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Results of the IPCS collaborative study on complex mixtures

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Summary

The International Programme on Chemical Safety (IPCS) sponsored a collaborative study to examine the intra- and inter-laboratory variation associated with the preparation and bioassay of complex chemical mixtures. The mixtures selected were National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs). 20 laboratories worldwide participated in the collaborative trial. The participating laboratories extracted the organic portion of two particulate samples – an air-particulate sample and a diesel-particulate sample – and bioassayed the extracts. The laboratories simultaneously bioassayed a NIST-prepared extract of coal tar and two control compounds (benzo[*a*]pyrene, and 1-nitropyrene). The bioassay method used was the Salmonella/mammalian microsome plate-incorporation test using strains TA98 and TA100. Study design also allowed for a comparison of sonication and Soxhlet extraction techniques. The mean extractable masses for the air particles and diesel particles were approximately 5% and 17.5%, respectively. The particulate samples were mutagenic in both strains with and without activation in all 20 laboratories. For TA100 the with and without activation slope values for the air particulate were 162 and 137 revertants per mg particles, respectively. For TA98 the respective diesel slope values were 268 and 269. The mutagenicity slope values for the diesel particles ranged from 3090 (TA98, +S9) to 6697 (TA100, +S9) revertants per mg particles. The coal tar solution was negative for both strains when exogenous activation was not used but was mutagenic in both strains with

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exogenous activation. The benzo[*a*]pyrene and 1-nitropyrene were used as positive controls and gave results consistent with the literature. This paper provides a complete summary of the data collected during the collaborative study. Companion papers provide further analysis and interpretation of the results.

Studies of the genotoxicity of complex mixtures from air, water, soil, foods, and other environmental sources continue to use short-term mutagenicity assays. The most commonly used assay is the *Salmonella typhimurium* mutagenicity assay as described by Maron and Ames (1983) which is used by laboratories throughout the world.

When data generated by different studies are highly variable, as they often are, one presently cannot know what factors contribute most to the variability. For example, both intra- and inter-laboratory variability in mutagenicity for airborne organic samples may be due to the types of chemicals within the collected organics, to the chemical extraction and sample storage methods used, to the bioassays employed, or to a combination of multiple factors. As described more fully in the overview paper for this IPCS/CSCM study (Lewtas et al., 1992) the purposes of the required portion of the study were:

(1) To determine the interlaboratory variation associated with the extraction and the bioassay of complex environmental mixtures, and

(2) To determine if one or more complex mixtures from the U.S. National Institute of Standards and Technology (formerly the National Bureau of Standards) can be used as a routine reference material in bioassay studies of complex environmental mixtures.

This manuscript describes the specific design of the required portion of the IPCS/CSCM study and the results as they were received from the participating laboratories. Other papers within this volume provide greater detail as to the concepts and objectives of the study (Lewtas, et al., 1992), the samples used (May et al., 1992), the statistical design and analysis of results (Krewski et al., 1992) and the final conclusions of the study (Claxton et al., 1992).

Study design

The purpose of the study design was to accomplish the objectives of the collaborative portion of the study as described by Lewtas et al. (1992). The required portion of the study had two major components: (1) the extraction and/or solvent exchange of complex mixtures for bioassay, and (2) the bioassay of these solubilized extracts of complex mixtures. Two of the complex mixtures (the diesel particles and the air particles) were supplied to the participating laboratories as solid material. Each laboratory extracted the solids and aliquoted them for chemical analysis and bioassay. The third complex mixture was a NIST-prepared organic extract in toluene containing the polycyclic aromatic hydrocarbons from coal tar. Each laboratory solvent exchanged this mixture into dimethyl sulfoxide (DMSO) for bioassay.

The study design provided for the determination of the contribution to total experimental error associated with the estimates of the slopes of the dose–response curves for the mixture assayed and the percentage of total error attributed to laboratories, extraction of organics, and the bioassay itself. As indicated in Fig. 1, this was accomplished by having each laboratory replicate the extraction process and by conducting replicate bioassays on each of the two extracts. Since the coal tar sample required only solvent exchange rather than extraction prior to bioassay, replicate extracts were not required for the coal tar samples.

Each bioassay involved five dose levels in addition to the DMSO control. Two plates were used at each dose. The doses used were specified and were the same for all laboratories although a small number of assays were performed, at the individual investigator's discretion, with additional alternate doses. Dose–response curves

were also generated for two control chemicals – benzo[*a*]pyrene and 1-nitropyrene. Doses were based upon preliminary data developed in the laboratories of the Technical Steering Group (data not presented).

The study design also allowed a direct comparison between Soxhlet and sonication extraction methods. This was accomplished by having half of the laboratories use Soxhlet extraction and half to use sonication extraction methods. Other ancillary information was provided by each of the participating laboratories. The additional information included the percentage (by weight) of total organic material extracted from the particles, historical bioassay control averages, counting and colony counter calibration methods, and auxiliary controls employed.

The statistical analysis methods employed and the final analysis and interpretation of test data are discussed in a separate report (Krewski et al., 1992). This paper summarizes the bioassay data and the ancillary data. The final conclusions of both the required and optional portions of the study are presented by Claxton et al. (1992).

Materials and methods

Test substances

Three complex mixtures from the National Institute of Standards and Technology (NIST) and two compounds purchased as positive controls were supplied to each of the participating laboratories. The three complex mixtures are designated by NIST as:

(1) Standard Reference Material (SRM) 1649, urban dust/organics,

(2) Standard Reference Material 1650, diesel particulate matter, and

(3) Standard Reference Material 1597, complex mixture of polycyclic aromatic hydrocarbons from coal tar.

SRMs 1649 is referred to as the air-particulate sample, while SRMs 1650 and 1597 are referred to as the diesel particulate and coal tar samples, respectively. These standard reference materials originally were intended by NIST for use in the evaluation and validation of chemical analytical methods. A more complete description is found in the publication of May et al. (1992). The air-

STEP	PARTICULATE 1		PARTICULATE 2		LIQUID	CONTROL 1	CONTROL 2
EXTRACTION							
DAY 1	P1E1		P2E2				
DAY 2		P1E2		P2E2			
ALIQUOTS FROM EXTRACTION[a]	P1E1A1	P1E2A1	P2E1A1	P2E2A1	S1A1	C1A1	C2A1
	P1E1A2	P1E2A2	P2E1A2	P2E2A2	S1A2	C1A2	C2A2
	P1E1A3	P1E2A3	P2E1A3	P2E2A3	S1A3	C1A3	C2A3
	P1E1A4	P1E2A4	P2E1A4	P2E2A4	S1A4	C1A4	C2A4
	P1E1A5	P1E2A5	P2E1A5	P2E2A5			
ALIQUOTS FOR GRAVIMETRIC ANALYSIS	P1E1A5	P1E2A1	P2E1A1	P2E2A2			
ALIQUOTS RETURNED TO ORGANIZERS	P1E1A1	P1E2A3	P2E1A2	P2E2A1	S1A2		
	P1E1A2	P1E2A5	P2E1A4	P2E2A5	S1A4		
ALIQUOTS SOLVENT EXCHANGED	P1E1A4	P1E2A4	P2E1A3	P2E2A4	S1A1		
	P1E1A3	P1E2A2	P2E1A5	P2E2A3	S1A3		
ALIQUOTS BIOASSAYED							
ROUND 1	P1E1A4		P2E2A3		S1A1	C1A4	C2A4
ROUND 2		P1E2A2		P2E2A4		C1A1	C2A2
ROUND 3	P1E1A3		P2E1A5		S1A3	C1A3	C2A3
ROUND 4		P1E2A4		P2E2A3		C1A2	C2A1

[a]Aliquots used for different purposes were randomized separately for each laboratory.

Fig. 1. Sample preparation and bioassay testing scheme for the required portion of the IPCS collaborative study on complex mixtures using a sample of the randomized aliquoting. P1 E1 A1 would represent Coded Particulate Sample Number 1, Extract Number 1, and Aliquot Number 1.

TABLE 1

EXTRACTION INFORMATION FOR THE AIR PARTICLES AND DIESEL PARTICLES USED IN THE IPCS STUDY

Lab.	Description of extraction method	Percent extractable			
		Air Particles (SRM 1649)		Diesel (SRM 1650)	
		Extract 1	Extract 2	Extract 1	Extract 2
1	Sonication: 40-ml sample sonicated for 3 min at 33% power (45 KHz, 50 W). Three cycles used. Sample kept below 30 °C. Branson Sonic Co. Sonifer B12 used. Filter samples using glass fiber filter. Used nitrogen purge/rotovap for solvent reduction.	4.76	5.80	19.30	19.90
2	Sonication: 50-ml sample sonicated twice for 2.5 min at 100% power (300 W). Temperature range was between 4 °C and 10 °C. Used an Artek Sonic 300 sonicator. Filtered samples using 0.5 μm Teflon filter (Millipore FHUP 04700). Used nitrogen purge/rotovap for solvent reduction.	4.66	5.03	21.67	20.20
3	Sonication: total volume of solvent used 1500 ml. Six cycles of 5 min each using a Bransonic B92 sonicator set at 50.55 kHz (425 W). Sample was filtered using fritted glass fiber filter and solvent reduction was with nitrogen purge/rotovap.	4.89	5.12	18.89	20.29
4	Sonication: 8 ml DCM added, sonicated 30 min using Branson B-220 sonication bath at 50/60 Hz (125 W). Followed by centrifugation and filtration (Millex filter). Repeated 3 times. Nitrogen purge used for solvent reduction.	5.21	4.70	17.14	29.58
5	Sonication: 15 ml DCM added for extraction. Sonicated (Ultrasonic A350 G) 3 times 2 min each and solvent removed. Repeated. Solvents pooled and sample centrifuged. Filtered through Whatman 9F/A and 9F/C filter papers (0.45 μm). Nitrogen purge used for solvent reduction.	3.00	3.70	13.70	11.60
6	Sonication: 200 ml DCM added for extraction. Sonicated once for 20 min using 100% power (Nippon Seiki Seisakusho Co. model NS 300 sonicator (28 kHz, 300 W). Temperature range between 23 °C and 28 °C. Filtered using Toyo filter Paper No. 5C. Solvent reduction using nitrogen purge/rotovap.	4.42	4.64	24.26	19.12
7	Sonication: 500 ml DCM added for extraction. Used 3 cycles of extraction, each with 10 min duration using Yamato Bransonic-B521 sonicator (60 Hz, 200 W). Temperature kept between 27 °C and 30 °C. Sample filtered using Toyo Filter paper No. 2. Solvent using argon purge/rotovap.	5.65	5.53	23.00	21.00
8	Sonication: 43 ml DCM added for extraction. Used 2 cycles each at 15 min duration using Branson B32 sonicator (55–60 kHz, 150 W). Samples filtered using Whatman No. 1 filter paper. Solvent reduction done with nitrogen purge only.	7.17	4.58	8.58	12.05
9	Sonication: 120 to 1800 ml DCM added for extraction each cycle. Sonicated 20 min each cycle (5 cycles) using Branson B-220H sonicator (45 kHz, 60 W). Temperature kept at 25 °C. Filtered using Toyo Roshi No. 5C filter paper. Solvent reduction with nitrogen purge/rotovap.	5.50	6.70	31.6	25.3
10	Sonication: 1000 ml DCM added for extraction. 15 min sonication	9.31	9.66	113	73

	for 2 cycles using Bransonic B 2200 R-I sonicator (60 Hz, 100 W). Filtered using 0.45 μm filter. Solvent reduction with nitrogen purge.				
	Mean percentage of extractables (sonication) =	5.50 ± 0.51		19.84 ± 1.82	
11	Soxhlet: extraction was done under nitrogen using 350 ml of DCM. Solvent cycle time was 32 min; thimble volume was 200 ml; and duration of extraction was 1440 min. 275 ml final volume. Filtered using Whatman #4 paper. Solvent reduction by nitrogen purge/rotovap.	6.48	6.60	20.20	16.00
12	Soxhlet: extraction was done using 70 ml of DCM. Solvent cycle time was 3.11 min; thimble volume was 26 ml; and duration of extraction was 480 min. 30.5 ml was the final volume. Filtered using ACRO LC PVDF (0.45 μm) filter from Gelman. Solvent reduction by nitrogen purge/rotovap.	1.15	1.95	10.20	13.90
13	Soxhlet: extraction was done using 165 ml of DCM. Cycle time was 5 min; thimble volume was 35 ml. A 24-h extraction was used giving a final volume of 10 ml. No filtering was used and solvent reduction was by nitrogen purge only.	1.89	2.13	12.93	9.93
14	Soxhlet: two extractions (65 ml DCM each) were combined. Cycle time was 63 s, thimble volume was 6 ml, and each extraction was for 120 min. Final combined volume was 94 ml. No filtering was done. Solvent reduction done by nitrogen purge/rotovap.	4.84	5.57	21.30	20.80
15	Soxhlet: extraction used 300 ml DCM. Cycle time was 15 min, thimble volume was 100 ml, and extraction time was 1140 min. Final volume was 250 ml. No filtration was done. Solvent reduction done with nitrogen purge/rotovap.	8.22	0.87	19.20	3.14
16	Soxhlet: extraction used 150 ml DCM. Cycle time was 6.59 min. Thimble volume was 104 ml. Extraction time was 240 min. Final volume was 135 ml. Solution was filtered using Millipore PTFE filter (1.0 μm). Nitrogen purge was used for solvent reduction.	5.17	5.19	21.70	22.60
17	Soxhlet: extraction used 125 ml DCM. Cycle time was 16 min. Thimble volume was 70 ml. Extraction time was 960 min. Final volume was 100 ml. No filter was used. Solvent volume reduced using nitrogen purge only.	1.03	0.86	2.50	3.60
18	Soxhlet: Extraction used 65 ml DCM. Cycle time was 4 min. Thimble volume was 30 ml. Extraction time was 420 min. Final volume was 33 ml. No filter was used, and solvent reduction was by rotovap.	4.40	4.10	17.70	17.80
19	Soxhlet: extraction used 200 ml DCM. Cycle time was 5 min. Thimble volume was 100 ml. Extraction time was 1000 min, and final volume was 150 ml. No filtration was used. Solvent reduction was by nitrogen purge.	6.00	9.00	7.00	7.00
20	Soxhlet: extraction used 300 ml DCM. Cycle time was 20 min. Thimble volume was 150 ml and duration of extraction was 960 min. Final volume was 250 ml. No filtration was used. Both nitrogen purge and rotovap were used for solvent reduction.	7.40	5.90	34.00	26.00
	Mean percentage extractable organics (Soxhlet) =	4.44 ± 0.72		15.38 ± 2.52	
	Mean percentage extractable organics (combined) =	4.97 ± 0.43		17.49 ± 1.54	

particulate and diesel-particulate samples required both extraction and Soxhlet exchange before bioassay. The coal tar sample is a liquid sample for which only solvent exchange was required by participating laboratories.

The two control chemicals used were benzo-[α]pyrene (BaP) and 1-nitropyrene (1-NP). The BaP was obtained from the Community Bureau of Reference (CBR), Brussels. The BaP, a CBR Certified Reference Material (Mo. 051R), is certified to be greater than 99.3% pure. Analysis by NIST using differential scanning calorimetry indicated that the BaP was > 99.8% pure. The 1-NP was obtained from Midwest Research Institute, Kansas City, Kansas (U.S.A.). Differential scanning calorimetry by NIST showed the 1-NP to be greater than 99.8% pure. The 1,3-, 1,6- and 1,8-isomers of dinitropyrene were each present in less than 0.02%. The BaP was used as a control for both *S. typhimurium* TA98 and TA100 when exogenous activation conditions (with S9) were present. The 1-NP was the direct-acting mutagen used as a control when S9 was absent.

Samples in containers with dry ice were shipped to the laboratories by the most rapid available means. Generally, shipments were received within 32 h although in a some overseas shipments the time was extended.

Extraction and solvent exchange

The two particulate samples required extraction before bioassay. 10 of the laboratories used a Soxhlet extraction while the other 10 laboratories used sonication. In both cases the extraction solvent was dichloromethane (DCM). The precise method of Soxhlet or sonication extraction was left to the discretion of each laboratory and was the same as done historically by the laboratory. Extracted material was evaporated to close to dryness and then solvent exchanged into DMSO. The coal tar sample is a liquid solution with toluene as its solvent base; therefore, it also was evaporated to close to dryness and solvent exchanged into DMSO. Table 1 documents the essential information supplied by each laboratory.

Bioassay methods

The bioassay procedure used was the *Salmonella typhimurium* plate-incorporation protocol as described by Maron and Ames (1983). *S. typhimurium* strains TA98 and TA100 supplied by Dr. Bruce Ames, Berkeley, CA, were used both with and without exogenous activation conditions. The study did stipulate that the minimal media plates were to be made with Difco agar and that the plates were to contain 30 ± 1 ml of base layer agar. The exogenous activation system (S9) was an Aroclor-1254 induced rat-liver homogenate as described by Maron and Ames (1983). Duplicate plates were used at each dose. Laboratories were asked to use the same staff members for each round of testing, to complete all bioassay rounds within a 2-week period, and to record their data on standardized sheets provided by the U.S. EPA. The participating laboratories used their own stocks of bacteria and S9 and were allowed to use their in-house controls in addition to the control compounds supplied. The actual dates for performing the required rounds were determined independently by each laboratory. All procedures were to follow the guidelines given by Claxton et al. (1987). Results were analyzed according to the methods of Krewski et al. (1992). Results were required to show a significant slope value according to Krewski et al. (1992) and to fulfill other established criteria for a mutagenic response (Claxton et al., 1987) before being considered mutagenic. Table 2 provides bioassay information associated with each laboratory.

Results

All of the 20 participating laboratories were able to complete the required collaborative portion of the IPCS/CSCM project. Laboratories completed two major components during the required portion of the study:

(1) extraction and solvent exchange of the particulate samples and (2) bioassay. 10 laboratories extracted the samples using Soxhlet extraction, and 10 laboratories used sonication extraction methods. Next, each laboratory bioassayed the extracts of the particulate samples, of a solvent exchanged coal tar extract, and two control compounds. Table 1 provides a summary of both the extraction procedures used and the results of those procedures. Both the sonication and solvent extraction methods were quite variable. Dif-

TABLE 2

SUMMARY OF COLONY COUNTING METHODS, MEDIA, INCUBATION CONDITIONS FOR THE REQUIRED PORTION OF THE IPCS/CSCM COMPLEX MIXTURE STUDY

Lab.	Counting method [a]			Hand counts [b]			Machine counts [c]			Broth culture incubation [d]					Petri dish size (mm)	S9 characterized by [e]:	Lighting during pouring (h)	Plate incubation time (h)
	H	E	B	Accurate to:	Range counted:	Notes:	Counter:	Accuracy range:	Notes:	Amount (ml)	Time (h)	Temp (°C)	Shaker (rpm)	Shaker Volume/shape				
1			B	200	0– 200	–	B	100–1500	C	50	14	37	225	250 E	90	P	Dark	48
2		E		–	–	–	N	0–1200	F(9%)	125	8.5	37	130	250 B	100	PB	Red. White	46
3	H			2000	0–2000	–	–	–	–	50	15	37	200	250 E	90	PB	Yellow	48
4		E		–	–	–	A	0–1500	–	5	14.5	37	80	18×150 T	100	PEB	Yellow	48
5		E		–	–	–	AM	0–1000	–	50	10	37	100	175 B	90	PB	Normal	72
6		E		–	–	–	T	50– > 1000	C	10	8	37	67	30 L	90	P	Yellow	48
7	H			2000	–	–	–	–	–	10	14	37	15	30 L	90	B	Yellow	48
8		E		–	–	–	A	75– 750	F(12%)	25	16	37	200	250 E	100	PE	Yellow	48
9			B	2000	1583–3000	–	C	0–1583	C	10	16	37	16	30 L	90	P	Dark	48
10			B	750	> 2500	P(25%)	A	0– 530	F(16%)	250	10	37	95	250 E	100	PE	Yellow	48
11		E		–	–	–	A	0–2000	–	250	10	37	120	250 E	100	PEB	Yellow	48
12	H			1000	–	P(10%)	–	–	–	250	16	37	150	250 E	90	B	Yellow	48
13		E		–	–	–	B	10–1000	–	250	12	37	150	250 E	100	PEB	Yellow	48
14			B	500	0– 500	–	A	500–1000	F(35%)	40	18	37	wo	250 E	90	P	Yellow	48
15	H			1200	–	P(25%)	–	–	–	25	8	37	100	250 E	90	PE	Normal	48
16		E		–	–	–	N	1–1000	–	250	16	37	150	100 E	90	PEB	Normal	48
17		E		–	–	–	T	–	–	12	8	37	w	30 L	90	P	Yellow	48
18			B	500	1– 500	–	B	1–2000	–	250	12	37	200	250 E	90	PE	Yellow	48
19			B *	–	25–2000	P(25%)	N	25–2000	–	50	16	37	120	500 B	90	B	Yellow	72
20			B	100	1– 100	–	B	100–2000	F(12%)	250	12	37	110	250 E	100	P	Yellow	48

[a] Colony counts were done either by hand (H), electronically (E), or using both (B) methods.

[b] Hand counts could have been assisted by a non-electronic means. Principal investigators reported the maximum hand count considered to be accurate within 5%, the range in which hand counts were used, and the method used when counts were above a normal maximum (e.g., 500). P(25%), for high counts, counted 25% of plate and calculated total number; P(10%), counted 10% of plate and calculated total.

c For electronic machine counts the following was recorded: type of machine (A, Artek; AM, AMS; B, BioTran; C, C.A.S.; N, New Brunswick; T, Toyo), range in which principal investigators believe counts are accurate within 5%, and the type of correction factor used (C, formula-calculated correction; F, percent factor set on machine, –, no correction factor used). * Due to counter malfunction, lab 19 used electronic counts on rounds 1, 2, and 3, but had to use hand counts on round 4.

[d] Incubation conditions for bacterial culture to be used in test giving: amount of broth used to grow culture, incubation time in hours, incubation temperature, shaking rate (number = rpm, w = used, wo = without shaking), volume or size of container and type of container (B, bottle; E, Erlenmeyer; L, L-shaped tube, T, test tube). Broth volume and container size lack of correlation may be due to number of containers used.

[e] P, protein content; E, levels of specific enzymes; B, bioassay results with control chemicals.

ferences centered around the amount of extraction solvent, use of filtration methods, and method of solvent reduction. Values for Laboratory 10 were excluded from all subsequent extractable mass comparisons because the high values were considered to be outliers. When examining the mean values for percent of extractable mass, one sees that sonication (5.5% for air particles and 19.8% for diesel particles) tended to provide a larger percent extractable mass than Soxhlet methods (4.4% for air particles and 15.4% for diesel particles). Overall, the air particulate sample had approximately 5% extractable mass and the diesel particles about 17.5% extractable mass.

Although all laboratories followed the bioassay methods of Maron and Ames (1983) and the guidelines of Claxton et al. (1987), there were differences in the details of the methods used.

TABLE 3

SUMMARY OF SALMONELLA MUTAGENICITY [a] FOR THE IPCS SAMPLES

Sample extraction method		TA100 +S9	TA100 −S9	TA98 +S9	TA98 −S9
Air particles	G. Mean	121.9	111.6	222.7	266.6
(SRMs 1649)	A. Mean	201.6	139.4	288.9	291.8
sonication	STD	250.4	98.3	183.3	127.2
	CV	1.24	0.70	0.63	0.44
Air particles	G. Mean	84.7	94.4	205.6	208.7
(SRMs 1649)	A. Mean	121.4	135.2	246.8	245.2
Soxhlet	STD	98.5	124.9	128.4	122.6
	CV	0.81	0.92	0.52	0.50
Air particles	G. Mean	102.0	103.0	214.0	237.0
(SRM 1649)	A. Mean	162.0	137.4	268.4	269.1
both extractions	STD	195.3	111.7	160.3	127.2
together	CV	1.21	0.81	0.60	0.47
Diesel particles	G. Mean	5072.9	4490.0	2285.0	2743.8
(SRM 1650)	A. Mean	6595.7	5012.8	2664.7	2882.3
sonication	STD	4412.7	2482.4	1600.1	927.6
	CV	0.67	0.50	0.60	0.32
Diesel particles	G. Mean	4070.1	3129.2	2243.3	2849.9
(SRM 1650)	A. Mean	6817.1	4167.4	3538.5	3823.9
Soxhlet	STD	5246.1	2637.9	2720.5	2423.8
	CV	0.77	0.63	0.77	0.63
Diesel particles	G. Mean	4585.0	3766.0	2265.0	2794.0
(SRM 1650)	A. Mean	6697.4	4601.0	3090.4	3334.8
both extractions	STD	4814.9	2594.0	2260.4	1868.5
together	CV	0.72	0.56	0.73	0.56
Coal tar PAH	G. Mean	144.0		60.0	
solution	A. Mean	173.6		67.4	
(SRM 1597)	STD	126.1		45.9	
	CV	0.73		0.68	
Benzo[*a*]pyrene	G. Mean	405.0		199.0	
	A. Mean	552.2		247.7	
	STD	388.9		176.5	
	CV	0.70		0.71	
1-Nitropyrene	G. Mean		243.0		2539.0
	A. Mean		579.0		6609.0
	STD		1418.9		11450.0
	CV		2.45		1.73

[a] Expressed as revertants/mg for SRM 1649 and SRM 1650, as revertants/μl for SRM 1597, and revertants/μg for compounds. G. Mean, Geometric mean; A. mean, Arithmethic mean; STD, Standard deviation; CV, coefficient of variation.

Differences existed for the methods and accuracy of counting revertant colonies, the amount of broth culture used to incubate the original culture of bacteria along with the time of incubation and the rate of culture shaking (Table 2). Investigators tended to agree that colony counts were accurate until counts between 1000 and 2000 were reached. This agrees with the published works of Claxton et al. (1984). None of the alternative methods appeared to have a definable effect upon the results.

Because a companion paper provides a thorough statistical analysis of the required portion of the study, only a general summary of the bioassay results is provided within this paper. The bioassay data was analyzed by the method of Krewski et al. (1992) and summarized with the calculated slope value. Table 3 provides a summary of the bioassay results for the required testing. The Appendix of an EPA report contains all of the Salmonella bioassay data and is available upon request (Claxton et al., 1992). Dosing was based upon the amount of the starting material. In other words, particulate sample doses are milligrams of starting particulate mass and the coal tar solution doses are microliters of starting solution.

The air-particulate extracts were mutagenic in all laboratories in strains TA100 and TA98 both with and without exogenous activation. Strain TA98 consistently gave a slope value that was approximately twice the slope value generated with strain TA100. When one averages the mean slope values from all laboratories, it is evident that similar slope values are obtained both with and without exogenous activation. For TA100, the with and without activation slope values average 162 and 137 revertants/mg; and for TA98 the respective mean slope values are 268 and 269. The coefficient of variation, however, was between 47% and 121%.

The diesel-particulate extracts, similarly were positive in both strains with and without exogenous activation. In this case, however, strain TA100 gave slope values higher than strain TA98. The respective slope values were 6697 (TA100, +S9), 4601 (TA100, −S9), 3090 (TA98, +S9), and 3335 (TA98, −S9). The coefficient of variation for the laboratory means was in a range similar to the air-particulate extracts, ranging from 56% to 73%.

The coal tar PAH mixture was negative in both strains when S9 was absent. When S9 was present, TA100 gave slope values greater than those of TA98 except in two cases. Again, the coefficient of variation was within a range similar to the other complex mixtures.

The control compounds, BAP and 1-NP, gave results in line with those of the complex mixtures. BAP was mutagenic to both strains when S9 was used. Except for results in one laboratory, TA100 gave higher mean slope values (revertants/μg) than TA98. The slope values averaged for all laboratories were 552 for TA100 and 248 for TA98. 1-NP used as the control without S9 also was mutagenic in every assay; however the mean slope values for 1-NP, in every laboratory, were greater for strain TA98. The values for 1-NP were 579 and 6609 for TA100 and TA98 respectively. Laboratory 3, however, appeared to have slope values at least 10-fold greater than most of the other laboratories for both strains as did laboratories 13 and 20 for TA98. If one averages the remaining laboratory means, the average slope values for 1-NP was 356 for TA100 and 1854 for TA98.

In most cases, the mean slope values for laboratories using sonication was higher than the mean values for the laboratories that used Soxhlet extraction. However, the control compounds also gave a higher mean slope values in the sonication laboratories. Also, the differences between the two groups of laboratories are not appreciable.

The statistical analyses and the final conclusions drawn by the final meeting participants concerning many of these observations are discussed in companion papers (Krewski et al., 1992; and Claxton et al., 1992).

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