

Precision of Measurement of Tissue Concentrations by RLG

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Existing investigations about the precision of radioluminography (RLG) are restricted to descriptive analysis of the tissue samples. The aim of the present experiments was to obtain a general prospective statement about the precision that the RLG method can achieve. Several pharmaceutical companies in Europe participated in the experiments. Albino rats of various strains were dosed with various ¹⁴C-labeled compounds. Whole-body sections were produced, and blood calibration scales were set up with standard radioactivity sources of dog or rat blood. Photostimulated luminescence was detected using Fuji imaging plate BAS-III. For each organ separately, variability was investigated on each of the levels: rat, section of rat, region within section, and residual, with the help of variance components. The producing company was seen as a fixed factor and adjusted for. A mixed linear model was fitted to the log-transformed data. The variance component (SD estimate) for the residual term gave the desired prospective statement about the achievable precision of the RLG method. Exponential back transformation from the logarithmic to the natural scale transformed the SD estimates to multiplication factors. In total, 29 organs were investigated. The RLG method was comparable in precision to the dissection/combustion method. © 2000 Academic Press

INTRODUCTION

Radioluminography (RLG) is proposed as a technique to quantify the concentration of radioactivity in the tissues of animals which are used for whole-body autoradiography (WBA). RLG has been automatized in the BAS system (Hamaoka, 1990; Miyahara, 1989; Motoji *et al.*, 1995) which is already being used by numerous laboratories throughout the world.

To validate the RLG method, it is necessary to compare its results with those of the liquid scintigraphy of dried organs as well as to estimate its precision.

Investigations already exist about the precision of the RLG method; these are however restricted to descriptive analysis of the tissue samples (Tanaka, 1993).

We now aim at a general prospective statement about the precision that the RLG method can achieve.

MATERIALS AND METHODS

Animal Experiments

Several pharmaceutical companies in Europe participated in the experiments.

Albino rats of various strains were dosed by single intravenous or oral administration with various ¹⁴C-labeled compounds. Whole-body sections were produced in a company-specific manner (thickness 25–50 μm). After freeze-drying for about 24 h, one to three sections per rat were used for RLG. Blood calibration scales, consisting of 6–16 points, were set up with standard radioactivity sources of dog or rat blood.

Equipment and Performance of Measurement

Exposure time was 4 h to 2 days at 4–6°C (refrigerator) or at room temperature (shielding box). Photostimulated luminescence (PSL) was detected using Fuji imaging plate BAS-III. Regions for PSL measurements were identified within organs manually and documented on the sections. Radioluminography and a subsequent image-processing step were performed using the system Fuji BAS 2000 including the Fuji software Image Analyse Version 3.0 or Raytest TINA.

Calibration was undertaken by linear regression after log transformation. The limit of quantification was 50 Bq/ml, representing 2× background level.

Circulation of Samples and Data Handling

Five companies, including Bayer (W. Steinke), Hoechst (J. Maas), Knoll (R. Binder), Merck (K. Steiner), and Sanofi (P. Dupont), produced sections. The original producers measured their sections first. The sections were then circulated within all measuring companies; these included the first five and Schering (C. Günther), Novartis-Agriculture (S. Hassler), Bayer-Agriculture (C. Anderson), Pharmacia (R. d'Argy), and Boehringer Ingelheim (U. Busch). Each measuring

TABLE 1
Intercompany Variability of Measurement
in Predefined Regions for Various Organs

	<i>N</i>	Mean (dpm/g)	CV (%)	Min (dpm/g)	Max (dpm/g)
Kidney	7	4,205,840	5	3,985,416	4,532,726
Spleen	7	783,678	8	734,520	880,344
Bone marrow I	7	651,277	9	576,440	732,830
Bone marrow II	7	691,446	15	466,457	797,688
Skeletal muscle I	7	821,301	9	695,161	940,176
Skeletal muscle II	7	782,306	9	697,758	880,698
Liver I	7	1,725,744	7	1,602,489	1,957,710
Liver II	7	1,631,840	9	1,512,734	1,859,756
Liver III	7	1,938,429	10	1,790,016	2,284,778
Blood I	7	210,370	9	184,380	233,347
Blood II	7	436,795	21	331,537	592,814
Thymus	7	354,835	9	320,324	402,959
Myocardium I	7	941,902	11	840,189	1,106,514
Myocardium II	7	945,162	7	888,194	1,076,268
Lung	7	875,420	11	777,724	1,013,154
Brain	7	25,313	24	19,015	34,200
Brain (cerebellum)	7	29,996	15	22,296	35,940
Harder's gland	7	994,100	14	792,028	1,212,145
Salivary gland	7	720,117	10	658,906	825,556
Pancreas	7	1,499,274	7	1,403,870	1,654,398

Note. Example: producing company no. 2, rat 1, section "thyr 2."

company investigated 8–16 organs; in total 29 organs were investigated.

Statistical Analysis

Description. For each produced section and investigated organ, the measured values obtained by the companies in the circle are reported descriptively (mean, SD, coefficient of variation (CV, 100% × SD/mean)). Relationships are investigated for each section and organ between the data of the original producing

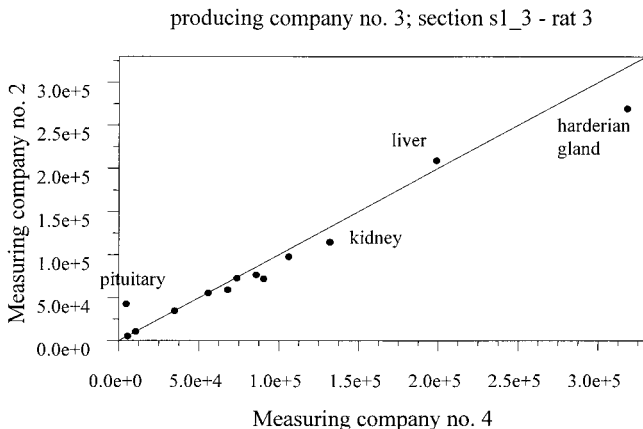


FIG. 1. Example for relationship between two measuring companies: Mean concentration (dpm/g). Producing company no. 3, rat 3, section s1_3, region not prespecified, measuring companies no. 2 and 4.

company and those obtained by other measuring companies.

Analysis of precision. The aim is to obtain a general prospective statement about the precision of the RLG method; however, this is done for each organ separately. Variability is investigated on each of the levels: rat, section of rat, region within section, and residual, with the help of variance components (Winer, 1971). The producing company is seen as a fixed factor and adjusted for. A mixed linear model is fitted to the data from each organ investigated by means of the program SAS version 6.12 (SAS, 1996a). Confidence intervals of 90% for the variance components are calculated with the help of the normal distribution and the observed Fisher information matrix for the variances (Wolfinger *et al.*, 1994) and a basic Satterthwaite approximation (SAS, 1996b), followed by square-root transformation. The variance components (SD estimates) that can be attributed to production of the sections (those assigned to rat, section of rat) are distinguished from those that reflect selection of region (if applicable, region within section) and from those that reflect imprecision of the measurement. The linear model for a measured value y obtained from producing company i , rat j , section k_j , region l_{kj} , measuring company m is

$$y_{ijklm} = \mu_i + \alpha_j + \beta_{kj} + \epsilon_{lm}$$

for the model without preselection of regions (see Table 5) and

$$y_{ijklm} = \mu_i + \alpha_j + \beta_{kj} + \gamma_{lkj} + \epsilon_m$$

for the model with preselection of regions (see Table 6), where α , β , γ and ϵ are independently normally dis-

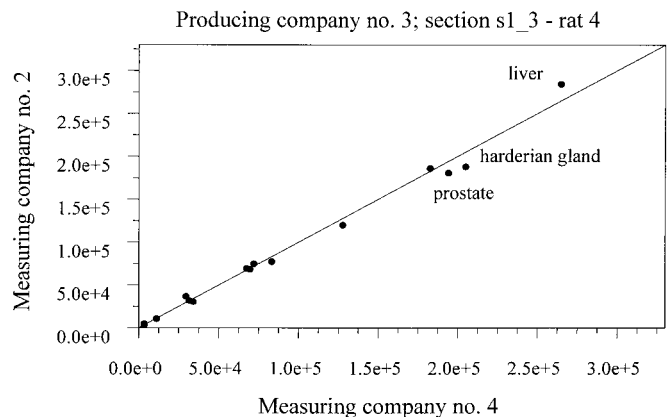


FIG. 2. Example for relationship between two measuring companies: Mean concentration (dpm/g). Producing company no. 3, rat 4, section s1_3, region not prespecified, measuring companies no. 2 and 4.

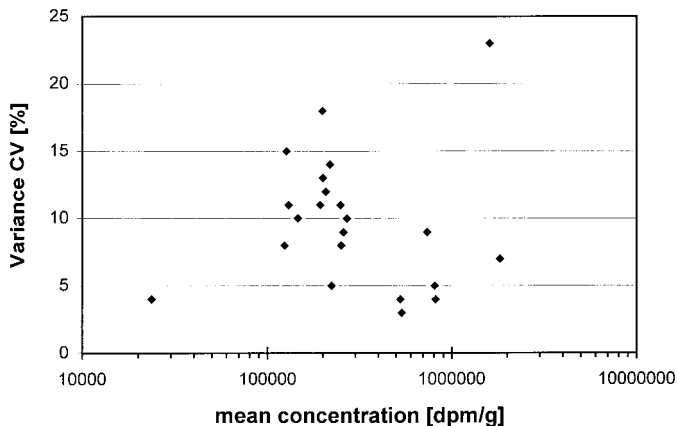


FIG. 3. Relationship between absolute radioactivity level (mean concentration) and intercompany CV of measurement (descriptive) without prespecification of regions, for one organ: liver.

tributed with mean values of 0 and possibly distinct SDs.

Because it became evident that variability was approximately proportional to the mean values, all analyses of variance are performed on the logarithmic scale, and the precision on the different levels is then expressed on the natural scale as a multiplication factor.

The variance component for the residual term gives the desired prospective statement about the achievable

precision of the RLG method. The errors related to the factors that had not been systematically considered in the trial design (substance, dose, route, time relative to administration) are integrated into the residual error, as far as they have not been accounted for by adjusting for the producing company as a fixed factor.

RESULTS

Description

In accordance with the above-mentioned lack of prescriptions for section production and region selection, multiple quantification of an organ occurred. In single cases, an organ was quantified in two serial sections of one rat or in two to three neighboring regions of the same organ in one section.

For a given region and section of a given rat, the intercompany variability of quantitation can be described by the CV for the respective organ. Examples are shown in Table 1.

The distribution of the precision for all producing companies, rats, and sections, with and without prespecification of regions, is shown in Tables 2 and 3.

Examples for the relationship between measurement of two measuring companies (one of them being the producing company) are shown in Figs. 1 and 2.

TABLE 2

Intercompany Variability of Measurement without Prespecification of Regions for One Organ: Liver

Producing company No.	Rat	Section	N	Mean (dpm/g)	CV (%)	Min (dpm/g)	Max (dpm/g)
1	r150/1	s4	10	819,774	4	774,600	894,234
1	r150/1	s5	10	792,870	5	747,224	843,066
1	r151/1	s13	10	222,567	5	206,170	240,397
1	r151/1	s4	10	249,488	11	217,016	285,473
2	1	thyr 1	24	1,830,951	7	1,682,435	2,134,504
2	1	thyr 2	21	1,608,898	23	203,294	2,064,940
3	3	s1_3	29	199,197	18	49,729	241,213
3	3	s2	57	218,587	14	151,931	273,962
3	3	s3	35	193,715	11	155,857	225,002
3	4	s1_3	48	251,437	8	191,750	289,591
3	4	s2	25	258,653	9	216,540	290,970
3	4	s3	64	270,042	10	204,506	317,023
3	6	s1	36	126,957	15	90,923	157,265
3	6	s2	49	146,193	10	110,302	168,715
3	6	s3	43	130,525	11	100,870	155,040
4	1	s3	6	809,018	5	755,756	861,288
4	1	s6	11	735,679	9	613,173	829,012
4	2	s3	6	535,946	3	508,833	549,210
4	2	s5	6	526,747	4	499,222	548,580
5	K15-12	K15	8	23,739	4	22,323	25,062
5	K23-39	K23	14	200,361	13	146,899	238,059
5	K28-37	K28	20	207,349	12	126,728	241,433
5	K31-19	K31	15	123,689	8	103,582	141,662
Total			557				

TABLE 3
Intercompany Variability of Measurement in Predefined Regions for One Organ: Liver

Producing company No.	Rat	Section	Region	N^a	Mean (dpm/g)	CV (%)	Min (dpm/g)	Max (dpm/g)
1	r150/1	s4	-1	2	819,685	4	794,852	844,517
1	r150/1	s4	26	7	826,253	5	779,009	894,234
1	r150/1	s5	27	9	797,942	4	759,070	843,066
1	r151/1	s13	27	9	222,481	5	206,170	240,397
1	r151/1	s4	-1	1	247,733	—	247,733	247,733
1	r151/1	s4	26	8	253,767	11	222,216	285,473

Note. Example: Producing company no. 1, rats r150/1 and r151/1 with two sections per rat.

^a In 4 additional cases (1 per section), the measured values could not be clearly allocated to regions. Taking all five producing companies together, this occurred in 88 cases (15.8% of all 557 readings). These 88 cases must be missing in the evaluation of the precision after preselection of the region (see below, Table 6).

Evaluation of Precision

The evaluation of the precision of the method is based on the data shown in Table 4.

For the other organs—in total 29 organs were investigated—the database is not sufficient to deliver generalizable results.

Table 5 shows the general variability of the BAS system. Variabilities on the different levels (variance components) are given as optimized estimates and 90% confidence intervals. Selection of region is integrated in the residual term.

For, e.g., the liver, the factor of 1.17 in Table 5 describes the overall variability of the measured data, including all contributions to variance. In particular low variability was found for thymus (factor 1.09) and lung (factor 1.11).

Considering the region also as given, we obtain the variability of the BAS system in direct comparison with that of the dissection/combustion method, which is also preceded by a selection of regions (see Table 6).

It is important to note that the variability ranges between 1.07 and 1.42 (accumulation at 1.11–1.22) in case of preselection of the region. The variability becomes higher (factors 1.09–1.62 with accumulation at 1.12–1.26) if no region is prespecified within an organ.

TABLE 4
Available Radioluminography Data per Organ

Organ measured	Total number of			
	Rats	Sections	Regions	Readings
Liver	12	23	122	557
Spleen	8	9	17	77
Lung	10	18	21	164
Myocardium	11	20	31	200
Thymus	9	17	17	142
Pancreas	3	4	5	31
Skeletal muscle	9	20	37	252
Brain	10	18	19	150

For, e.g., the liver, the imprecision factor in Table 6 (1.15) is smaller than in Table 5 (1.17). The difference is however small. This indicates that the selection of regions has an impact, however only a small one, on the results, which can be explained by the internal inhomogeneity of the liver. In contrast, the imprecision factor before and after region selection for, e.g., spleen is 1.12 and 1.07, respectively. This is consistent with the observation that the distribution of the radiolabeled compounds in the spleen was less homogeneous and the selection of regions was therefore more critical. Lung, myocardium, and thymus present themselves also as homogeneous organs (imprecision factors 1.11

TABLE 5
Variability of Measurements, Expressed as Multiplication Factors

Organ measured	General variability: SD estimates as multiplication factors (90% CI)		
	Between rats	Between sections	Residual
Liver	2.15 1.71–4.00	1.07 1.05–1.12	1.17 1.16–1.18
Spleen	2.33 1.69–11.98	— ^a	1.12 1.11–1.15
Lung	2.07 1.63–4.62	1.07 1.05–1.16	1.11 1.10–1.12
Myocardium	1.63 1.40–2.66	1.13 1.09–1.24	1.15 1.13–1.16
Thymus	1.71 1.41–3.58	1.03 1.02–1.15	1.09 1.08–1.10
Pancreas	1.27 1.11–>1000 ^a	— ^a	1.62 1.49–1.86
Skeletal muscle	1.61 1.36–3.22	— ^a	1.45 1.41–1.49
Brain	1.74 1.45–3.42	1.13 1.09–1.34	1.26 1.23–1.29

Note. Regions not preselected.

^a Difficulties occurred during the optimization process for finding the optimal values or the confidence intervals of the variance component.

TABLE 6
Variability of Measurements in Preselected Regions

Organ measured	Variability of measurements in preselected regions: SD estimates as multiplication factors (90% CI)			
	Between rats	Between sections	Between regions	Residual
Liver	2.14 1.71–4.00	1.06 1.04–1.14	1.08 1.07–1.11	1.15 1.14–1.16
Spleen	2.37 1.71–12.71	— ^a —	1.07 1.04–1.24	1.07 1.06–1.09
Lung	2.07 1.63–4.66	1.04 1.02–>1000 ^a	1.04 1.02–>1000 ^a	1.11 1.10–1.12
Myocardium	1.62 1.39–2.65	1.13 1.08–1.30	1.08 1.05–1.18	1.14 1.12–1.16
Thymus	1.70 1.41–3.55	— ^a —	— ^a —	1.09 1.08–1.11
Pancreas	1.97 1.41–>1000 ^a	— ^a —	— ^a —	1.42 1.32–1.62
Skeletal muscle	1.61 1.36–3.21	1.05 1.02–5.73	— ^a —	1.32 1.29–1.36
Brain	1.70 1.42–3.24	1.13 1.08–1.36	— ^a —	1.22 1.20–1.26

^a Difficulties occurred during the optimization process for finding the optimal values or the confidence intervals of the variance component.

and 1.11, 1.15 and 1.14, and 1.09 and 1.09, respectively). In contrast, pancreas, skeletal muscle, and brain show a marked inhomogeneity (imprecision factors 1.62 and 1.42, 1.45 and 1.32, and 1.26 and 1.22, respectively).

It remains to be investigated whether the precision (represented as imprecision factors) could be related to the absolute level of measured radioactivity.

The data of the liver (see above, Table 2) were used here. No clear relationship is detectable (see Fig. 3).

Different organs are compared with each other in Table 7.

Generally, higher absolute levels are associated with higher precision. An exception is the pancreas. This may, however, have been caused by the low number of measurements.

TABLE 7
Comparison between Organs: Measured Absolute Radioactivity Level and Precision

Organ	N	Overall quantitative mean (dpm/g)	Residual imprecision factor	
			Without preselection of regions	With preselection of regions
Pancreas	31	332,243	1.62	1.42
Spleen	77	255,378	1.12	1.07
Liver	557	250,060	1.17	1.15
Thymus	142	123,699	1.09	1.09
Lung	164	120,691	1.11	1.11
Myocardium	200	105,931	1.15	1.14
Skeletal muscle	252	69,037	1.45	1.32
Brain	150	7,296	1.26	1.22

DISCUSSION

Limitations in Study Design

The study was designed in a way that each producing company sent its sections to the measuring companies in a predefined circle; these circulations started simultaneously so that each measuring company was involved in early and late measurements. Thus, any confounding with aging of the sections was avoided, as well as any interaction between measuring company and section age. However, the following design elements, related to production, were not prespecified at the beginning of the study: substance administered, dose, route, and time of sacrifice after administration. Each producing company handled the animal experiments according to its own rules. The errors related to these elements were therefore all integrated into the residual error. The production of sections in the five companies covered the variations in terms of exposure time, section thickness, etc., which occur in general during preparation of typical autoradiographs. The investigated collection of typical autoradiographs from these five producing companies appears to be representative for WBA production in pharmaceutical companies.

We included in our investigation only preparations which were technically faultless. We did not explore the limits of the technical acceptability of the WBA preparations.

Also, the selection of investigated organs was left to the measuring companies. This led to incomplete data, because, naturally, the exact position of the sections differed between producing companies. A total of 29

organs were identified in the sections. Of these, only 8 had sufficient data for quantitative evaluation of precision; it can however be said that all major organs are covered.

The relationship between the areas of organ images or of regions and measured values could not be evaluated because the sizes were not systematically collected.

Statistical Inference

Classical external quality control assessments for laboratory measurements are evaluated in a way that is comparable to our "description." This is completely adequate when all circumstances of the measurement apart from the to-be-measured concentration are kept constant and measurement by different companies is considered as independent repetition.

We investigated this situation for one organ, the liver, and obtained 23 values for the variability which ranged from 3 to 23%. Generally, we have, however, a more complex situation. Not only the last step, RLG, is subject to variability, but also the production process that delivers the to-be-investigated sections, organ images, and regions. For the general case, the mixed linear model with multilevel variability is therefore necessary.

Generalizability of Results

The present investigation covers a range of production conditions of WBA preparations. This is accounted for by the specific methodology adopted which delivers an estimate of the precision, on the background of a whole spectrum of possible production conditions. The conditions used in our trial were representative for WBA preparations occurring in the pharmaceutical industry. Therefore, the investigation permits a general statement about the precision of the BAS 2000 for the organs liver, spleen, skeletal muscle, brain, lung, myocardium, pancreas, and thymus.

CONCLUSION

For the organs that admitted quantitative investigation, the RLG method is comparable with the dissec-

tion/combustion method with respect to precision. Previous investigations (Zane *et al.*, 1997) led to values of the CV (in a descriptive manner) of 0.5–10%, which are comparable with imprecision factors of 1.005–1.10. We have obtained slightly greater values because we came to a general prospective statement. The imprecision factors are generally in the range 1.12–1.26 (myocardium, brain, spleen, liver) and in single cases can be as high as 1.45–1.62 (skeletal muscle, pancreas). Particularly low variability was found for thymus (factor 1.09) and lung (factor 1.11).

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