond the external surface of the particle (beyond Edge R in Fig. 3), but these high counts may have been due to high PSS loading on the external particle surface or to irregular X-ray scattering arising from surface roughness, which can be seen in the SEM image. The X-ray emission profile for a blank PAC-T particle did not show higher X-ray emission counts outside the particle relative to the counts inside; the emission count profile was roughly flat for the blank PAC-T particle (Fig. 4).

3.2. *Statistical hypothesis testing*

Although the FE-SEM/EDXS results (Figs. 3 and 4) supported the SAM, analytical errors were associated with the data. To verify the SAM, we prepared additional PSS-loaded PAC-T and blank PAC-T particles, cut the particles with the FIB, conducted EDXS line-scan analyses, and measured X-ray counts for the shell regions (0.024–0.400 μm from the outer surface) and the inner regions (2.024–2.400 μm from the outer surface).

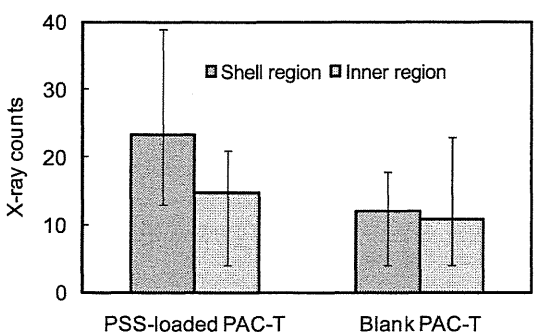

Fig. 6 – Mean X-ray counts in shell and inner regions of PSS-loaded PAC-T (n = 9) and blank PAC-T (n = 7) particles. Error bars indicate maximum and minimum values.

Comparison of the X-ray counts for the shell region and the inner region of each PSS-loaded PAC-T particle (Fig. 5, left panel) indicated that the X-ray counts for each particle were always higher in the shell region than in the inner region. For the blank particles, the X-ray counts in the shell region were not consistently higher or lower than the counts in the inner region (Fig. 5, right panel). Note that for the PSS-loaded PAC samples, the X-ray counts in the shell regions of some of the particles were lower than the counts in the inner region of other particles: for example, the X-ray counts in the shell region for PSS-loaded PAC particle "c" were lower than the X-ray counts in the inner region for PSS-loaded PAC particle "a"; that is, the ranges for the shell region and the inner region overlapped each other (Fig. 6). Therefore, we cannot definitively say that the X-ray counts in the shell region were higher than the counts in inner region. However, the overlap may have resulted from data scattering arising from the low sensitivity of S in EDXS.

To determine whether the difference between the X-ray counts in the shell region and the inner region was statistically significant, we conducted statistical hypothesis tests. The tests indicated that for PSS-loaded PAC-T, the mean number of X-ray counts in the shell region was 23.4, whereas the number of counts in the inner region was 10.2 (Fig. 6). For blank PAC-T particles, in contrast, the mean number of X-ray counts in the shell region was 12.1, whereas the number of counts in the inner region was 10.9. The statistical significance of the difference between the two mean counts was evaluated by means of the equal-variance Student's T-test after the homogeneity of variances was tested by means of the F-test. For PSS-loaded PAC-T, the P-value for the null hypothesis H_0 (that is, the hypothesis that there was no difference in the variance of counts between the shell and inner regions) was 12.9%, and the corresponding P-value for blank PAC-T particles was 75.1% (Table 1). Therefore, the null hypothesis could not be rejected for either PAC sample. By assuming the variances of counts were the same for the shell region and the inner region, we conducted the equal-variance

Table 1 – Results of F-test.

	PSS-loaded PAC-T		Blank PAC-T	
Variance of counts	Shell region	86.3	Outside	31.1
	Inner region	27.7	Inside	40.8
H_0: Null hypothesis	No difference in mean counts			
F-value	3.12		1.31	
P-value (%)	12.9		75.1	

Student's T-tests for the null hypothesis H_0 (that is, the hypothesis that there was no difference in the population means of counts between the shell and inner regions). The null hypothesis was rejected at the 0.2% level for the PSS-loaded PAC-T, whereas the null hypothesis could not be rejected for the blank PAC-T particles (P-value = 69.5%; Table 2). In summary, the statistical tests clearly indicated that the X-ray counts were different between the shell region and the inner region of PSS-loaded PAC-T particles, but for the blank PAC-T particles, the difference between the X-ray counts in the shell and inner regions was not statistically significant. Thus, we conclude that for the PSS-loaded PAC-T particles, the solid-phase concentration of PSS was higher on the outside than in the inside of the particles. Therefore, the results of the EDXS line-scan analysis clearly supported the hypothesis that the PSS was adsorbed mainly in the vicinity of the outer surface. Consequently, PAC particles, which have less outer surface area than SPAC particles, can be expected to show reduced adsorption capacity.

3.3. *Comparison with shell adsorption model*

The SAM assumes a pattern of adsorbate concentration profile described by two parameters: δ, which is the thickness of the shell (penetration depth), and *p*, which is a dimensionless parameter that defines availability of internal porous structures for adsorption. Once these parameter values are known, the solid-phase adsorbate concentration profile can be depicted. The determination of these parameter values requires the isotherm data for adsorbent particle of different sizes. In this study, the isotherm data for SPACb-T, SPACc-T and SPACd-T (Fig. 1S in the supplementary information) as well as SPACa-T and PAC-T (Fig. 2) were obtained with same PSS solution prepared for the analysis of the PAC-T samples by FE-SEM/EDXS, and the isotherm data were analyzed by means of the SAM (Matsui et al., submitted for publication). The PSS concentration profile in a PAC-T particle is depicted in Fig. 7 (solid line). The SAM results suggest that the shell was thin (shell thickness, 0.16 μm) and that the PSS load was high in the shell region. From the profile of the X-ray counts for S, however, we could not confirm the thinness of the shell, because of the low density of the data (as mentioned earlier, the X-ray emission counts were summed for each 0.376-μm interval). For comparison, we determined a moving-average PSS concentration profile for 0.376-μm intervals so that we could compare the results of the SAM analysis and the X-ray counts over the same data-acquisition interval. The moving-average PSS profile was similar to the characteristic X-ray profile, but because of data scattering and the small number of data points over the distance, we could not unequivocally conclude that the two profiles were consistent. If the two profiles were different, there are two possible explanations. First, PSS molecules may have diffused into the inner region during preparation of PAC-T samples and the FIB cutting process. Second, the effect of the large evolution area of the characteristic X-rays must be considered (Lee et al., 2006); the large area means that the EDXS method is center-weighted average metering scheme rather than spot metering scheme and the obtained X-ray counts were inevitably area-averaged counts (Castaing, 1960).

Table 2 – Results of student's T-test.

	PSS-loaded PAC-T		Blank PAC-T	
Mean counts	Shell region	23.4	Outside	12.1
	Inner region	10.2	Inside	10.9
H_0: Null hypothesis	No difference in mean counts			
Pooled variance	57.0		36.0	
T-value	3.72		0.40	
P-value (%)	0.2		69.5	

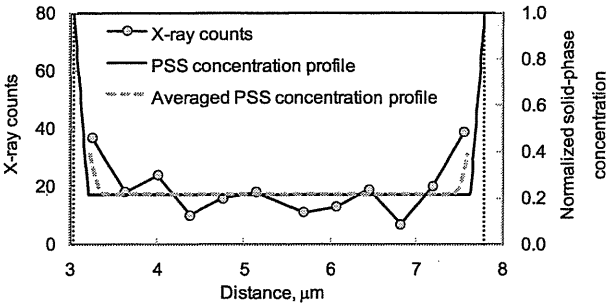

Fig. 7 – Comparison of characteristic X-ray profile (see Fig. 3) and PSS solid-phase concentration profiles. Circles: characteristic X-ray profile for S. Solid line: PSS solid-phase concentration profile obtained by SAM and PSS adsorption isotherm data. Dashed gray line: Moving-average profile of the PSS solid-phase concentration. Vertical dotted lines: Particle boundaries.

4. Conclusions

PSS-loaded and blank PACs were cut by FIB, and the solid-phase PSS concentration profiles of the particle cross-sections were directly observed by means of FE-SEM/EDXS line-scan analysis. PSS was adsorbed mainly in the shell region close to the outer surface of the particles and less so in the inner region. These results confirmed that the shell adsorption mechanism can explain the higher adsorption capacity of SPAC relative to that of PAC.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2010.08.050

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Original Article

Influence of coefficient of variation in determining significant difference of quantitative values obtained from 28-day repeated-dose toxicity studies in rats

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ABSTRACT — In order to understand the influence of coefficient of variation (CV) in determining significant difference of quantitative values of 28-day repeated-dose toxicity studies, we examined 59 parameters of 153 studies conducted in accordance with Chemical Substance Control Law in 12 test facilities. Sex difference was observed in 12 parameters and 10 parameters showed large CV in females. The minimum CV was 0.74% for sodium. CV of electrolytes was comparatively small, whereas enzymes had large CV. Large differences in CV were observed for major parameters among 7-8 test facilities. The changes in CV were grossly classified into 11. Our study revealed that a statistical significant difference is usually detected if there is a difference of 7% in mean values between the groups and the groups have a CV of about 7%. A parameter with a CV as high as 30% may be significantly different, if the difference of the mean between the groups is 30%. It would be ideal to use median value to assess the treatment-related effect, rather than mean, when the CV is very high. We recommend using CV of the body weight as a standard to judge the adverse effect level.

Key words: Coefficients of variation, Repeated-dose study, Quantitative value, Standard deviation, Chemical substance control law

INTRODUCTION

Repeated dose toxicity studies with rodents are usually conducted with a minimum of three treatment doses and a control (OECD, 1995). The quantitative data obtained from these studies are subjected to statistical analysis, using parametric or nonparametric statistical tools. If the data do not show heterogeneity and show a normal distribution, a parametric statistical tool is used, otherwise a nonparametric statistical tool. When the individual values of a parameter distribute in a wider range, it is most likely that the data show heterogeneity in variance. Distribution of data around mean can be estimated in terms of standard deviation and coefficient of variation (CV). CV is a numerical value where the proportion of the standard deviation in the mean value is shown as a percentage. Generally, the distribution of the quantitative values is broad for serum enzymes and narrow for electrolytes. Statistical significant difference of a parameter between groups is influenced by the difference of means between the groups, variance of the data and number of animals of the groups. A large difference observed between the mean values of control and dosage groups may not be statistically significant, if the variance of one or more groups explodes in a wider range.

Reports on the influence of CV in determining significant difference of quantitative values obtained from toxicity studies are rare. Matsuzawa *et al.* (1993) analyzed historical control data of clinical pathology testing provided by 67 member companies of the Japan Pharmaceutical Manufacturers Association covering study populations of approximately 14,000 rats, 10,000 dogs and 1,400 mon-

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keys. The authors assessed the potential factors contributing to variation in reference data based on weighted means and standard deviations. The authors described that the serum assay values showed greater variation than the plasma values.

In the present investigation, we examined the data of control groups of 28-day repeated-dose toxicity studies in rats performed according to the Chemical Substance Control Law (CSCL) in 12 test facilities. We examined 59 parameters of 153 studies. The number of animals per group, administration period and other factors were standardized according to the guidelines of CSCL. The distribution for each quantitative item (males and females separately) was converted into CV, and the influence of it in determining the significant difference between the groups was studied.

MATERIALS AND METHODS

The data investigated (Table 1) were from male and female SD rats of the control groups in the repeated-dose 28-day toxicity studies (Opening 153; MHLW, 2009) conducted by CSCL (NITE, 2007). CAS (Chemical Abstracts Service) numbers of the test substance administered in these studies are shown in Table 2. In these animals the vehicle was administered by oral gavage using a stomach tube. The number of animals in the group was 5-7 in most of the clinical examinations and organ weight determination and 10-12 in body weight and the feed consumption measurements. Most of the data were obtained on day 28 of the experimental period. However, few data pertaining to body weight, feed consumption, urinalyses and water consumption were obtained on days 29, 22-28, 26 and 24-26, respectively, during the experiment. The organ weight/body weight ratio was not included in the present investigation. CV (%) was calculated for each parameter using the standard formula.

The cluster analysis was conducted using the SAS JMP software (ver. 5.0; SAS Institute, Cary, NC, USA). The analysis of variance (ANOVA) and *t*-test were performed using software by Aoki (Aoki, 2010). Sex differences in CV were analyzed by the *F*-test for homogeneity of variance. When the variances were homogeneous at a significance level of 5%, Student's *t*-test was performed. When the variances were not homogeneous, Welch's *t*-test was performed. These *t*-tests were two-sided.

Items examined were (1) sex differences in CV of each quantitative value, (2) rank order of the parameters based on CV, (3) classification of CV of parameters by cluster analysis, (4) changes in CV of parameters in different test facilities, and (5) significant difference detection pattern when the difference between two groups was set constant and CV was changed.

RESULTS

CV for each quantitative item

Sex differences in CV of the 59 quantitative values are shown in Table 3. Statistically significant differences were observed in 12 items. Among them, large CVs were observed for prothrombin time (PT) and adrenal weights in the males compared with females (Table 4).

Rank order of parameters with regard to their CVs

CVs of the parameters of males and females were

Table 1. Parameters investigated

Item	Parameter
Animal care	Body weight (BW), Feed consumption (FC), Water consumption (WC)
Urinalyses	Urine volume (UV), Specific gravity (SG/urine), Osmotic pressure (OP/urine), *etc.*
Hematology	White Blood cell (WBC), Differential lymphocyte ratio (Lymph), Differential neutrophil ratio (Neut-seg), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet (PLT), Reticulocyte (RET), Fibrinogen (Fib), Prothrombin time (PT), Activated partial thromboplastin time (APTT), Methemoglobin, *etc.*
Blood chemistry	Total protein (TP), Albumin (Alb), Albumin/Globulin ratio (A/G), Total cholesterol (Cho), Total bilirubin (Bili), Triglyceride (TG), Glucose (Glu), Blood urea nitrogen (BUN), Creatinine (CRN), Triglyceride (TG), Phospholipid (PL), Alkaline phosphate (ALP), Lactic dehydrogenase (LDH), Cholinesterase (ChE), Creatine phosphokinase (CPK), Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), γ Glutamyl transpeptidase (γ-GTP), Calcium (Ca), Inorganic phosphorus (IP), Sodium (Na), Potassium (K), Chloride (Cl), *etc.*
Absolute organ weights	Brain, Thymus, Thyroids, Heart, Lungs, Liver, Spleen, Kidneys, Adrenals, Testes, Epididymis, Ovaries, *etc.* The right and left attached table record adopts the right.

Table 2. CAS number of test substance administered in the 28-day repeated-dose toxicity studies

95-64-7	99-71-8	96-29-7	83-32-9	842-18-2	84-51-5	657-84-1	109-59-1	111-17-1
100-61-8	106-37-6	96-69-5	87-59-2	1066-40-6	88-18-6	824-78-2	109-64-8	112-26-5
103-69-5	121-47-1	111-41-1	95-33-0	16219-75-3	88-89-1	1328-53-6	1552-42-7	1025-15-6
105-99-7	526-78-3	119-47-1	97-52-9	25321-09-9	95-50-1	1333-16-0	3846-71-7	27676-62-6
88-53-9	1806-54-8	126-33-0	100-54-9	79-39-0	95-57-8	5039-78-1	4286-23-1	102-81-8
140-66-9	7756-94-7	526-73-8	100-69-6	80-09-1	96-76-4	6505-28-8	7803-57-8	620-92-8
538-75-0	26967-76-0	585-07-9	103-83-3	88-19-7	102-06-7	6731-36-8	12033-89-5	96-45-7
544-76-3	583-39-1	626-17-5	108-69-0	121-45-9	106-48-9	26471-62-5	26630-87-5	77-90-7
629-62-9	623-26-7	1570-64-5	123-30-8	793-24-8	108-39-4	76-83-5	56539-66-3	96-49-1
4390-04-9	1477-55-0	1843-05-6	1241-94-7	2416-94-6	109-70-6	98-51-1	80-51-3	100-47-0
5460-09-3	56-93-9	2216-69-5	3586-14-9	5707-44-8	110-30-5	108-73-6	95-32-9	107-95-9
86-87-3	87-02-5	3319-31-1	101-83-7	38640-62-9	123-07-9	5124-25-4	97-39-2	108-87-2
95-64-7	87-84-3	3648-21-3	127-68-4	51-28-5	591-27-5	6099-57-6	97-99-4	118-75-2
100-61-8	88-44-8	25154-52-3	130-13-2	75-59-2	599-64-4	79-27-6	99-94-5	121-60-8
103-69-5	95-63-6	78-51-3	135-51-3	80-43-3	620-17-7	101-72-4	100-74-3	134-62-3
141-02-6	141-17-3	461-72-3	517-23-7	1314-98-3	2580-78-1	3710-84-7	4435-53-4	5468-75-7
9014-90-8	9016-45-9	20679-58-7	25791-96-2	40766-31-2	112-18-5	29836-26-8	118-91-2	95-68-1

combined, arranged in order and are given in Table 5. The smallest CV was 0.74% for Na and next in order were Na, Cl, SG-urine, MCHC, Ca, MCV, MCH, HGB, brain weight, HCT, Alb, TP and RBC (up to 4% CV). The CV was between 4 and 10% for PT, lymph, K, lungs weight, Fib, testes weight, A/G ratio, IP, APTT, submaxillary gland weight, heart weight, kidneys weight, epididymis weight, PLT, liver weight and CRN. For Glu, BW, FC, adrenals weight, pituitary weight, BUN, Bili, spleen weight, ASAT, PL, ovaries weight, ALAT, thyroid weight, Cho, thymus weight and RET the CV was between 10-20%. For ALP, prostate gland weight, WC, ChE, WBC, OP-urine, LDH, uterus weight, methemoglobin and ã-GTP the CV varied between 20-30%, whereas for TG, UV, CPK and Neut-seg it varied between 30-40%.

The parameters with CV similar to body weight (7.09%) were PT, Fib, lungs weight, testes weight and submaxillary glands weight. Electrolytes showed smaller CVs. On the other hand, enzymes, urine volume, Neut-seg and methemoglobin showed larger CVs.

Classification of CV of quantitative values by cluster analysis

Mean, S.D., 95% confidence limits to mean (upper and lower), median, and maximum and minimum values for the 59 parameters of males and females were combined and cluster analysis was carried out using these parameters. The results of cluster analysis are shown in Table 6 and Fig. 1. There were 11 clusters. In cluster 1, 14 parameters were classified, whereas the number of parameters classified in clusters 2, 3, 4 and 5 were 12, 14, 6 and 7, respectively. In clusters 6-11, one parameter each having larger CV was classified.

Changes in CV of parameters with regard to the test facility

Several factors, like methodology employed, equipment used, expertise of personnel, laboratory environment, etc. to quantitatively determine a parameter in laboratory animals may differ from one test facility to the other. An analysis was carried out to understand the influence of the above factors on CVs of few selected parameters among the test facilities which carried out the 28 day repeated dose toxicity studies examined in the present investigation. The parameters selected and rationale for their selection is given in Table 7. Eight test facilities, viz., A1, B2, C3, D4, E5, F6, G7 and H8, which conducted 10 or more than 10 studies, were selected for the analysis. Differences among the facilities were analyzed by ANOVA. The parameter with minimum CV was considered as control. Comparison with control was done by a one-sided Student's or Welch's *t*-test. Test for homogeneity of variance (*F*-test) was conducted before the *t*-tests. Changes in CV of quantitative values according to the test facility are shown in Table 8.

Body weight: ANOVA did not indicate significant difference among the eight test facilities. However, the minimum CV value of F6 was significantly different from H8, E5, A1 and C3.

Feed consumption, Urine volume and Lymphocyte: ANOVA revealed a significant difference among the groups. The value of these parameters with the minimum

Table 3. Sex difference in CV (%) for each parameter

Parameter	Male					Female					Sex diff., *P*
	Mean ± S.D. (*N*)	95% confidence limit of mean	Median	Min.	Max.	Mean ± S.D. (*N*)	95% confidence limit of mean	Median	Min.	Max.	
BW	7.03 ± 1.77 (151)	6.75–7.32	6.91	3.22	12.8	7.15 ± 1.71 (151)	6.87–7.42	8.05	2.5	13.1	NS
FC	10.1 ± 2.90 (150)	9.66–10.5	10.2	1.56	20.0	12.3 ± 4.54 (150)	11.5–13.0	12.4	2.53	27.7	$P < 0.05$
WC	21.9 ± 9.18 (42)	15.0–24.7	20.1	8.33	39.6	24.6 ± 13.5 (42)	20.4–28.9	21.6	4.81	83.5	NS
UV	35.8 ± 13.7 (107)	33.2–38.4	34.6	10.8	81.8	39.5 ± 14.6 (109)	36.7–42.2	37.5	9.52	83.5	NS
SG-urine	1.45 ± 1.43 (82)	1.13–1.76	1.23	0.39	13.5	1.54 ± 1.12 (84)	1.29–1.78	1.43	0.45	10.4	NS
OP-urine	26.6 ± 9.01 (24)	22.8–30.4	25.8	8.87	46.3	26.5 ± 8.35 (24)	23.0–30.1	25.4	9.49	41.8	NS
HCT	3.48 ± 1.50 (150)	3.24–3.72	3.30	1.06	9.52	3.68 ± 1.51 (150)	3.44–3.93	3.42	0.64	9.35	NS
HGB	3.57 ± 2.37 (150)	3.19–3.96	3.24	0.64	20.8	3.46 ± 1.40 (150)	2.24–3.69	3.34	0.71	8.85	NS
RBC	4.16 ± 1.69 (150)	3.89–4.43	3.89	1.52	12.3	4.16 ± 1.48 (150)	3.92–4.40	4.05	1.1	8.98	NS
MCV	2.77 ± 1.13 (149)	2.59–2.95	2.73	0.44	6.66	2.55 ± 0.88 (149)	2.41–2.69	2.37	0.80	7.76	NS
MCH	2.89 ± 1.47 (149)	2.74–3.21	2.90	0.93	15.2	2.72 ± 0.94 (149)	2.57–2.87	2.61	0.50	5.39	NS
MCHC	1.62 ± 1.37 (149)	1.40–1.83	1.46	0.29	15.3	1.64 ± 1.13 (149)	1.46–1.82	1.44	0.29	10.2	NS
RET	17.7 ± 9.07 (119)	16.1–19.4	15.3	4.30	65.3	22.7 ± 11.4 (119)	20.6–24.7	20.8	1.22	72.2	$P < 0.05$
PLT	8.95 ± 3.58 (150)	8.38–9.53	8.31	1.94	28.3	9.64 ± 4.74 (150)	8.88–10.3	9.04	2.04	34.5	NS
WBC	24.6 ± 8.78 (150)	23.2–26.1	23.6	6.92	50.0	26.8 ± 8.04 (150)	25.5–28.1	26.4	6.38	50.0	$P < 0.05$
Neut-scg	39.8 ± 17.2 (150)	37.1–42.6	38.2	7.69	116	42.7 ± 17.1 (150)	39.9–45.4	41.5	0.00	95.0	NS
Lymph	4.88 ± 2.46 (150)	4.48–5.27	4.44	1.11	15.7	5.38 ± 3.17 (150)	4.87–5.89	4.76	1.07	22.5	NS
PT	8.63 ± 6.95 (150)	7.52–9.75	6.15	1.43	6.15	4.00 ± 2.20 (150)	3.65–4.36	3.55	0.57	16.7	$P < 0.05$
APTT	7.87 ± 3.07 (150)	7.38–8.36	7.40	2.51	17.6	7.51 ± 3.68 (150)	6.92–8.10	7.33	1.31	24.6	NS
Fib	6.40 ± 2.31 (37)	5.63–7.17	6.19	1.86	12.0	7.56 ± 2.94 (37)	6.58–8.55	7.32	2.27	13.6	NS
Methemoglobin	33.6 ± 20.2 (6)	12.3–54.9	34.3	12.7	56.0	34.9 ± 21.8 (6)	12.0–57.9	29.0	11.1	6.33	NS
BUN	13.3 ± 5.30 (150)	12.4–14.1	12.4	0.00	37.5	14.4 ± 6.25 (150)	13.4–15.4	13.3	4.54	13.7	NS
CRN	10.8 ± 6.26 (150)	9.83–11.8	10.6	0.00	10.6	10.9 ± 6.66 (150)	9.90–12.0	9.81	0.00	9.81	NS
Cho	16.8 ± 7.27 (151)	15.6–17.9	15.7	2.56	45.0	18.6 ± 7.55 (151)	17.4–19.8	18.0	6.67	53.3	$P < 0.05$
Bili	16.9 ± 20.8 (118)	13.3–20.6	11.7	0.00	100	16.0 ± 15.1 (118)	13.2–18.7	14.2	0.00	100	NS
TP	3.80 ± 1.98 (151)	3.48–4.12	3.69	0.00	20.7	4.26 ± 1.60 (151)	4.00–4.51	3.92	0.90	11.1	$P < 0.05$
Alb	3.70 ± 1.66 (151)	3.43–3.96	3.32	0.00	9.37	5.11 ± 2.40 (151)	4.72–5.49	5.06	0.00	13.5	$P < 0.05$
A/G	7.32 ± 3.19 (149)	6.81–7.83	7.29	1.06	19.1	8.26 ± 4.71 (149)	7.50–9.02	7.20	2.16	7.20	$P < 0.05$
Glu	10.2 ± 4.00 (150)	9.62–10.9	10.2	2.93	24.6	9.97 ± 3.62 (150)	9.39–10.5	10.1	2.58	18.2	NS
TG	34.8 ± 13.4 (151)	32.6–36.9	33.4	9.75	75.9	34.0 ± 17.1 (151)	31.3–36.7	32.3	7.40	96.4	NS
PL	12.7 ± 4.57 (25)	10.9–14.6	13.0	4.39	25.6	15.1 ± 5.95 (25)	12.6–17.6	15.2	6.61	26.3	NS
AST	13.4 ± 6.38 (151)	12.4–14.4	12.7	2.08	34.3	14.0 ± 6.52 (151)	12.9–15.0	13.1	4.34	42.1	NS
ALT	15.7 ± 6.65 (151)	14.6–16.7	15.3	3.57	40.7	16.8 ± 8.63 (151)	15.4–18.2	15.1	1.50	61.2	NS
ALP	18.8 ± 7.12 (150)	17.7–19.9	18.3	6.46	46.8	22.5 ± 7.31 (150)	21.3–23.6	22.2	6.68	45.1	$P < 0.05$
LDH	31.3 ± 15.8 (52)	26.9–35.7	26.1	10.3	78.4	27.7 ± 12.9 (52)	24.1–31.3	24.8	5.55	70.8	NS
γ-GTP	41.1 ± 48.2 (138)	33.1–49.2	27.9	0.00	210	51.4 ± 60.8 (142)	41.4–61.4	33.1	0.00	318	NS
CPK	48.3 (2)					36.3 (2)					
ChE	21.9 ± 11.3 (27)	17.4–26.3	22.2	5.50	51.8	26.4 ± 11.7 (27)	21.8–31.1	24.6	9.16	57.5	NS
Na	0.73 ± 0.30 (149)	0.68–0.77	0.69	0.06	1.99	0.76 ± 0.32 (149)	0.71–0.81	0.70	0.13	1.70	NS
K	6.06 ± 4.99 (149)	5.25–6.86	5.02	1.03	41.0	6.47 ± 5.74 (149)	5.55–7.39	5.57	0.00	50.6	NS
Cl	1.34 ± 0.60 (149)	1.24–1.44	1.21	0.37	4.68	1.30 ± 0.65 (149)	1.20–1.40	1.19	0.37	6.03	NS
Ca	2.70 ± 1.14 (149)	2.51–2.88	2.38	0.61	8.79	2.73 ± 1.12 (149)	2.55–2.91	2.47	0.86	7.77	NS
IP	6.33 ± 3.08 (149)	5.83–6.82	5.66	1.16	22.7	9.24 ± 3.56 (149)	8.67–9.81	8.82	1.36	23.7	$P < 0.05$
Brain weight	3.41 ± 1.40 (150)	3.24–3.69	3.33	0.47	9.26	3.54 ± 1.25 (150)	3.34–3.74	3.35	0.82	7.21	NS
Submaxillary gland weight	9.31 ± 2.39 (3)	3.36–15.2	10.7	6.55	10.7	5.83 ± 2.71 (3)	0.00–12.5	7.31	2.7	7.5	NS
Pituitary weight	12.0 ± 3.92 (42)	10.8–13.2	11.9	4.2	20.6	13.8 ± 4.27 (42)	12.5–15.1	12.4	6.20	24.9	$P < 0.05$
Thyroid weight	16.6 ± 5.14 (51)	15.2–18.1	16.1	5.00	28.4	15.8 ± 5.08 (51)	14.4–17.2	15.9	3.37	15.9	NS
Thymus weight	18.2 ± 5.95 (124)	17.2–19.3	17.2	5.79	46.4	18.1 ± 6.68 (124)	16.9–19.2	17.7	4.72	49.3	NS
Heart weight	8.25 ± 3.43 (108)	7.60–8.90	7.62	2.83	20.6	8.85 ± 4.41 (108)	9.02–9.68	8.17	3.03	8.17	NS
Lungs weight	7.16 ± 4.28 (49)	5.92–8.39	6.65	1.45	31.6	7.16 ± 3.43 (49)	6.17–8.14	6.66	0.99	23.2	NS
Liver weight	9.95 ± 3.53 (150)	9.39–10.5	9.63	2.46	19.4	9.19 ± 3.26 (150)	8.67–9.71	8.75	2.63	18.4	NS
Kidneys weight	8.51 ± 3.69 (150)	7.92–9.10	8.11	1.55	32.4	7.92 ± 2.59 (150)	7.50–8.33	7.73	1.91	15.6	NS
Spleen weight	13.9 ± 5.80 (133)	12.9–14.1	13.0	2.73	33.3	13.3 ± 4.89 (150)	12.4–14.1	12.7	0.16	28.9	NS
Adrenals weight	13.1 ± 4.75 (150)	12.3–13.8	12.9	3.12	24.7	11.8 ± 4.81 (150)	11.0–12.6	11.5	2.66	27.2	$P < 0.05$
Testes v	7.44 ± 4.16 (150)	6.77–8.10	6.93	1.43	35.4						
Epididymis weight	8.88 ± 4.33 (88)	7.96–9.80	8.17	1.88	28.2						
Prostate weight	8.88 ± 4.33 (88)	7.96–9.80	8.17	1.88	28.2						
Ovaries weight						14.7 ± 4.93 (145)	13.9–15.5	14.4	3.40	25.7	
Uterus weight						27.6 ± 13.7 (16)	20.3–34.9	26.3	6.21	55.5	

NS, not significant difference. Vide Table 1 for abbreviations.

Table 4. Parameters that showed changes in CV with regard to sex

Sex	Parameter that increased compared to the other sex
Male	PT, Adrenals
Female	FC, RET, WBC, Cho, TP, Alb, A/G, ALP, IP, Pituitary

CVs were different from those respective parameters of 7 test facilities.

GOT and Sodium: Differences were observed among the test facilities. Compared to the value of G7, GOT was different in the other test facilities, whereas sodium was different in 5 facilities compared to the value of C3.

Brain, liver and spleen weights: These parameters were not statistically different among the facilities as per ANOVA. However, the value with smallest CV was significantly different in 6 facilities in the case of brain weight, 4 facilities in the case of liver weight and 3 facilities in the case of spleen weight as per t test.

Rank order of test facilities with regard to the parameters evaluated is given in Table 9. The mean CV ranks in the increasing order were D4, F6, A1, H8, G7, C3, E5, and B2.

Next, a cluster analysis was performed using the data given in Table 9 to understand the features of each test facility. Test facility G7 was excluded because data on urine volume were not available in this test facility.

The clusters were grossly divided into two bunches (Fig. 2). One bunch consisted of D4 and F6, with several parameters having small CV. The second bunch included five test facilities that had two sub-bunches, A1, H8, and E5 in the first bunch and C3 and B2 in the second bunch. The distance (difference) between D4 and B2 was the largest.

Significant difference detection pattern when the difference of mean between two groups is set constant and the CV changed

Changes in body weight are considered as the most important index in toxicity studies (MHLW, 2009). In the present study, the power of detection pattern of statistically significant difference as result of change in CV was assessed using the body weight values. In a study (CAS No. 26471-62-5), the value of 10 males in the control group was 336 g (100%) at 4 weeks after the test substance administration. A normal distribution was assumed for these rats with a CV of 7.7%. The mean value of the dosage group was set as constant (312 g, 92.8%). The CV of both the groups was changed in the range of 2 to 45%. These data were analyzed by a two-sided Student's *t*-test.

A significant difference ($P < 0.05$) was observed if the

Table 5. Rank order of parameters with regard to CV (%)

Rank order	Parameter	Mean ± S.D. (Number of study*)	95% confidence limit in mean	Median
1	Na	0.74 ± 0.31 (298)	0.71–0.78	0.69
2	Cl	1.32 ± 0.63 (298)	1.25–1.39	1.25
3	SG/urine	1.49 ± 1.28 (166)	1.30–1.69	1.33
4	MCHC	1.63 ± 1.26 (298)	1.49–1.77	1.44
5	MCV	2.66 ± 1.02 (298)	2.55–2.78	2.54
6	Ca	2.72 ± 1.13 (298)	2.59–2.84	2.46
7	MCH	2.85 ± 1.24 (298)	2.71–2.99	2.87
8	Brain	3.51 ± 1.32 (300)	3.36–3.66	3.35
9	HGB	3.52 ± 1.94 (300)	3.30–3.74	3.27
10	HCT	3.58 ± 1.51 (300)	3.41–3.75	3.37
11	TP	4.03 ± 1.82 (302)	3.82–4.23	3.85
12	RBC	4.16 ± 1.59 (300)	3.98–4.34	3.97
13	Alb	4.40 ± 2.18 (302)	4.16–4.65	3.73
14	Lymph	5.13 ± 2.84 (300)	4.81–5.45	4.55
15	K	6.26 ± 5.38 (298)	5.65–6.87	5.48
16	PT	6.32 ± 5.64 (300)	5.68–6.96	4.27
17	Fib	6.98 ± 2.69 (74)	6.36–7.61	6.88
18	Lungs	7.16 ± 3.86 (98)	6.38–7.93	6.65
19	BW	7.09 ± 1.74 (302)	10.7–11.6	10.7
20	Testes	7.44 ± 4.16 (150)	6.77–8.10	6.93
21	Submaxillary gland	7.57 ± 2.98 (6)	4.48–10.7	7.40
22	APTT	7.69 ± 3.39 (300)	7.31–8.07	7.33
23	IP	7.78 ± 3.63 (298)	7.37–8.20	7.31
24	A/G	7.79 ± 4.05 (298)	7.33–8.25	7.24
25	Kidneys	8.22 ± 3.20 (300)	7.85–8.58	7.89
26	Heart	8.55 ± 3.95 (216)	8.02–9.08	7.87
27	Epididymis	8.88 ± 4.33 (88)	7.96–9.80	8.17
28	PLT	9.28 ± 4.21 (300)	8.82–9.77	8.70
29	Liver	9.57 ± 3.41 (300)	9.18–9.96	9.19
30	Glu	10.1 ± 3.81 (300)	9.69–10.5	10.2
31	CRN	10.9 ± 6.45 (300)	10.1–11.6	10.0
32	FC	11.2 ± 3.96 (300)	10.7–11.6	10.7
33	Adrenals	12.4 ± 4.81 (300)	11.9–13.0	12.1
34	Pituitary	12.9 ± 4.17 (84)	12.0–13.8	12.2
35	Spleen	13.6 ± 5.36 (266)	12.9–14.2	12.8
36	ASAT	13.7 ± 6.44 (302)	13.0–14.4	12.8
37	BUN	13.8 ± 5.81 (300)	13.2–14.5	12.6
38	PL	13.9 ± 5.38 (50)	12.4–15.4	13.3
39	Ovaries	14.7 ± 4.90 (145)	13.9–15.5	14.4
40	Thyroid	16.2 ± 5.10 (102)	15.2–17.2	16.1
41	ALAT	16.2 ± 7.71 (302)	15.4–17.1	15.2
42	Bili	16.4 ± 17.8 (236)	14.2–18.7	12.6
43	Cho	17.7 ± 7.46 (302)	16.8–18.5	16.8
44	Thymus	18.1 ± 6.31 (248)	17.4–18.9	17.6
45	Prostate	18.9 ± 9.74 (7)	9.92–27.9	20.5
46	RET	20.2 ± 10.5 (238)	18.9–21.5	18.4
47	ALP	20.6 ± 7.43 (300)	19.8–21.5	20.2
48	WC	23.3 ± 11.5 (84)	20.7–25.8	21.5
49	ChE	24.2 ± 11.6 (54)	21.0–27.3	23.0
50	WBC	25.7 ± 8.47 (300)	21.8–26.7	25.1
51	OP/urine	26.6 ± 8.59 (48)	24.1–29.1	25.5
52	Uterus	27.6 ± 13.7 (16)	20.3–34.9	26.3
53	LDH	29.5 ± 14.5 (104)	36.7–32.3	25.8
54	Methemoglobin	34.3 ± 20.1 (12)	21.5–47.1	29.0
55	TG	34.4 ± 15.4 (302)	32.7–36.1	33.0
56	UV	37.9 ± 14.2 (216)	35.7–39.6	35.3
57	Neut-seg	41.3 ± 17.1 (300)	39.3–43.2	40.0
58	CPK	42.3 ± 16.3 (4)	16.3–68.2	36.3
59	γ-GTP	46.3 ± 55.1 (280)	39.9–52.8	30.0

* Values given in parentheses are number of observations from both males and females.

difference in body weight mean was about 7% between the groups and the CV was 7-8% (Table 10). A significant difference was observed for the γ-GTP, CPK, neutrophil cell, urine volume, and triglyceride levels which showed a CV of 30% or more and a 30% difference of mean between groups.

Table 6. Classification of CVs of 59 parameters by cluster analysis

Custer No.	Parameter
1	BW, Fib, Submaxillary gland weight, PLT, Epididymis weight, Kidneys weight, Heart weight, APTT, IP, A/G ratio, Lungs weight, Testes weight, PT, K
2	FC, Adrenals weight, Gul, Liver weight, BUN, Spleen weight, ASAT, CRA, PL, Pituitary weight, Thyroid weight, Ovaries weight
3	SG-urine, MCHC, Na, Cl, HCT, Brain weight, RBC, MCV, Ca, MCH, HGB, TP, Alb, Lymph
4	WC, ChE, LDH, Uterus weight, OP-urine, WBC
5	RET, Cho, ALAT, ALP, Thymus weight, Prostate gland weight
6	Bili
7	UV, TG
8	Methemoglobin
9	Neut-seg
10	CPK
11	γ-GTP

DISCUSSION

We calculated CV for 59 quantitative values obtained from the 153 numbers of 28-day repeated-dose toxicity studies carried out in accordance with the CSCL in 12 test facilities. We conclude the following from our findings:

1. Sex difference in CV was observed in 12 out of 59 items including body weight. Larger CV was observed in 10 items of male.

2. The quantitative value with the smallest CV was 0.74% for sodium. Electrolytes and calculated hematology values showed smaller CVs, whereas, enzymes showed larger CVs.

3. The values based on their CVs were classified into 11 clusters, which were grossly divided into 4 to 5 bunches.

4. CVs for liver, brain and spleen weights were similar in all the test facilities studied. However, large differences in body weight and some clinical laboratory tests were observed in few test facilities. Therefore it is most likely that statistically significant differences could be detected for the data with small CVs in some test facilities. Similarly, no statistical significant difference will be shown by the data with large CVs in some test facilities.

5. A significant difference is seen at 5% probability level, if the difference of the mean value between the groups is about 7% and CV 7%-8%.

6. A statistically significant difference can be detect-

Table 7. Influence of laboratory factors on CVs of parameters analysed. List of parameters selected and the rationale for their selection

Parameter	Rationale for selection
Body weight	Toxicity of a chemical is primarily reflected on body weight. Several factors like poor animal husbandry, animal house condition and improper handling of the animals can affect body weight.
Feed consumption	The factors that may affect body weight can affect feed consumption of the animals. Accurate measurement of feed consumption is not an easy task because of spillage. Feed hoppers vary from one laboratory to the other.
Urine volume	Metabolic cages are used for urine collection. These cages differ from one testing facility to the other.
Lymphocyte	Lymphocyte count usually done under a microscope, varies among the technicians who perform the count.
GOT	Variation in GOT estimation may occur with regard to the analytical reagents, methodology, instrument calibration and the nature of the plasma/serum sample (hemolysed samples may show erratic GOT values).
Sodium	Fluctuation in sodium is the least compared to other clinical chemistry parameters.
Brain weight	Absolute weight of the brain can be determined accurately in all the test facilities
Liver weight	Onslaught of toxicity effect of a chemical is reflected on liver and variation in absolute weight of this organ is usually seen.
Spleen weight	Most of the test facilities determine absolute weight of the spleen accurately.
Other parameters	Instruments used to determine other hematology and blood chemistry parameters are not different from those used to determine above parameters. Enzymes that show very larger variations are not also not included. Erythrocyte indices *etc.* have a lot of calculation values. However, these parameters were seemed no large changes.

Table 8. Changes in CV of quantitative values according to test facilities

Parameter	Test facility	Mean ± S.D. (%)	No. of study (male + female)	*P* ANOVA	*P* *t*-test
Body weight	F6	6.36 ± 1.81 (100)	20	0.485	vs. F6
	D4	6.81 ± 1.54 (107)	40		0.156
	G7	6.98 ± 1.45 (109)	36		0.084
	B2	7.08 ± 1.83 (111)	28		0.091
	H8	7.17 ± 1.50 (112)	30		0.045
	E5	7.18 ± 1.27 (112)	26		0.038
	A1	7.31 ± 1.87 (114)	48		0.028
	C3	7.32 ± 2.14 (115)	50		0.040
Feed consumption	D4	7.00 ± 2.11 (100)	40	0.000	vs. D4
	B2	10.6 ± 4.03 (152)	28		< 0.01
	A1	10.7 ± 3.04 (153)	48		< 0.01
	G7	11.4 ± 3.25 (163)	34		< 0.01
	C3	12.2 ± 3.55 (174)	49		< 0.01
	H8	12.6 ± 3.74 (180)	30		< 0.01
	E5	12.8 ± 4.92 (183)	26		< 0.01
	F6	13.2 ± 4.16 (189)	20		< 0.01
Urine volume	F6	1.43 ± 0.40 (100)	20	0.000	vs. F6
	D4	19.3 ± 17.2 (1349)	14		< 0.01
	C3	29.0 ± 10.8 (2027)	36		< 0.01
	H8	35.5 ± 12.0 (2482)	28		< 0.01
	A1	35.9 ± 15.6 (2510)	46		< 0.01
	E5	42.4 ± 16.5 (2965)	26		< 0.01
	B2	44.4 ± 13.8 (3104)	28		< 0.01
Lymphocyte	E5	3.10 ± 0.99 (100)	26	0.000	vs. E5
	H8	4.32 ± 1.97 (139)	28		< 0.01
	A1	4.35 ± 2.65 (140)	48		< 0.01
	D4	4.44 ± 1.72 (143)	40		< 0.01
	H8	5.49 ± 1.94 (177)	36		< 0.01
	C3	6.16 ± 3.52 (198)	50		< 0.01
	B2	6.49 ± 3.05 (209)	28		< 0.01
	F6	7.38 ± 4.04 (238)	20		< 0.01
GOT	G7	10.1 ± 4.36 (100)	36	0.001	vs. G7
	H8	12.8 ± 5.78 (126)	30		0.022
	C3	12.9 ± 8.29 (127)	50		0.026
	A1	12.9 ± 6.39 (127)	48		0.012
	F6	14.9 ± 4.95 (147)	20		< 0.01
(Continued)					
	D4	15.3 ± 4.25 (151)	40		< 0.01
	E5	15.8 ± 7.08 (156)	26		< 0.01
	B2	16.0 ± 6.41 (158)	28		< 0.01
Sodium	C3	0.61 ± 0.26 (100)	50	0.000	vs. C3
	F6	0.62 ± 0.21 (101)	20		0.452
	D4	0.65 ± 0.19 (106)	40		0.184
	A1	0.72 ± 0.29 (118)	48		0.023
	B2	0.79 ± 0.25 (129)	28		< 0.01
	H8	0.81 ± 0.28 (132)	30		< 0.01
	E5	0.82 ± 0.43 (134)	26		0.015
	G7	0.89 ± 0.29 (145)	34		< 0.01
Brain weight	F6	2.79 ± 0.87 (100)	20	0.081	vs. F6
	D4	3.22 ± 1.12 (115)	38		0.074
	A1	3.40 ± 1.20 (121)	48		0.022
	H8	3.45 ± 1.09 (123)	30		0.014
	G7	3.56 ± 1.49 (127)	36		< 0.01
	E5	3.58 ± 1.17 (128)	26		< 0.01
	C3	3.79 ± 1.33 (135)	50		< 0.01
	B2	3.83 ± 1.72 (137)	28		< 0.01
Liver weight	F6	8.17 ± 2.41 (100)	20	0.726	vs. F6
	D4	9.35 ± 3.67 (115)	38		0.102
	A1	9.46 ± 3.88 (121)	48		0.052
	H8	9.53 ± 2.41 (123)	30		0.028
	E5	9.61 ± 3.31 (127)	26		0.054
	B2	9.69 ± 3.19 (128)	28		0.040
	G7	9.70 ± 2.74 (135)	36		0.021
	C3	10.0 ± 4.05 (137)	50		0.010
Spleen weight	F6	11.4 ± 4.49 (100)	20	0.549	vs. F6
	D4	12.6 ± 3.60 (110)	30		0.212
	A1	13.1 ± 5.63 (114)	48		0.129
	G7	13.6 ± 5.12 (119)	28		0.072
	E5	13.9 ± 6.06 (121)	20		0.081
	H8	13.9 ± 4.47 (121)	30		0.032
	B2	14.1 ± 5.44 (123)	28		0.042
	C3	14.3 ± 6.40 (125)	38		0.042

Table 9. Rank order of test facilities with regard to the parameters evaluated. Ranking is done from smallest to largest CV

Parameter	Test facility							
	D4	F6	A1	H8	G7	C3	E5	B2
Body weight	2	1	7	5	3	8	6	4
Feed consumption	1	8	3	6	4	5	7	2
Urine volume	2	1	5	4		3	6	7
Lymphocyte	4	8	3	2	5	6	1	7
GOT	6	5	4	2	1	3	7	8
Sodium	3	2	4	6	8	1	7	5
Brain weight	2	1	3	4	5	7	6	8
Liver weight	2	1	3	4	7	8	5	6
Spleen weight	2	1	3	6	4	8	5	7
Mean rank	2.6	3.1	3.8	4.3	4.6	5.4	6.6	7.1

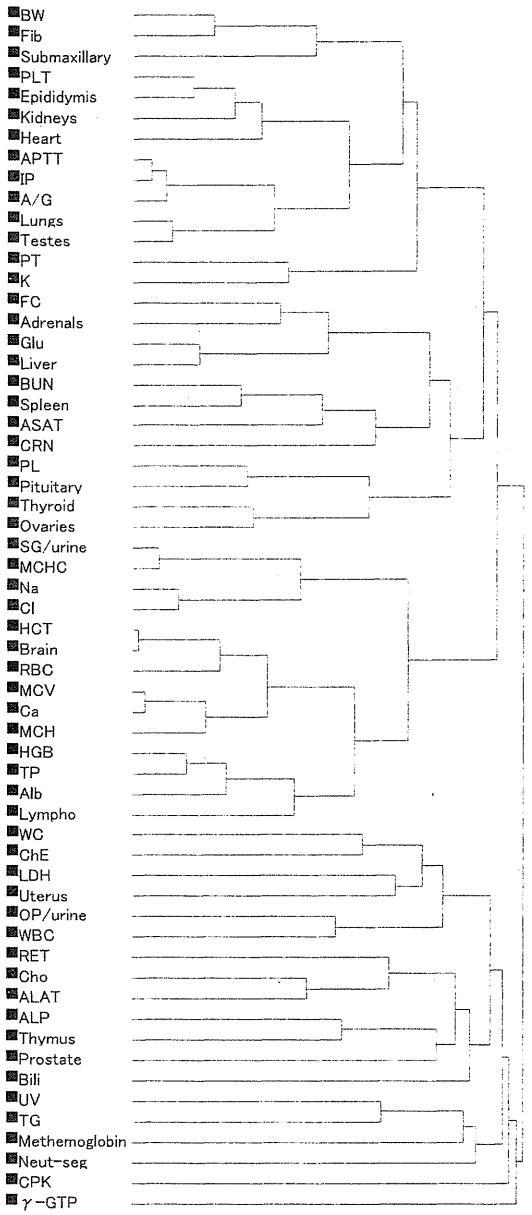

Fig. 1. Classification of CV of quantitative value by cluster analysis.

ed for parameters with extremely small CVs even for a smaller difference in mean values.

Information on the influence of CV in determining significant difference of quantitative parameters obtained from animal toxicity studies is scarce. Aoyama (2005) suggested that when the number of animals is adjust-

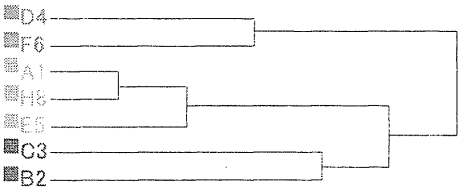

Fig. 2. Classification of CV of test facilities by cluster analysis.

Table 10. Significant difference power pattern when the body weight difference between two groups is set to a constant and CV is changed

Control group: 336 g vs. dosed group: 312 g	
Coefficient of variation (%) of two groups	*P* value
2	0.000
3	0.000
4	0.000
5	0.003
6	0.012
7	0.029
8	0.053
9	0.082
10	0.115
12	0.184
14	0.252
16	0.314
18	0.369
20	0.418
25	0.516
30	0.587
35	0.641
40	0.683
45	0.717

ed, the decentralization of data, like body weight and the organ weight, becomes comparatively smaller, and a CV of about 10% is obtained. CV for blood levels of various hormones, even data in the control group are large. Often, the standard deviation exceeds the mean value by more than 50% for these parameters. Present study also reveals similar findings. CVs have greater influence in determining the significant difference of a parameter in repeated dose toxicity studies and the CVs vary considerably for certain parameters in different test facilities. Thus priority should be given to a judgment of toxicological effects, not to a statistically significant difference when such a dif-

ference is smaller than that based on CV of body weight measurements.

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（1） ヨード造影剤の琵琶湖・淀川水系及び塩素処理過程における挙動

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ヨード造影剤は水環境に多く残存する医薬品の一種である．また，既知の有機ヨウ素系消毒副生成物は毒性が高い傾向がある．ヨード造影剤は塩素処理過程において，有機ヨウ素系消毒副生成物の前駆体となる可能性がある．そこで，琵琶湖・淀川水系におけるヨード造影剤と吸着性有機ヨウ素（AOI）の濃度を調査した．ヨード造影剤の濃度は流下とともに増加する傾向がみられ，AOIに対するヨード造影剤の寄与率も流下とともに増加し，最大20%程度となった．また，ヨード造影剤と塩素との反応性について評価した．その結果，物質により異なる反応性を示すことがわかった．さらにイオパミドールと塩素の反応速度定数を推定し，速度論的に塩素処理過程でイオパミドールは他物質へ変換されうることを指摘した．

Key Words : *iodinated X-ray contrast media, absorbable organic iodine, water chlorination, iodinated disinfection byproducts*

1. はじめに

様々な医薬品が使用され，水系に流入している．医薬品自体は，高用量での安全性は確認されており，仮に水道水中に残存したとしても，ヒトに対する健康リスクは十分低いとされている．しかし，この評価は，浄水処理過程における酸化・ハロゲン化反応など他物質への変換過程を十分に考慮していない．すなわち，残留医薬品の消毒副生成物前駆体としての重要性についても評価が必要となるが，このような調査は十分なされていない．

残留医薬品の1つとしてヨウ素を分子内に含むヨード造影剤があげられる．ヨード造影剤は環境水中にμg/Lのレベルで検出され[1,2]，医薬品の中では比較的高濃度で存在する．ヨード造影剤と酸化剤の反応については，オゾンとの反応に関する知見はあるが[3]，塩素との反応に関する知見は皆無である．このため，ヨード造影剤が消毒副生成物の前駆体となる可能性を否定できない．

ハロ酢酸のように既知の有機ヨウ素系消毒副生成物は塩素や臭素を含む有機消毒副生成物よりも単位濃度あたりの毒性が高い傾向があるとされており，低濃度であっても毒性への寄与率としては無視できない可能性がある[4]．塩素処理中でのヨウ化物イオンの挙動は明らかにされており，有機ヨウ素系化合物への経路とヨウ素酸イオンへの経路が競合している[5-7]．そのため，ヨウ化物イオン由来の有機ヨウ素系消毒副生成物の生成は限定的と考えられる．しかし，ヨード造影剤のように分子内にすでにヨウ素を含む化合物が塩素と反応して有機ヨウ素系消毒副生成物が生成する経路が見落とされている可能性がある．そのため，水道原水中の有機ヨウ素系化合物の内訳，挙動の把握は重要である．

そこで本研究では，ヨード造影剤に由来するヒトに対する健康リスク評価のための基礎的情報の収集として，琵琶湖・淀川水系を対象としたヨード造影剤の水系での分布調査と吸着性有機ヨウ素に対する寄与を明らかにした後，塩素との反応速度定数の推定と反応生成物の探索を目的に実験的検討を行った．なお，吸着性有機ヨウ素とは吸着性有機ハロゲンの測定方法で測定できる有機ヨウ素化合物のことである．

本論文は上記の目的に対応して，琵琶湖・淀川水系におけるヨード造影剤と吸着性有機ヨウ素の調査と，塩素処理過程におけるヨード造影剤の挙動の2部構成とした．

2. 調査・実験方法

本論文は琵琶湖･淀川水系における調査と4つの実験的検討で構成される．まず，琵琶湖・淀川水系におけるヨード造影剤と吸着性有機ヨウ素（AOI）の分布調査を行った．次に個々のヨード造影剤の塩素との反応性を確認した後，塩素との反応速度定数の推定を行い，評価した．この後，pHの影響について評価した後，反応生成物の探索を行った．以下この順で調査と実験の方法を述べる．ただし，分析方法については共通部分が多いため，(3)にまとめて記述した．また，実験，分析には特に断りがない限り，試薬は和光純薬製試薬特級を用いた．水溶液の調製や希釈にはMILLIPORE社製のMilli-Q Academic A10（0.22μm Millipak-20 Express）で精製した超純水を用いた．

(1) 琵琶湖・淀川水系におけるヨード造影剤と吸着性有機ヨウ素（AOI）の分布

a) 調査対象

本研究で対象とするヨード造影剤は，日本で一般的に使用されているものとして，薬事工業生産動態統計年報の特掲医薬品に掲載されているイオパミドール（IPD），イオヘキソール（IHX）とした[9]．IPDの構造式を図-1に，IHXの構造式を図-2に示す．また，ヨード造影剤の吸着性有機ヨウ素への寄与を評価およびヨウ素の挙動の把握のために吸着性有機ヨウ素（AOI）とヨウ化物イオン(I^-)も測定対象とした．

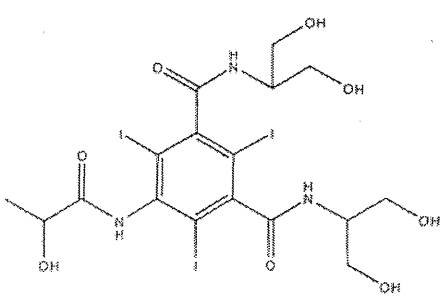

図-1　IPDの構造式

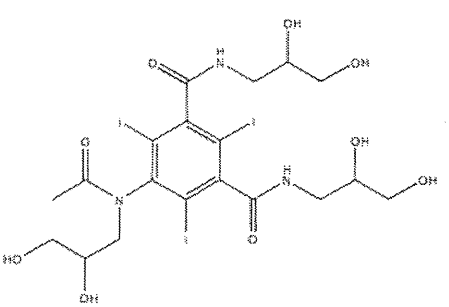

図-2　IHXの構造式

b) 調査地点

流域内でのヨード造影剤の全体的な分布が把握できるように採水地点を選定した．上流部では人為起源の排水の影響のない最源流部で採水を行った．都市部では下水処理施設放流水や河川の合流の影響を把握するため，下水処理水や河川の合流部を重点的に採水した．採水地点を図-3に示す．

c) 採水方法

採水にあたっては日間変動を最小限にするため全地点について一日で採水を行った．また，ヨード造影剤が使用されると考えられる医療機関が診察を行っている平日に採水を行った．試料は超純水で洗浄したガラスビンに，共洗い後採水した．採水した試料は冷蔵保存の上，実験室に持ち帰り，直ちにろ過（ADVANTEC GA-100 90mm）した．採水は2010年11月15日，11月30日，12月9日の計3回行った．

(2) 塩素処理過程におけるヨード造影剤の挙動

a) ヨード造影剤の塩素との反応性

各ヨード造影剤と塩素との反応性を把握するために比較的高濃度の実験条件でIPDとIHXの濃度変化を追跡した．具体的にはヨード造影剤の濃度を50 μg/L，塩素濃度を10 mg/Lとし，12，24，36，48時間pH 7の1 mMのリン酸緩衝液中で反応させた．これらの試料のヨード造影剤の濃度を測定した．

b) 反応速度定数の推定

1 μg/LのIPDと1，2，3 mg/Lの塩素をpH 7の1 mMリン酸緩衝液中で反応させ，0，6，12，18，24，36，48時間後にチオ硫酸ナトリウム（和光純薬製容量分析用）で反応を停止し，ヨード造影剤の濃度を測定した．

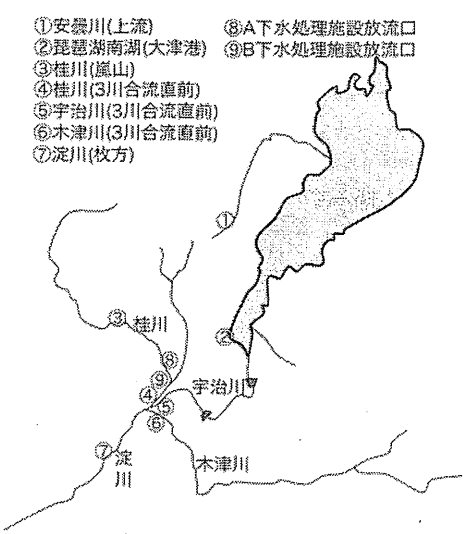

図-3　採水地点

c) pHの影響評価

pHの影響を評価するため1 μg/LのIPDと1 mg/Lの塩素をpH 6，7，8の1 mMのリン酸緩衝液中で反応させ，0，24，48時間後にチオ硫酸ナトリウムで反応を停止し，ヨード造影剤の濃度を測定した．

d) 反応生成物の探索

反応生成物の探索の対象物として，ジクロロヨードメタン（DCIM），ヨード酢酸，クロロヨード酢酸を選択した．100 μg/LのIPDと1，10，100 mg/Lの塩素をpH 7の1 mMのリン酸緩衝液中で24時間反応させ，DCIM，ヨード酢酸，クロロヨード酢酸の測定を行った．

(3) 分析方法

a) ヨード造影剤の測定方法[9]

まず，3 mol/Lの硫酸で各試料をpH 3に調整した．次に，0.1%ギ酸メタノール，メタノール，超純水それぞれ10 mLでコンディショニングを行ったInertSep Slim RP-2（GLサイエンス社製）に10 mL/minで試料を通水した．調査では試料を400 mL，(2)のb)，c)では試料を50 mL通水した．その後，カートリッジに超純水10 mLを通水し，窒素ガスを吹き付けて40分間乾燥させた．メタノール10 mLで溶出後，窒素ガスで乾固し，内標準物質100 μg/Lを含む超純水1 mLで再溶解した．この試料をLC/MSで測定した．LC部はWaters 2695 Separation Moduleを，MS部はmicromass ZMDを使用した．分析条件を表-1に，グラジエント条件を表-2に，モニタリングイオンの質量数と保持時間を表-3に示す．標準品は，IPDには和光純薬製薬理研究用を，IHXにはLKT Laboratories製を用いた．また，内標準物質には3つの水素が重水素に置換されたイオパミドール（Toronto Research Chemicals製）を用いた．移動相のギ酸，アセトニトリルはともに和光純薬製試薬LC/MS用を用いた．なお，定量限界はIPDは1 μg/L，IHXは2 μg/Lであったが，日によってLC/MS感度が変動したため，測定ごとに定量限界を求めた．また，標準品の回収率はIPDは20%，IHXは35%であった．

b) 吸着性有機ヨウ素（AOI）の測定方法

まず，濃硝酸で試料をpH 2に調整した．この試料100 mLを全有機ハロゲン吸着装置（TX-3AA，三菱化学製）で，2本直列に接続した活性炭プリパックドカラム（TXAPCC，三菱化学アナリテック製，一本あたりの活性炭充填量は40 mg）に通水した．通水後に濃硫酸でpH 2に調節した硝酸カリウム溶液（濃度8.2 g/L）を10 mL通水し無機ヨウ素を除去した．その後，TOX10Σ（三菱化学製）を用いて，酸素気流中で900 ℃で10分間活性炭を燃焼させた．燃焼炉の排ガス出口には超純水10 mLを入れた小型のインピンジャーを装着し，AOIが燃焼してヨウ化水素に変化したものを超純水に溶解させた．この試料

表-1 ヨード造影剤LC/MS分析条件

移動相	0.05% ギ酸
	アセトニトリル
分析カラム	Atlantis dC18 2.1×150 mm 3 μm (Waters)
測定時間	40 min
イオン化法	ESI+
カラム温度	30 ℃
注入量	10 μL
流量	0.2 mL/min
キャピラリ電圧	3.5 kV
コーン電圧	30 V
Source Temp.	100 ℃
Desolvation Temp.	350 ℃

表-2 ヨード造影剤LC/MSのグラジエント条件

時間(min)	移動相	
	0.05%ギ酸	アセトニトリル
0	95%	5%
8	95%	5%
13	1%	99%
15	1%	99%
15	95%	5%

表-3 モニタリングイオンの質量数と保持時間

測定物質	保持時間(min)	質量数(m/z)
IPD	4.08	778
IHX	5.72	822
内標準物質	4.08	781

表-4 ヨウ化物イオン濃度分析条件

システム	SHIMADSU LCsolution
溶離液	35 mM NaOH
分析カラム	DIONEX IonPac AS16 4×250 mm
ガードカラム	DIONEX IonPac AG16 4×50 mm
検出器	紫外可視吸光検出器 SPD-10
測定波長	226 nm
流量	1.0 mL/min
カラム恒温槽温度	30 ℃
測定時間	30 min

をイオンクロマトグラフでヨウ化物イオン濃度として測定した．分析条件を表-4に示す．ヨウ化物イオンの標準的な溶出時間は9 minであった．なお，標準品の回収率は55%であった．

c) ヨウ化物イオン（I⁻）の測定方法

ヨウ化物イオンはイオンクロマトグラフで測定した．分析条件は(3)のb)の表-4と同様である．なお，定量限界は1 μg/Lであった．

d) DCIMの測定方法

DCIMの測定は液液抽出を行った後にGC/MSで行った．まず，試料40 mLを50 mLグラスバイアルに移し，塩化アンモニウムを約3 mg加えた．その後硫酸（1＋1）を30 μL加え，pH 2以下に調節した．続いて，16 gの塩化ナトリウムを加え，次いで内標準を含んだヘキサンを4 mL加え，振とう機を用いて250 回/minで3分間振った．内標準物質としてブロモジクロロメタンを用いた．その後，上層のヘキサン層から約3 mLを遠沈管にとり，硫酸ナトリウムを流動するようになるまで加えて脱水した．ヘキサン層約2 mLをバイアルに移し測定した．測定にはGC/MS（SHIMADZU QP2010 Plus）を用いた．分析カラムにはJ&W Science社製のDB-5MS（30 m×0.32 mm i.d. 膜厚0.25 μm）を用いた．GC/MSの分析条件を表-5に，モニタリングイオンの質量数を表-6に示す．DCIMの標準試料はCansyn製を用いた．なお，予備実験で10 μg/Lまで測定できることを確認した．

e) ヨード酢酸とヨードクロロ酢酸の測定方法

ヨード酢酸とヨードクロロ酢酸の測定はLC/MSで行った．分析条件を表-7に，グラジエント条件を表-8に，モニタリングイオンの質量数と保持時間を表-9に示す．なお，クロロヨード酢酸の標準品はCansyn製を用いた．

表-5　GC/MSの分析条件

キャリアーガス	He
流量	73.9 cm/sec
気化室温度	200 ℃
モード	スプリットレス
サンプリング時間	0.45 min
注入量	1 μL
パージ流量	4.5 mL/min
スプリット比	30
オーブン温度	30 ℃(6 min)→5 ℃/min→60 ℃ →25 ℃/min→150 ℃(2 min)
イオン源温度	200 ℃
インターフェイス	280 ℃
測定モード	SIM

なお，ヨード酢酸は10 μg/L，クロロヨード酢酸は5μg/Lまで定量できることを確認した．

3. 琵琶湖・淀川水系におけるヨード造影剤と吸着性有機ヨウ素の分布

測定結果を表-10，表-11，表-12に示す．なお，検出限界値はヨウ化物イオン濃度では1 μg/L，AOIでは0.17 μg/L，IPDは第1回，第2回調査では0.13 μg/L，第3回調査では0.013 μg/L，IHXは第1回，第2回調査では0.071 μg/L，第3

表-6　GC/MSのモニタリングイオンの質量数と保持時間

測定物質	保持時間(min)	質量数(m/z)
DCIM	5.4	83
ブロモジクロロメタン	2.4	83

表-7　ヨード酢酸およびクロロヨード酢酸測定時のLC/MSの分析条件

移動相	0.05% ギ酸 アセトニトリル
分析カラム	Atlantis dC18 2.1×150 mm 3 μm(Waters)
測定時間	30 min
イオン化法	ESI-
カラム温度	30 ℃
注入量	10 μL
流量	0.2 mL/min
キャピラリ電圧	1.5 kV
コーン電圧	20 V
Source Temp.	120 ℃
Desolvation Temp	350 ℃

表-8　ヨード酢酸，クロロヨード酢酸のグラジエント条件

時間(min)	移動相	
	0.05%ギ酸	アセトニトリル
0	95%	5%
8	95%	5%
8	0%	100%
18	0%	100%
18	95%	5%

表-9　ヨード酢酸，クロロヨード酢酸のモニタリングイオンの質量数と保持時間

測定物質	保持時間(min)	質量数(m/z)
ヨード酢酸	5.83	185
クロロヨード酢酸	4.85	219

表-10　第1回調査結果（2010年11月15日採水）

水系	採水場所	AOI濃度(μg I/L)	IPD濃度(μg/L)	IHX濃度(μg/L)	ヨード造影剤寄与率(%)
安曇川	上流部	ND	ND	ND	ND
琵琶湖南湖	大津港	1.13	ND	ND	ND
宇治川	3川合流直前	2.03	0.59	0.097	16.4
桂川	嵐山	4.60	0.35	0.16	5.3
	3川合流直前	8.92	0.90	0.22	6.1
木津川	3川合流直前	3.98	0.15	0.099	3.0
淀川	枚方	5.69	0.88	0.19	9.1
A処理施設放流口	放流口	15.2	0.82	0.14	3.1

表-11　第2回調査結果（2010年11月30日採水）

水系	採水場所	AOI濃度(μg I/L)	IPD濃度(μg/L)	IHX濃度(μg/L)	ヨード造影剤寄与率(%)
琵琶湖南湖	大津港	1.54	ND	ND	ND
宇治川	3川合流直前	2.03	0.54	0.22	17.9
桂川	嵐山	1.86	0.20	0.12	8.1
	3川合流直前	8.98	1.86	1.30	16.9
木津川	3川合流直前	1.32	0.18	0.16	12.4
淀川	枚方	4.02	1.29	0.25	18.6
A処理施設放流口	放流口	23.6	3.23	1.62	9.9

表-12　第3回調査結果（2010年12月5日採水）

水系	採水場所	I⁻濃度(μg I/L)	AOI濃度(μg I/L)	IPD濃度(μg/L)	IHX濃度(μg/L)	ヨード造影剤寄与率(%)
安曇川	上流部	ND	ND	ND	ND	ND
琵琶湖南湖	大津港	1.60	1.86	0.12	0.050	4.4
宇治川	3川合流直前	ND	3.38	0.80	0.30	15.8
桂川	嵐山	1.20	1.73	0.59	0.24	23.1
	3川合流直前	3.04	14.7	4.51	1.84	20.9
木津川	3川合流直前	1.83	3.39	0.35	0.27	8.7
淀川	枚方	1.19	5.43	1.76	0.59	21.0
A処理施設放流口	放流口	4.09	29.3	5.81	1.56	12.2
B処理施設放流口	放流口	5.23	46.6	10.1	6.71	17.3

回調査では0.014 μg/Lであった．これらの結果のうち，ヨウ化物イオン濃度のまとめを図-4に示す．ヨウ化物イオンの濃度範囲は河川水ではND~3.04 μg/L，下水処理施設放流水では4.09~5.23 μg/Lであった．下水処理施設放流水のヨウ化物イオン濃度は河川水と比較すると高濃度であった．また，下水処理施設が上流にあり，下水処理施設放流水の影響を受ける桂川3川合流直前地点ではヨウ化物イオン濃度が高くなった．

AOI濃度のまとめを図-5に，IPD濃度のまとめを図-6に，IHXのまとめを図-7に示す．AOIの濃度範囲は河川水でND~14.7 μg I/L，下水処理施設放流水で15.2~46.6 μg I/L，

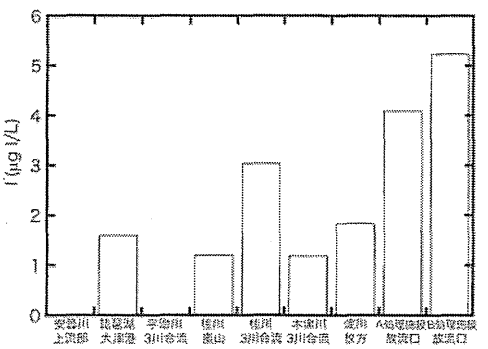

図-4　ヨウ化物イオン濃度のまとめ

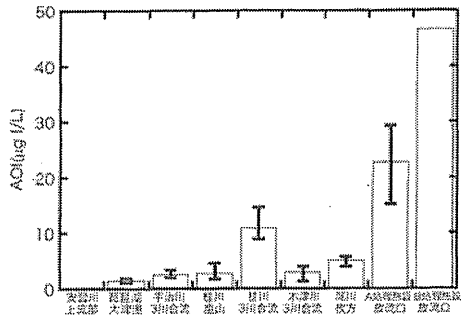

図-5　AOI 濃度のまとめ

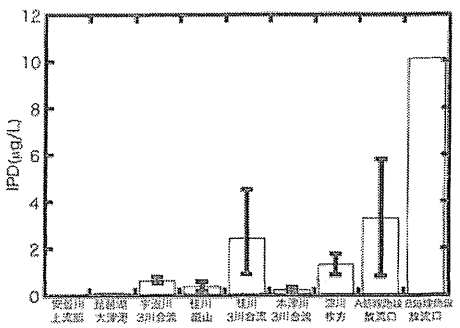

図-6　IPD 濃度のまとめ

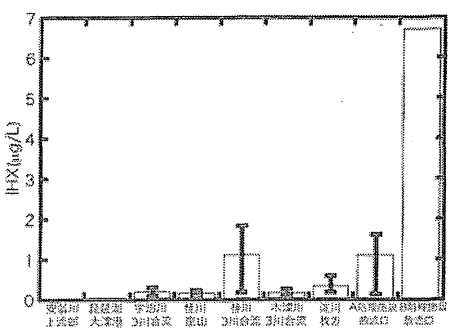

図-7　IHX 濃度のまとめ

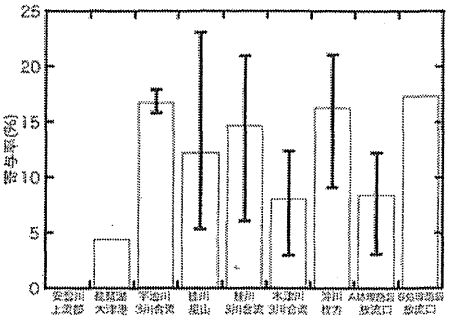

図-8　ヨード造影剤の AOI への寄与率のまとめ

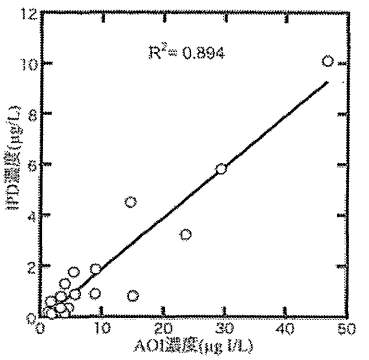

図-9　IPD と AOI の関係

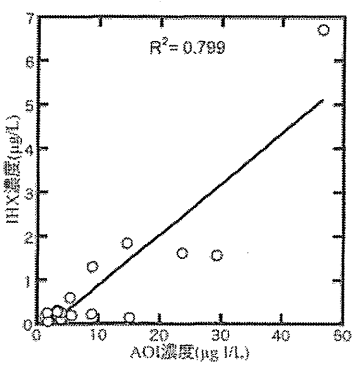

図-10　IHX と AOI の関係

IPDの濃度範囲は河川水でND~4.51 μg/L，下水処理施設放流水で0.82~10.1 μg/L，IHXの濃度範囲は河川水でND~1.84 μg/L，下水処理施設放流水で0.14~6.71 μg/Lであった．いずれも源流部では濃度が低く，下流に進むにつれ濃度が上昇した．特に下水処理施設放流水合流後に濃度が高いことが確認された．このため，都市排水の影響が大きいことが示唆された．また，IPDとIHXの濃度を比較するとIPDの濃度の方が高かった．

次に，IPDとIHXのAOIへの寄与率について図-8にまとめた．同じ河川の寄与率を比較すると，流下と共に寄与率が上昇する傾向が見られた．このことから，AOIの増加に少なからずヨード造影剤が寄与していることが示唆された．また，河川水での寄与率は最大20%程度，下水処理施設放流水での寄与率は最大17%となった．ヨード造影剤以外にも人為起源のAOI排出源が存在することが考えられた．

また，ヨード造影剤濃度とAOI濃度の関係を把握するためAOI濃度に対してIPDとIHX濃度を表示した（図-9, 10）．ヨード造影剤濃度とAOI濃度は一定の相関がある

ことがわかった.

次に，測定結果を用いて下水処理施設におけるヨード造影剤の排出負荷量を試算した．試算には，12月9日の濃度を用いた．下水処理水量は平成20年度下水統計の値を用いた[10]．表-13に計算結果を示す．また，ヨード造影剤の使用量，総人口，処理区域内人口からA下水処理施設とB下水処理施設における予想負荷量を算出し，結果を表-14に示した．ヨード造影剤使用量は薬事工業生産動態統計年報に掲載されている数量と数量単位[8]，イオパミロン添付文章[11]とイオパーク添付文章[12]に掲載されているヨード造影剤濃度を用いて算出した．また，総人口は平成22年に国勢調査おける人口速報値128,767,994人を[13]，処理区域内人口は平成20年下水統計の値を用いた[10]．測定結果から算出した値と予想負荷量を比較するとIPDにおいては予想負荷量と同程度であるが，IHXにおいては大幅に低い値となった．IHXはIPDより生分解されやすいという報告があり[14]，IHXが下水処理や生分解により分解されたことが考えられる．

また，これらの結果を用いて淀川中流(枚方大橋付近)における人為起源のAOI，IPD，IHXと琵琶湖南部より上流起源のAOI，IPD，IHX量をいくつかの仮定の下に試算した．枚方大橋付近の淀川の河川流量を最も近い流量観測所である高浜観測所における河川流量と等しいと仮定した．上流起源のAOI，IPD，IHXの量を琵琶湖・淀川水系上流部のそれぞれの濃度と淀川の河川流量との積で近似した．上流部のそれぞれの濃度は琵琶湖南湖における濃度を用いた．また，下水処理施設からの負荷量は琵琶湖南部より下流かつ枚方大橋より上流にある下水処理施設の総処理水量(1,508,389 m^3/日)と下水処理施設放流水のそれぞれの濃度の積とした．下水処理施設放流水のそれぞれの濃度は測定した下水処理施設放流水の濃度と処理水量から存在量を求め，2つの下水処理施設の総処理水量で除したものを用いた．それぞれの総負荷量は枚方大橋における濃度と河川流量の積で表されると仮定した．ただし，AOIでは下水処理施設放流水の負荷量と上流由来の負荷量の和が総負荷量を越えたため，下水処理施設放流水の負荷量と上流由来の負荷量の和を総負荷量と仮定した．起源不明のそれぞれの負荷量の推定値は総負荷量から上流由来の負荷量と下水由来の負荷量を引いたものとした．AOIでは起源不明の負荷量はなかった．なお，AOI，IPD，IHXの濃度は12月9日の測定結果を，河川流量は高浜観測所のは国土交通省近畿地方整備局淀川河川事務所調査課に問い合わせ，2010年12月9日の日流量の暫定値114.34m^3/sを[15]，下水処理施設の処理量は平成20年度下水統計の値を用いた[10]．この試算において，AOI，IPD，IHXは河川中では生分解されないと仮定した．

AOIの試算結果を図-11に，IPDの試算結果図-12に，

表-13　下水処理施設におけるAOIとヨード造影剤の負荷量

処理施設	晴天時日平均下水量 (m^3/日)	AOI負荷量(g/日)	ヨード造影剤負荷量(g/日)	
			IPD	IHX
A下水処理施設	558130	16034	3245	873
B下水処理施設	141083	6578	1427	947

表-14　下水処理施設におけるヨード造影剤の予想負荷量

処理施設	処理区位置内人口(人)	予想負荷量(g/日)	
		IPD	IHX
A下水処理施設	772800	3959	31488
B下水処理施設	340413	1744	13870

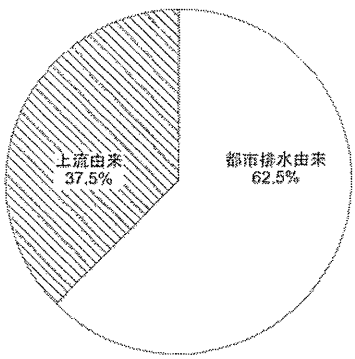

図-11　枚方大橋でのAOIの起源の内訳

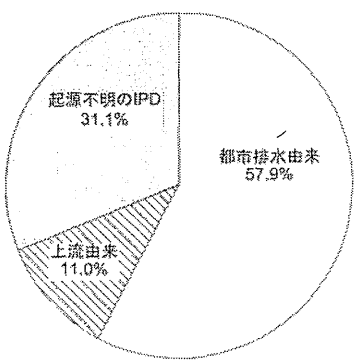

図-12　枚方大橋でのIPDの起源の内訳

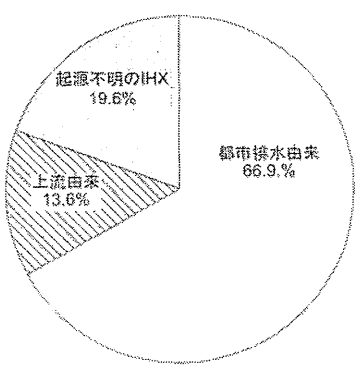

図-13　枚方大橋でのIHXの起源の内訳