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Mutagenicities of Workplace Chemicals in Korea

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ABSTRACT : **Bacterial reverse mutation assays were performed for 20 workplace chemicals in Korea, which were selected among workplace chemicals under the Korea Industrial Safety and Health Act (KISHA) with the occupational exposure levels (OELs). The assays were carried out by using the pre-incubation method (37°C, 20 min) with and without metabolic activation using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvrA*. The chemicals were tested at 5 concentrations both in the preliminary and the second assays. Despite the cell toxicities, there were no chemical-induced mutagenicities with or without metabolic activation in any of 20 chemicals.**

Keywords : **Bacterial reverse mutation assay, Korean workplace chemicals**

Introduction

For the appropriate management of chemicals, Korea has been conducting the work for hazard assessment of existing chemicals to keep pace with recent trends on the chemical programs of USA, EU, and OECD. The Ministry of Environment (MOE) has assessed the existing chemicals since 1988 and the Ministry of Agriculture & Forestry (MOAF) since 1992 (Lee, 1997). Approximately 35,000 chemicals are listed in the existing chemical inventory in Korea (MOL, 1996). Among them 698 chemicals are regulated under the OSHA with OELs. A few chemicals, however, had full sets of data including physicochemical properties, acute and chronic toxicity data, and human exposure data for the better risk assessment. Thus most chemicals has been used without knowing potential hazards in workplaces. From 1996, the Ministry of Labor (MOL) legislated to furnish the material safety data sheets (MSDS) of chemicals for the workers health protection, but several kinds of toxicity data are apt to be omitted in MSDSs possibly because of absence of data or unreporting of negative data of toxicity tests. To avoid duplication of experiments among ministries, we had a meeting with researchers from the MOE and MOAF to arrange subject chemicals to be tested. Center for Occupational Toxicology, Occupational Safety and Health Research Institution, Korea Occupational Safety & Health Agency together with MOL initiated an existing chemical risk assessment program (ECRAP) in 1997. The program was directed to test 10 chemicals annually to provide mutagenicity data (Lee, 1997). The criterion in selecting chemicals for the mutagenicity test was choosing workplace chemicals of which mutagenicities were not known in NIOSH RTECS (NIOSH, 1997), KOSHA-NET MSDS database, IPCS lists (IFCS, 1997), and Databook of Korean Ministry of Environment (MOE, 1997). In this paper, we report the results of ECRAP from 1997-1998. The output will be used in hazard assessment of existing chemicals and MSDS revision.

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Materials and Methods

Chemicals

Selected 20 chemicals (Table 1) were divinyl benzene, isophrone diisocyanate, 2-chlorostyrene, 3-chlorostyrene, 4-chlorostyrene, 2-chlorotoluene, diisobutyl ketone, diphenylamine, acetic anhydride, 2-aminopyridine, ethyl amyl ketone, methyl n-amyl ketone, methyl isoamyl ketone, oxalic acid, n-amyl acetate, D-α-chloropropionic acid, 2-N-dibutylaminoethanol, ethyl formate, methyl ethyl ketone, and 4-vinylcyclohexene. They were purchased from Aldrich (USA), FLUKA (Switzerland), Mallinkrodt (Mexico), Sigma (USA), TCI (Japan), WAKO (Japan), and Yakuri (Japan). Liver homogenate (S9) for the assays was purchased from Mortox (USA) and Oriental Yeast Co (Japan), which was prepared from male Sprague-Dawley rats (7-weeks old with body weight 180- 221g) injected with Aroclor 1254 or phenobarbital/5,6-benzoflavone as drug-metabolizing inducers. NADPH, NADH, glucose-6-

Table 1. Lists of 20 industrial chemicals tested

Chemical Name (abbreviation)	CAS No.	Manufacturer	Lot No.
1. Divinyl benzene (DB)	1321-74-0	TCI	GG01
2. Isophrone diisocyanate (ID)	4098-71-9	TCI	GG02
3. 2-Chlorostyrene (2-CS)	2039-87-4	Sigma	73H3720
4. 3-Chlorostyrene (3-CS)	2039-85-2	Sigma	63H3583
5. 4-Chlorostyrene (4-CS)	1073-67-2	Sigma	36H3522
6. 2-Chlorotoluene (2-CT)	95-49-8	TCI	GA01
7. Diisobutyl ketone (DK)	108-83-8	TCI	FAX01
8. Diphenylamine (DA)	122-39-4	FLUKA	RA10198
9. Acetic anhydride (AA)	108-24-7	Mallinkrodt	2420MVMZ
10. 2-Aminopyridine (2AP)	504-29-0	TCI	GE01
11. Ethyl amyl ketone (EAK)	106-68-3	WAKO	150-01452
12. Methyl n-amyl ketone (MAK)	110-43-0	WAKO	137-01943
13. Methyl isoamyl ketone (MIK)	110-12-3	WAKO	014-03882
14. Oxalic acid (OA)	144-62-7	YAKURI	25805510
15. n-Amyl acetate (nAA)	628-63-7	Sigma	35H3699
16. D-α-Chloropropionic acid (CP)	598-78-7	Sigma	31H0378
17. 2-N-Dibytylaminoethanol (DAE)	102-81-8	WAKO	040-19512
18. Ethyl formate (EF)	109-94-4	WAKO	TPRI521
19. Methyl ethyl Ketone (MEK)	78-93-3	YAKURI	22505701
20. 4-Vinylcyclohexene (VC)	100-40-3	Aldrich	11140-6

phosphate (G-6-P), and DMSO were purchased from Sigma Chemical Co.USA.

Mehods

Bacterial mutation assays (Maron & Ames, 1983; OECD, 1993) were carried out by using the pre-incubation method (37°C, 20 min) with and without S9 mix using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA*. These bacteria were kindly supplied by Korea Research Institute of Chemical Technology, Daejon, Korea. Oxoid nutrient broth was used to preculture the bacteria, and Difco Bacto agar was used to make 2 ml of top agar. The top agar layer contained 0.1 μmole of L-histidine and biotin, or 0.1 μmole of L-tryptophan. The S9 mix contained 4 mM of NADPH, 4 mM of NADH, 5 mM of G-6-P, 8 mM of $MgCl_2$, 33 mM of KCl, 100 mM of sodium phosphate buffer (pH7.4) and 10% S9.

The chemicals were tested at 5 concentrations: 5,000, 1,000, 500, 100 and 50 μg/plate in the preliminary assay. The concentrations for the second assay were based on the cell toxicity as results of the preliminary assay. Duplicated plates were used at each concentration of chemicals, while 3-4 plates were used for negative (DMSO) and positive controls. The revertant colonies per plate were counted with an automatic colony counter (Unitron, ImageTeck System, IA100, USA).

Results & Discussion

From the results of the preliminary tests, growth inhibitory concentrations were different depending on the types of chemicals: Divinyl benzene = isophrone diisocyanate (100 μg/plate)<2-chlorostyrene = 3-chlorostyrene = 4 chlorostyrene = 2-chlorotoluene = diisobutyl ketone = diphenylamine (500 μg/plate) < acetic anhydride = 2-aminopyridine = ethyl amyl ketone = methyl n-amyl ketone = methyl isoamyl ketone = oxalic acid (5,000 μg/plate) < n-amyl acetate = D-a-chloropropionic acid = 2-N-dibutylaminoethanol = ethyl formate = methyl ethyl ketone = 4-vinylcyclohexene (>5,000 μg/plate). The results of the second assay were presented in Table 2-8. Despite the positive results of positive controls in each test, there were no 2-fold over increase in the number of histidine+-revertant at each dose, when compared with negative control values with or without metabolic activation. During the course of these research projects, the experiment for each chemical was accomplished independently, which resulted in a little variations of the numbers in controls.

In this study, 20 chemicals tested were in negative in the bacterial reverse mutation assays. Some chemicals, 4-vinylcyclohexene and diphenylamine have been reported as carcinogenic in the literatures (EPA, 1988; Kiligerman *et al.*, 1996), but they were negative in our study. Thus some chemicals need to be examined further using other genotoxic tests.

Our mutagenicity tests for industrial chemicals will be continued annually. Currently we have a list of 50 chemicals to be tested by the year 2003. Although some of them were reported as highly toxic chemicals, and tumorigenic in some experimental animals, these negative bacterial

Table 2. Results of the bacterial reverse mutation assay of divinyl benzene (DB) and Isophrone diisocyanate (ID)

Dose (μg/plate)	S9 mix	*Number of revertants/plate									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA		TA98		TA1537	
Chemicals	+/–	DB	ID	DB	ID	DB	ID	DB	ID	DB	ID
0**	–	137	107	15	11	37	18	34	39	20	20
3.15	–	171	117	17	7	38	19	40	38	25	18
6.25	–	138	105	13	9	26	13	37	28	27	28
12.5	–	167	91	15	16	31	19	37	44	28	23
25	–	142	54	14	8	37	22	41	32	26	21
50	–	100	32	14	10	30	23	28	14	24	4
AF2 0.01	–	580	583			146	198				
0.1	–							496	545		
NaN_3 0.5	–			163	101						
9AA80	–									468	463
0**	+	185	140	15	15	46	23	40	35	20	21
3.15	+	161	143	16	15	51	24	45	44	32	26
6.25	+	191	139	19	13	49	27	33	40	30	22
12.5	+	170	145	15	12	46	20	34	46	20	27
25	+	177	129	13	9	49	25	38	49	25	29
50	+	196	109	19	11	42	18	40	45	20	24
2AA 1.0	+	683	816								
2.0	+			147	146					154	259
10	+					654	534				
0.5	+							309	371		

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls: AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide,; 9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

Table 3. Results of the bacterial reverse mutation assay of 2-chlorostyrene (2-CS), 3-chlorostyrene (3-CS), and 4-chlorostyrene (4-CS).

Dose (μg/plate)	S9 mix	*Number of revertants/plate														
		Base-pair substitution type									Frameshift type					
		TA100			TA1535			WP2uvrA			TA98			TA1537		
Chemicals		2-CS	3-CS	4-CS	2-CS	3-CS	4-CS	2-CS	3-CS	4-CS	2-CS	3-CS	4-CS	2-CS	3-CS	4-CS
0**	–	105	195	158	13	25	25	36	40	39	30	38	23	17	17	20
6.25	–	126	180	143	14	15	26	41	51	21	31	36	38	12	16	14
12.5	–	117	182	125	9	14	21	44	58	19	38	32	16	12	18	12
25	–	102	195	123	18	16	10	31	46	20	45	33	24	7	17	11
50	–	79	121	113	6	11	16	35	30	17	39	23	23	13	11	11
100	–	61	54	110	11	12	8	26	16	20	15	20	18	12	6	23
AF2 0.01	–	830	438	343				315	183	101						
0.1	–										547	489	408			
NaN_3 0.5	–				429	427	426									
9AA80	–													206	260	316
0**	+	119	143	156	21	14	24	29	40	24	42	40	26	16	20	19
6.25	+	149	151	157	22	11	26	31	44	24	50	38	19	18	13	30
12.5	+	129	148	152	15	12	28	33	47	22	50	43	35	15	23	23
25	+	129	144	130	11	17	14	31	46	22	51	46	19	16	21	30
50	+	131	137	117	23	19	16	37	51	19	46	56	38	18	19	22
100	+	114	172	113	20	13	17	30	41	21	49	40	29	17	16	33
2AA 1.0	+	879	983	747												
2.0	+				363	308	240							189	130	190
10	+							444	417	491						
0.5	+										408	524	484			

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls : AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide, ;9AA, 9-aminoacridine ; 2AA, 2-aminoanthracene.

Table 4. Results of the bacterial reverse mutation assay of 2-chlorotoluene (2-CT), diisobutyl ketone (DK) and diphenylamine (DA)

Dose (μg/plate)	S9 mix	*Number of revertants/plate														
		Base-pair substitution type									Frameshift type					
		TA100			TA1535			WP2uvrA			TA98			TA1537		
Chemicals		2-CT	DK	DA	2-CT	DK	DA	2-CT	DK	DA	2-CT	DK	DA	2-CT	DK	DA
0**	–	100	88	75	12	16	7	35	32	20	26	43	20	14	14	16
6.25	–	102	92	63	13	14	10	36	39	21	26	40	21	10	12	14
12.5	–	102	112	65	11	17	9	33	31	20	23	42	19	15	16	11
25	–	112	101	49	15	15	7	35	36	21	28	41	23	18	12	14
50	–	127	105	35	12	14	6	41	37	21	39	41	15	17	9	6
100	–	141	95	34	12	14	9	54	38	21	28	43	9	19	17	2
AF2 0.01	–	349	426	316				174	203	106						
0.1	–										355	248	258			
NaN_3 0.5	–				239	151	198									
9AA80	–													354	243	137
0**	+	97	111	90	10	18	7	43	41	30	40	42	24	20	20	17
6.25	+	109	105	86	12	15	11	38	39	28	43	39	25	26	19	25
12.5	+	99	108	80	14	18	9	37	43	32	40	45	27	29	22	17
25	+	95	108	84	10	17	10	42	36	25	36	38	27	34	17	18
50	+	118	118	60	11	18	12	56	36	28	41	38	22	30	15	28
100	+	146	79	30	9	15	10	68	38	21	46	38	19	35	18	11
2AA 1.0	+	574	409	459												
2.0	+				209	111	126							267	138	123
10	+							465	867	454						
0.5	+										433	159	262			

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls : AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide,; 9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

Table 5. Results of the bacterial reverse mutation assay of acetic anhydride (AA), 2-aminopyridine (2-AP), and ethyl amyl ketone (EAK)

Dose (μg/plate)	S9 mix	*Number of revertants/plate														
		Base-pair substitution type									Frameshift type					
		TA100			TA1535			WP2uvrA			TA98			TA1537		
Chemicals		AA	2-AP	EAK	AA	2-AP	EAK	AA	2-AP	EAK	AA	2-AP	EAK	AA	2-AP	EAK
0**	–	97	198	100	20	14	20	30	36	28	35	21	39	12	18	20
62.5	–	93	205	83	18	16	16	22	38	43	29	29	41	13	24	32
125	–	115	207	92	19	16	18	26	45	33	33	32	32	7	18	30
250	–	107	189	79	22	12	21	29	35	34	29	26	29	8	18	25
500	–	113	190	107	26	15	17	25	35	33	42	26	41	10	16	20
1000	–	113	191	64	26	16	4	23	37	17	42	23	33	13	19	18
AF2 0.01	–	357	467	349				121	164	203						
0.1	–										398	346	211			
NaN_3 0.5	–				138	150	404									
9AA80	–													404	239	325
0**	+	87	182	124	19	14	21	30	33	42	38	32	42	19	20	20
62.5	+	104	187	125	20	15	26	36	30	51	42	27	28	18	22	28
125	+	100	169	125	16	16	16	30	38	45	37	32	32	23	27	31
250	+	112	172	126	20	17	18	41	32	56	45	30	47	16	23	24
500	+	88	173	114	24	17	18	30	30	65	41	32	47	14	21	28
1000	+	95	162	100	18	17	18	37	32	55	43	29	48	27	24	22
2AA 1.0	+	670	474	310												
2.0	+				124	169	139									
10	+							673	629	790				355	207	209
0.5	+										450	176	157			

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls : AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide,; 9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

Table 6. Results of the bacterial reverse mutation assay of methyl n-amyl ketone (MAK), methyl isoamyl ketone (MIK), and oxalic acid (OA)

Dose (μg/plate)	S9 mix	*Number of revertants/plate														
		Base-pair substitution type									Frameshift type					
		TA100			TA1535			WP2uvrA			TA98			TA1537		
Chemicals		MAK	MIK	OA	MAK	MIK	OA	MAK	MIK	OA	MAK	MIK	OA	MAK	MIK	OA
0**	–	101	122	183	8	14	8	42	15	19	27	42	16	18	18	16
313	–	89	124	221	15	17	12	43	18	17	34	51	15	19	23	19
625	–	87	118	196	8	13	10	51	18	26	47	46	15	12	27	17
1250	–	95	121	217	13	15	14	32	19	35	26	37	23	17	24	15
2500	–	86	122	202	8	9	11	35	18	22	37	62	15	16	21	15
5000	–	43	113	220	4	16	17	36	23	26	38	49	22	8	14	13
AF2 0.01	–	436	428	617				357	443	154						
0.1	–										826	449	518			
NaN_3 0.5	–				279	184	174									
9AA80	–													218	221	423
0**	+	89	122	220	13	18	14	24	20	18	46	21	19	13	17	20
313	+	96	123	183	14	16	9	28	23	26	44	25	17	19	11	20
625	+	89	130	210	16	18	20	35	25	28	60	18	23	22	7	16
1250	+	96	114	205	15	19	16	30	15	27	58	22	22	20	7	33
2500	+	90	120	195	15	15	14	25	23	27	53	28	17	15	10	20
5000	+	51	120	189	16	17	16	26	25	20	42	28	24	8	11	16
2AA 1.0	+	429	334	341												
2.0	+				121	278	120									
10	+							421	470	1281	245	205	407	123	235	138
0.5	+															

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls : AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide,; 9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

Table 7. Results of the bacterial reverse mutation assay of n-amyl acetate (nAA), D-α-chloropropionic acid (CP), and 2-N-dibutylaminoethanol (DAE)

Dose (μg/plate)	S9 mix	*Number of revertants/plate														
		Base-pair substitution type									Frameshift type					
		TA100			TA1535			WP2uvrA			TA98			TA1537		
Chemicals		nAA	CP	DAE	nAA	CP	DAE	nAA	CP	DAE	nAA	CP	DAE	nAA	CP	DAE
0**	–	110	83	73	15	12	36	33	27	35	14	24	25	7	13	20
313	–	116	66	71	15	7	30	36	36	29	14	28	24	10	12	19
625	–	109	68	71	17	12	23	41	34	36	13	22	21	7	13	13
1250	–	114	74	64	22	11	21	12	28	20	12	32	20	10	19	18
2500	–	98	94	33	20	12	28	23	38	21	11	23	13	5	13	17
5000	–	105	53	13	10	17	19	9	35	10	10	18	7	2	12	15
AF2 0.01	–	345	356	396				220	154	167						
0.1	–										175	302	231			
NaN_3 0.5	–				214	238	518									
9AA80	–													230	85	90
0**	+	124	97	105	21	13	38	24	32	34	12	27	28	10	11	16
313	+	131	113	102	20	11	49	27	33	32	17	32	30	11	16	16
625	+	132	106	103	16	16	41	26	31	36	10	48	35	6	9	12
1250	+	119	107	106	15	17	41	27	33	35	15	40	28	5	12	9
2500	+	107	107	48	13	16	43	17	33	39	10	24	20	8	9	11
5000	+	103	104	45	12	14	15	10	33	26	10	34	16	10	9	10
2AA 1.0	+	414	428	486												
2.0	+				140	164	201							170	116	146
10	+							445	757	425						
0.5	+										190	349	178			

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls : AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide; 9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

Table 8. Results of the bacterial reverse mutation assay of ethyl formate (EF), methyl ethyl ketone (MEK), and vinylcyclohexene (VC)

Dose (μg/plate)	S9 mix	*Number of revertants/plate														
		Base-pair substitution type									Frameshift type					
		TA100			TA1535			WP2uvrA			TA98			TA1537		
Chemicals		EF	MEK	VC	EF	MEK	VC	EF	MEK	VC	EF	MEK	VC	EF	MEK	VC
0**	–	90	109	127	12	8	16	25	15	32	36	15	30	19	8	20
313	–	95	109	115	15	10	11	53	14	17	36	18	23	22	7	8
625	–	99	115	108	13	10	10	53	13	18	29	14	14	16	11	11
1250	–	105	112	114	15	8	12	51	11	17	28	16	19	9	8	13
2500	–	109	116	111	17	10	9	61	12	15	30	24	21	17	11	16
5000	–	94	114	114	19	8	7	57	13	14	40	26	20	15	8	13
AF2 0.01	–	660	397	480				362	195	188						
0.1	–										329	345	317			
NaN_3 0.5	–				465	124	550									
9AA80	–													165	285	299
0**	+	115	116	137	21	14	19	31	20	31	27	22	31	20	10	17
313	+	106	109	125	17	11	18	33	18	28	22	18	35	24	11	18
625	+	96	121	113	17	15	19	31	17	27	21	21	27	19	11	15
1250	+	91	119	113	11	13	17	35	16	20	19	26	31	24	7	15
2500	+	110	124	113	14	15	11	35	23	16	14	21	25	16	6	19
5000	+	114	118	111	18	10	12	33	15	14	22	24	30	24	8	10
2AA 1.0	+	714	470	450												
2.0	+				152	143	179							230		108
10	+							517	569	440						
0.5	+										228	226	222		153	

*indicates mean number from duplicated, tripricated or quadruplicated plates; **, negative control, DMSO; Positive controls : AF2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3, sodium azide; 9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

mutagenicity test results may be useful in in the chemical toxicity or risk assessment.

References

EPA (1988). GENETOX PROGRAM.

IFCS (1997). Chemicals assessed in 1994-1996 by IPCS/EHC. IFCS/Forum-II/97.06B

Kiligerman AD, Morgan DL, Doerr CL, Milholland V, Tennant AH (1996) Cytogenetic effects in mice of divinylbenzene-55 inhalation. Mutation Res 370, 107-113, 1996.

Lee SK, Kim JH, Kim K, Park JA, Kim HK, Yook HK, Chung YJ (1997) Establishment of existing chemical risk assessment program (ECRAP).

Maron DM, Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutation Res 113, 173-215.

Ministry of Environment (1997). 1997 Proposed Lists for Risk Assessment.

Ministry of Labor, Ministry of Environment (1996) Existing Chemical Inventory in Korea.

National Institute for Occupational Safety and Health (NIOSH, 1997). Registry of Toxic Effects of Chemical Substances (RTECS).

OECD (1993). OECD guidelines for testing of chemicals, Reverse Mutation Assay, No. 471, 472.