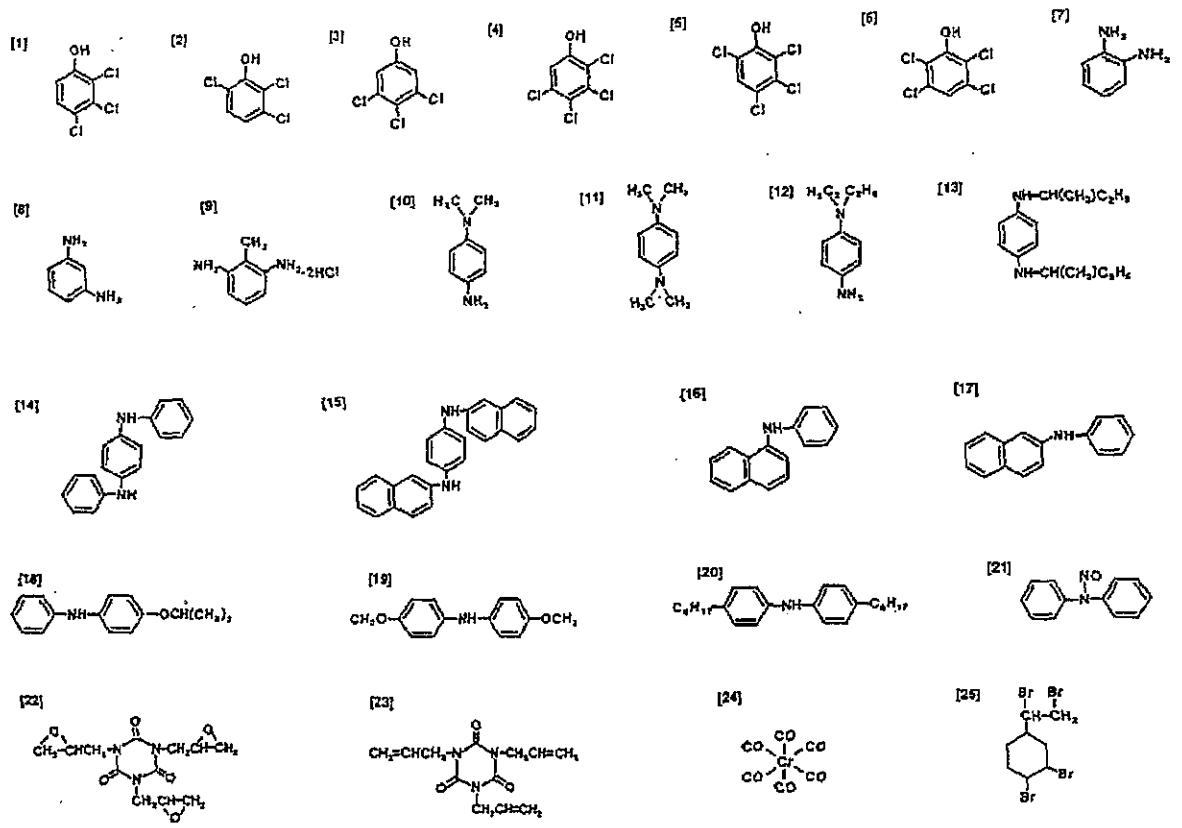

Fig. 1. Structures of test chemicals.

Chromosome preparations were made in the same way as in the test without metabolic activation.

One hundred well-spread metaphases per dose were observed under the microscope (at magnifications of 600–1000). The numbers of cells with chromosomal aberrations and polyploid cells were recorded. Different types of aberrations such as chromatid gaps (ctg) including chromosome gaps, chromatid breaks (ctb), chromatid exchanges (cte), chromosome breaks (csb), chromosome exchanges (cse) and 'others' including fragmentation (frg), were recorded. Any cell with 1 or more aberrations was counted as 1 aberrant cell.

The NIHS historical data base showed that the frequency of CHL cells with aberrations or polyploidy in both untreated and solvent-treated negative controls had not exceeded 4%. From the present study, the mean and range of aberrant cells in solvent-treated negative controls was as follows: ctg: 0.59% (0–3%); ctb: 0.25% (0–2%); cte: 0.14% (0–1%); frg: 0%; csb: 0.04% (0–1%); cse: 0.05% (0–1%); total (with ctg but without polyploid): 1.04% (0–4%); polyploid: 0.45% (0–4%). The final result of each test was decided for structural aberrations or polyploid cells, separately as follows: negative (−) if the frequency of aberrant cells (including gaps for structural aberrations) was less than 5%; inconclusive (±) if greater than 5% but less than 10%; and positive (+) if 10% or more. Overall evaluation for each chemical was made after the judgement of individual results at different dose groups. Weakly positive (+w) was used if the effect was found only at a relatively high concentration (about 2 mg/ml or more), or at a relatively low frequency (about less than 20%), or without a dose-related response (negative at 2 lower concentrations). If the outcome was evaluated as inconclusive or showed no dose-re-

TABLE 2

MAJOR PROTOCOL DIFFERENCES BETWEEN CHO AND CHL ASSAYS FOR CHROMOSOMAL ABERRATIONS

	CHL system	CHO system
Culture medium	Eagle's MEM with 10% calf serum	McCoy's 5A with 10% fetal calf serum
Dose determination	3 doses including one above and one below the 50% growth inhibition dose measured by the Monocellater	3 highest scorable doses selected from 10 doses spanning 4–5 orders of magnitude up to a maximum dose of 5 mg/ml
Treatment time		
Without S9 mix	24 and 48 h	8–10 h
With S9 mix	Treated for 6 h, followed by culture in fresh medium for 18 h	Treated for 2 h, followed by culture in fresh medium for 10–18 h
S9	From the livers of male Fischer rats pretreated with KC-400	From the livers of male Sprague–Dawley rats pretreated with Aroclor 1254
S9 mix [a]	50 µl/ml of S9, 0.16 mg/ml of HEPES, 0.17 mg/ml of $MgCl_2 \cdot 6H_2O$, 0.41 mg/ml of KCl, 0.25 mg/ml of G 6-P, 0.53 mg/ml of NADP in culture medium with 10% calf serum	15 or 20 µl/ml of S9, 2.4 mg/ml of NADP, 4.5 mg/ml isocitric acid in serum-free medium
S9 concentration [a]	5.0%	1.5%
Colcemid treatment	Added 2 h before harvesting	Added 2.0–2.5 h before harvesting (without S9 mix), the cells are washed and then treated with Colcemid in fresh medium
Cell harvest	Trypsin treatment	Mitotic shake-off
Judgement	Percent of cells with aberrations: negative if less than 5%, inconclusive if greater than 5% but less than 10%, positive if 10% or more	Percent of cells with aberrations: statistical significance using the binomial sampling assumption and the trend analysis
Achromatic lesions (gaps)	Included in analysis	Not included in analysis

[a] Concentrations in the final incubation mixture with a test chemical and cells.

lated response, the experiment was repeated. No statistical procedures were applied to the CHL data.

CHO system

The test method has been described by Galloway et al. (1985, 1987). Cloned Chinese hamster ovary cells (CHO-WBL), with a modal chromosome number of 21 (only cells with 19–23 chromosomes were scored), were cultured in McCoy's 5A medium supplemented with 10% fetal calf serum, L-glutamine and antibiotics. In the test without metabolic activation, the cells were treated with the test chemical for 8–10 h. They were then washed, reincubated with medium containing Colcemid and harvested 2.0–2.5 h later. In the test with metabolic activation, the cells were exposed to the test chemical and S9 mix in serum-free medium for 2 h. Following treatment, the cells were washed and reincubated in medium containing serum. The cells were allowed to grow for 10 h with Colcemid present for the final 2.0–2.5 h.

The S9 mix was prepared from the livers of male Sprague–Dawley rats pretreated with Aroclor 1254. The S9 mix consisted of 15 or 20 µl/ml of S9, 2.4 mg/ml of NADP, and 4.5 mg/ml isocitric acid in serum-free medium (the final concentration of S9 in the medium is estimated to be 1.5%). The cells were exposed to a range of 10 doses spanning 4–5 orders of magnitude up to a maximum dose of 5 mg/ml or to the limits of solubility in culture medium. Generally, the 3 highest doses with analyzable cells were scored.

If little or no delay was found in the cell cycle as indicated by a preliminary sister-chromatid exchange test, the cells were harvested 10–12.5 h after the beginning of treatment as stated above; if a delay was found, they were harvested at 17–20 h. Thus, harvest times ranged from 10 to 20 h (−S9) and from 12 to 20 h (+S9). Cells were

collected by mitotic shake-off as described by Galloway et al. (1985). Slides were prepared by the air-drying technique and stained with Giemsa. One hundred or 200 well-spread metaphases per dose were scored. Solvent-treated and/or untreated cultures served as solvent and negative controls, respectively. Triethylenemelamine (CAS No. 51-18-3) or mitomycin C (CAS No. 50-07-7) was used as the positive control in tests without metabolic activation. Cyclophosphamide (CAS No. 50-18-0) was the positive control in tests with metabolic activation.

Cells were scored for the following types of aberrations: 'simple' (chromatid or chromosome breaks, fragments, deletions and double minutes), 'complex' rearrangements (interstitial deletions, triradials, quadriradials, rings, and dicentrics) and 'other' (pulverized). Gaps and endoreduplications were scored, but not included in the analyses or in the data presented in the Appendix. All other categories were combined for the statistical analyses. The 'percent cells with aberrations' rather than the number of aberrations per cell was analyzed, so as not to distort the results in cases where there were a high number of aberrations in any one cell. For these 25 studies the mean and range of cells with aberrations in solvent control groups was 1.5% (0.0–3.0%) without S9 and 2.2% (0.0–6.0%) with S9.

The data were analyzed for trend and individual dose groups were compared with the concurrent solvent control. A linear regression of the log dose vs. the percent of cells with aberrations was used for the trend analysis. A binomial sampling assumption was used to examine increases over the solvent control for each dose point. The P value was adjusted by Dunnett's method to take into account the multiple-dose comparison.

A dose was considered positive if the adjusted P value was < 0.05 and the trend was significant if the P value was < 0.003. A positive test result was defined as a trial with 2 significant doses. If only 1 dose was significant and there was a significant trend, the result was weakly positive. In cases where there was only 1 significant dose and no trend, or only a significant trend, the chemical was concluded to be equivocal. Generally, trials concluded to be positive, weakly positive, or equivocal were repeated.

Results and discussion

The summary results obtained with 25 chemicals in CHL and CHO cells are shown in Table 3. The data and results for the individual trials are given in the Appendix. The chemical numbers used in Table 1 are used consistently throughout the other Tables and the Appendix. Usually the experiment was repeated for confirmation; however, only data which were considered representative (in most cases the data from the last experiment performed on each sample) are presented in the Appendix. In all cases the positive controls responded as anticipated and these data are not presented.

For the CHL system, both exposure and harvest times are constant throughout experiments. Tests without metabolic activation are designated as 24-0 or 48-0 (exposed for 24 h or 48 h with Colcemid present for the last 2 h and harvested immediately after the treatment). The tests with metabolic activation are designated as 6-18 (exposed for 6 h and grown for 18 h with Colcemid present the last 2 h). Cells treated only with the test agent (without S9 mix) for 6 h and harvested 18 h after the treatment were also examined in this test. In the Appendix, the frequencies of polyploid cells and cells with chromosome aberrations are presented.

For the CHO system, the exposure and harvesting time varied slightly among the 4 laboratories conducting the tests. The number of aberrations per cell and the frequency of cells with the simple and complex types of aberrations are presented.

When the overall results obained from tests both with and without S9 mix are considered, the 2 systems agree on 15 results: positive or weakly positive in 11 chemicals (Nos. 2, 4, 5, 6, 7, 8, 9, 11, 12, 19 and 22), and negative or inconclusive in 4 chemicals (Nos. 3, 13, 16, 20). The individual results of these chemicals are as follows.

No. 2 (2,3,6-trichlorophenol) was positive in both CHL and CHO cells in the presence of S9 mix. Positive results were obtained in CHL cells at doses of 200.0 μg/ml and higher (11.0% at 200.0 μg/ml, 15.0% at 250.0 μg/ml, 24.0% at 300.0 μg/ml), and in CHO cells at 375.0 μg/ml and higher (10.0% and 68.0% at 375.0 and 750 μg/ml, respectively). In the absence of S9 mix, it was

TABLE 3

SUMMARY RESULTS OF CHROMOSOMAL ABERRATION TESTS IN THE CHL AND CHO TEST SYSTEMS

Chemical No.	CHL system						CHO system			
	Without S9 mix				With S9 mix		Without S9 mix		With S9 mix	
	Dose [a]		Result [b]		Dose	Result	Dose	Result	Dose	Result
	(μg/ml)	(mM)	24 h	48 h	(μg/ml)	24 h [c]	(μg/ml)	(time)	(μg/ml)	(time) [c]
1	30.0	(0.15)	−	−	120.0	−	37.7	− (20 h)	70.2	+ (20 h)
2	180.0	(0.91)	−	±	200.0	+	175.0	+ (20 h)	375.0	+ (20 h)
3	15.0	(0.075)	−	−	30.0	±	30.0	− (20 h)	30.0	− (12 h)
4	30.0	(0.13)	−	−	60.0	+w	40.0	− (20.5 h)	29.8	+ (12 h)
5	90.0	(0.39)	±	±	250.0	+w	50.0	+w (20 h)	100.0	+ (20 h)
6	60.0	(0.26)	−	−	60.0	+	75.0	− (20 h)	175.0	+ (20 h)
7	3.0	(0.028)	+w	+	25.0	−	7.5	+ (20 h)	2513.0	+ (20 h)
8	15.0	(0.14)	±	+	125.0	+w	246.5	+ (20 h)	5000.0	− (12 h)
9	250.0	(1.28)	+	+	1500.0	+	1000.0	+ (17 h)	1600.0	− (13 h)
10	10.0	(0.073)	+	−	20.0	±	30.0	− (12.5 h)	160.0	− (15.5 h)
11	10.0	(0.061)	+	+	20.0	+w	50.0	+w (12.5 h)	50.0	+ (13.5 h)
12	2.5	(0.015)	+w	−	10.0	−	16.0	+ (12.5 h)	100.0	− (13.5 h)
13	2.0	(0.009)	±	−	4.0	−	5.0	− (12.5 h)	30.0	− (13.5 h)
14	1.8	(0.007)	+	+	15.0	−	30.0	− (12.5 h)	160.0	− (13 h)
15	500.0	(1.39)	+	+	1000.0	±	1600.0	− (12.5 h)	500.0	− (13 h)
16	30.0	(0.14)	−	−	15.6	−	29.9	− (10.5 h)	19.9	− (20 h)
17	30.0	(0.14)	−	−	60.0	+w	29.7	− (10.5 h)	29.7	− (12 h)
18	30.0	(0.13)	±	+w	31.3	±	49.8	− (10.5 h)	30.0	− (12 h)
19	30.0	(0.13)	+w	+	30.0	+w	2498.0	+w (20.5 h)	1320.0	± (12 h)
20	500.0	(1.27)	−	−	500.0	−	4450.0	− (10.5 h)	4450.0	− (12 h)
21	62.5	(0.32)	−	−	62.5	+	87.5	− (10.5 h)	1530.0	− (12 h)
22	1.2	(0.004)	+	+	5.0	−	3.0	+ (10.5 h)	10.0	+ (12 h)
23	1000.0	(4.01)	±	±	250.0	+	1460.0	− (10.5 h)	2910.0	− (12 h)
24	8000.0	(36.35)	−	−	2000.0	+w	128.0	− (10.5 h)	128.0	− (12.5 h)
25	120.0	(0.28)	−	−	125.0	+	100.0	− (10.5 h)	300.0	− (12 h)

[a] The minimum effective dose for 'positive' results or the maximum tested dose for 'negative' and 'inconclusive' results.
[b] Overall evaluation: +, clearly positive; +w, weakly positive; ±, inconclusive; −, negative.
[c] Harvest time.

positive only in CHO cells at more than 175.0 μg/ml (7.0% and 6.5% at 175.0 and 250.0 μg/ml, respectively). In CHL cells, it was negative at 24 h and inconclusive at the highest doses, 120 and 180.0 μg/ml at 48 h.

No. 4 (2,3,4,5-tetrachlorophenol) was positive with S9 mix in both cell lines; in CHL cells effects were observed at 60.0 μg/ml (12.0%) and at 120.0 μg/ml (15.9%). In CHO cells, doses of 29.8 μg/ml (5.5%), 45.2 μg/ml (12.0%) and 60.3 μg/ml (17.0%) were judged to be positive. Without S9 mix, it was negative in CHL and CHO cells, at doses up to 30.0 μg/ml and 40.0 μg/ml, respectively.

No. 5 (2,3,4,6-tetrachlorophenol) was positive or weakly positive with S9 mix in both cell lines. In CHO cells, it was clearly positive at doses of 100.0 μg/ml (20.5%) and 150.0 μg/ml (74.0%). In CHL cells, the overall conclusion was weakly positive, because it was evaluated as positive only at a single dose, 250.0 μg/ml (14.0%) and a dose-related effect was not found in 3 separate experiments. Without S9 mix, it was considered weakly positive (not because of a small effect but because aberrations were elevated only at the highest dose tested) in CHO cells with a significant effect only at 50.0 μg/ml (6.0%) and a repeat test gave a positive effect only at 125.0 μg/ml (64%). In CHL cells without S9 mix, it was inconclusive at doses up to 90.0 μg/ml.

No. 6 (2,3,5,6-tetrachlorophenol) was positive with S9 mix, but negative without S9 mix, in both cell lines. With S9 mix, it was positive in CHL cells at more than 60.0 μg/ml (11.0% at 60.0 μg/ml and 60.5% at 120.0 μg/ml), and in CHO cells at 175.0 μg/ml (28.0%) and at 262.5 μg/ml (80.0%) (cytotoxic at 350.0 μg/ml). Without S9 mix, it was negative in CHL cells at doses up to 60.0 μg/ml, as well as in CHO cells at doses up to 75.0 μg/ml (cytotoxic at 112.0 μg/ml).

No. 7 (*o*-phenylenediamine) was positive without S9 mix in both cell lines; a positive response was observed in CHL cells at 3.0 μg/ml (15.0% at 48 h) and at 6.0 μg/ml (14.0% at 24 h and 32.0% at 48 h). It was judged to be positive in CHO cells at 7.5 μg/ml (9.0%), 11.3 μg/ml (19.0%) and 15.0 μg/ml (23.0%). With S9 mix, the effect was reduced in CHO cells and positive results were obtained at relatively high doses, 2513 μg/ml and 5000 μg/ml. In CHL cells, it was negative up to 25.0 μg/ml, though the control for the S9 mix (treated for 6 h without S9 mix) showed a positive response at 25.0 μg/ml.

No. 8 (*m*-phenylenediamine) was positive without S9 mix in both cell lines: in CHL cells at 15.0 μg/ml (16.0%) and 30.0 μg/ml (21.0%) at 48 h, and in CHO cells at doses of 246.5, 502.5, and 747.0 μg/ml (31.0%, 32.0% and 64.0%, respectively). With S9 mix, it was positive in CHL cells only at 125.0 μg/ml (17.0%) (cytotoxic at 250.0 μg/ml), but negative in CHO cells at doses up to 5000 μg/ml.

No. 9 (2,6-toluenediamine dihydrochloride) was clearly positive without S9 mix in both cell lines: in CHL cells at 250.0 (23.0%) and 500.0 μg/ml (57.0%) at 48 h, and in CHO cells at 250.0 (8%), 500.0 (5%), 1000.0 (12.0%) and 1500.0 μg/ml (29.0%). With S9 mix, it was also positive in CHL cells at high dose levels (28.0% at 1500.0 μg/ml), but in CHO cells it was negative at doses up to 1600.0 μg/ml (cytotoxic at 3000.0 μg/ml).

No. 11 (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) was positive or weakly positive without S9 mix in both cell lines. In CHL cells, it was clearly positive at doses above 10.0 μg/ml (32.0% at 15.0 μg/ml at 24 h). In CHO cells, it was only positive at the highest dose, 50.0 μg/ml (6.5%), and was therefore considered weakly positive. With S9 mix, it was positive in CHL cells only at 20.0 μg/ml (18.0%), and in CHO cells at 50.0 (5.5%) and 100.0 μg/ml (20.0%), though no clear-cut dose response was seen in either cell line.

No. 12 (*N,N*-diethyl-*p*-phenylenediamine) was positive or weakly positive without S9 mix in both cell lines. In CHL cells, it was judged to be weakly positive, because it was positive only at 2.5 μg/ml (24.0%) at 24 h and showed no dose response. In CHO cells, it was positive at 16.0 and 20.0 μg/ml. With S9 mix, it was negative in both cell lines; in CHL cells it was tested at doses up to 10.0 μg/ml, and in CHO cells at doses up to 100.0 μg/ml (cytotoxic at 160.0 μg/ml). In CHL cells, it was positive in the cells treated for 6 h without S9 mix (24.7% at 10.0 μg/ml).

No. 19 (4,4′-dimethoxydiphenylamine) was positive without S9 mix in both cell lines. In CHL cells, it was clearly positive at 30.0 μg/ml (12.0% at 24 h and 31.0% at 48 h) (cytotoxic at 60.0 μg/ml). In CHO cells, it was positive only at the relatively high dose of 2498.0 μg/ml (7.5%). With S9 mix, it was positive in CHL cells at 30.0 μg/ml (21.0%). In CHO cells, the lowest dose tested (132.0 μg/ml) gave a positive effect, and in repeated tests single doses gave positive results; however, the results are considered inconclusive, because of lack of both reproducibility and a dose-related effect. In CHL cells, a slight increase in the frequency of polyploid cells was noted in cells treated for 6 h without S9 mix (12.0% at 30.0 μg/ml), and this was the only case of increased polyploid cells in the present study.

No. 22 (tris(2,3-epoxypropyl)isocyanurate) was clearly positive without S9 mix in both cell lines; in CHL cells at more than 1.2 μg/ml (74.0% at 24 h and 92.0% at 48 h) (no mitosis was observed at 2.5 μg/ml and 5.0 μg/ml at 48 h), and in CHO cells it was positive at 3.0–30.0 μg/ml (30.0–87.0%) (cytotoxic at 50.0 μg/ml). An additional experiment in CHO cells showed 100.0% of cells with aberrations at 19.0 μg/ml. With S9 mix, it was negative in CHL cells at doses up to 5.0 μg/ml, although it was positive in the cells treated for 6 h without S9 mix (48.0% at 1.2 μg/ml). In CHO cells, the effect was slightly reduced in the test using S9 mix, but still positive at the dose range of 10.0–100.0 μg/ml.

No. 3 (3,4,5-trichlorophenol) was negative without S9 mix in both cell lines: in CHL cells at

doses up to 15.0 μg/ml, and in CHO cells at doses up to 30.0 μg/ml. With S9 mix, it was inconclusive in CHL cells at 30.0 μg/ml; only gaps and breaks were increased. In CHO cells, it was negative at doses up to 30.0 μg/ml with S9 mix.

No. 13 (*N*,*N*′-di-*sec.*-butyl-*p*-phenylenediamine) was negative with S9 mix in both cell lines: in CHL cells at doses up to 4.0 μg/ml, and in CHO cells at doses up to 30.0 μg/ml. Without S9 mix, it was inconclusive in CHL cells at 2.0 μg/ml at 24 h, and negative in CHO cells at doses up to 5.0 μg/ml.

No. 20 (4,4′-dioctyldiphenylamine) was negative with and without S9 mix in both cell lines. In CHL cells, it was negative at doses up to 500.0 μg/ml both without and with S9 mix. In CHO cells, it was negative even at the relatively high dose of 4450.0 μg/ml both without and with S9 mix (precipitation of the chemical was noted at 4450.0 μg/ml without S9 mix and at 1340.0 μg/ml with S9 mix).

The remaining 10 chemicals gave qualitatively different results in the 2 test systems: 1 chemical was positive only in the CHO system, while 9 were positive only in the CHL system. These differences are as follows.

No. 1 (2,3,4-trichlorophenol) was positive only in CHO cells in the presence of S9 mix. A dose-related increase in the frequency of aberrant cells was observed over a dose range of 49.8–70.2 μg/ml. Negative results were obtained in the CHL system with doses up to 120 μg/ml with S9 mix. The difference in results is most easily attributable to the activity of the S9 fraction used in the CHO system; a higher concentration of the test chemical and a longer treatment time were employed in the CHL test.

No. 10 (*N*,*N*-dimethyl-*p*-phenylenediamine) was positive only in CHL cells in the absence of S9 mix. Positive results were obtained at 10 μg/ml using 24-h exposure and at 10 and 20 μg/ml following 6-h exposure. In all cases the frequency of aberrant cells was about 20%. Exposure of CHO cells for 12.5 h to doses of 5, 10 and 30 μg/ml gave a weak effect at 30 μg/ml in one test, but the effect could not be reproduced. Because an effect was observed in CHL cells following both 6-h and 24-h exposures, it is possible that the difference in effects between the 2 systems is attributable to a difference in the sensitivities of the 2 cell lines to this chemical. However, if harvest of CHO cells had been delayed to 18 or 24 h, an effect might have been observed.

No. 14 (*N*,*N*′-diphenyl-*p*-phenylenediamine) was positive only in CHL cells without S9 mix. Effects were observed at doses below 10 μg/ml following 24- and 48-h treatments. As an aromatic amine, this chemical should require metabolic activation for activity. It is possible that the inherent metabolic capacity of CHL cells activates the compound when there is sufficient exposure time. It is unexpected, however, that aromatic amines, compounds generally requiring metabolic activation for mutagenic activity, are positive in CHL cells in the absence of S9 mix (see also chemicals No. 10 and No. 15). Tests in CHO cells using extended exposure times should be conducted to determine if an effect similar to that in CHL cells is observed.

No. 15 (*N*,*N*′-di-2-naphthyl-*p*-phenylenediamine) produced results similar to those of chemical No. 14, although the effective doses were higher for chemical No. 15 (500–1500 μg/ml).

No. 17 (*N*-phenyl-2-naphthylamine) gave a positive result in CHL cells only with S9 mix. There was no evidence of a dose–effect relationship in repeat experiments and the effect was judged to be weakly positive. In CHO cells, elevated aberration frequencies were observed in tests with S9 mix but the solvent control value was higher than normal and the treatment effects were not sufficiently high to give a positive result. This compound appears to be a weak clastogen requiring metabolic activation and a longer exposure time or later harvest time, or higher S9 concentration than that used in the CHO test protocol.

No. 18 (*p*-isopropoxydiphenylamine) gave a weakly positive effect in CHL cells without S9 mix and an inconclusive result with S9 mix. It was judged to be negative in CHO cells even though one test with S9 mix gave 17.5% aberrant cells at a dose of 20.0 μg/ml; the result was not reproducible. An explanation for these conflicting results is not readily obvious. It could be concluded that chemical No. 18 possesses weak clastogenic activity, but that its effect is not easily detectable using the test systems employed in this study.

No. 21 (*N*-nitrosodiphenylamine) was positive

only in CHL cells with S9 mix and at lower doses than those used in the CHO tests. As with chemical No. 17, the clastogenic activity may only be evident following longer treatment times and/or later harvest times (with S9 mix) than used in the CHO system.

No. 23 (triallyl isocyanurate) was negative in CHO cells both with and without S9 mix while giving a clear positive result in CHL cells with S9 mix; dose ranges were similar in the 2 test systems. As discussed above, the difference in results may be due to the difference in treatment or harvest times.

No. 24 (chromium carbonyl) was negative in CHL and CHO cells without S9 mix. With S9 mix, it was weakly positive only in CHL cells at relatively high dose levels (2000.0 μg/ml). In the CHO test where much lower doses were used (75.0–128.0 μg/ml), results were negative.

No. 25 (1-(1,2-dibromoethyl)-3,4-dibromocyclohexane) was positive only in CHL cells in the presence of S9 mix; it induced a dose-related increase in the frequency of aberrant cells with doses of 62.5, 125.0 and 250.0 μg/ml. A similar dose range (30.0–300.0 μg/ml) gave no evidence of an effect in CHO cells. The simplest explanation for this difference in results is the longer treatment time used in CHL cells where exposure to the test chemical and S9 mix is 6 h as opposed to 2 h in the CHO system. It is also possible that the higher concentration of S9 fraction in the final reaction mixture (5% in CHL, 1.5% in CHO) and/or later harvest time (18 h in CHL, 10 h in CHO) may have contributed to the different results.

Of the 10 differences in qualitative results discussed above, 5 are positive in CHL cells only in the presence of S9 mix (Nos. 17, 21, 23, 24, 25). Fig. 2 shows the results of testing triallyl isocyanurate (No. 23) with S9 mix using the CHO cells in the CHL protocol. It should be noted that the CHO cells used in these tests were obtained from the ATCC and were not the same cells used by the NTP. It can be seen that positive results are obtained in CHO cells when they are exposed to chemical No. 23 for 6 h in the presence of S9 mix and harvested at 18 h. The dose effect is very similar to that seen with CHL cells using the same protocol. Thus, it appears that some test chemicals

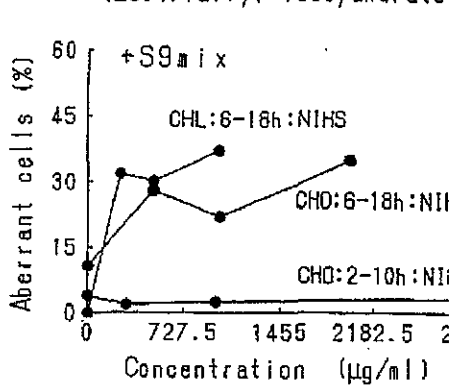

Fig. 2. Results of chromosomal aberration tests o isocyanurate (No. 23) in the presence of S9 mix. C were exposed for 6 h and grown in fresh medium berore harvesting (performed at NIHS). CHO cells posed for 2 h and grown in fresh medium for 10 harvesting (performed at BSC). An additional test wa out at NIHS using CHO cells (obtained from ATCC according to the CHL protocol (6-h exposure time recovery time).

only induce an increase in aberrations wl treatment and harvest times are longer tha h used in the standard CHO protocol. It assumed that this results from the rate at v test agent is metabolized to its genotoxi and/or the time required for an adequate the active form to reach the chromosomes.

There are 4 cases where the only positive come from an effect in CHL cells in the a of S9 mix (Nos. 10, 14, 15, 18). In all 4 ca test chemicals are aromatic amines whi known to require metabolic activation for genic activity. Fig. 3 gives results of *N* phenyl-*p*-phenylenediamine (No. 14) usi CHL protocol with CHO cells. Where cl No. 14 gave negative results in the standar test (12.5-h exposure), results from tests cor at NIHS were positive following 24-h ex using the same doses. As this phenylenec was negative in both test systems in the p of S9 mix, the exogenous metabolic act systems used are apparently not adequate t in the induction of chromosomal aberrati possible explanation for this is that the con is not activated by S9 mix or is activat rapidly detoxified such that the active for

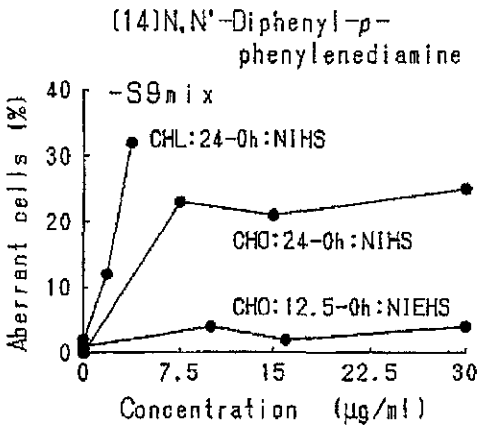

Fig. 3. Results of chromosomal aberration tests on *N,N'*-diphenyl-*p*-phenylenediamine (No. 14) in the absence of S9 mix. CHL cells were exposed for 24 h before harvesting (performed at NIHS). CHO cells were exposed for 12.5 h before harvesting (performed at EHRT). An additional test was carried out at NIHS using CHO cells (obtained from ATCC, U.S.A.) according to the CHL protocol (24-h exposure time).

not reach the cell nucleus. Given sufficient exposure time, the inherent metabolizing capacity of CHL and CHO cells may be sufficient to result in activation of the compound to a clastogen.

A similar phenomenon (a positive effect in CHO cells following long-term exposure in the absence of S9) was observed with *N,N'*-di-2-naphthyl-*p*-phenylenediamine (chemical No. 15) as seen in Fig. 4. In this case, the effect seen in CHO cells using the CHL protocol was apparent only at higher doses than used in CHL cells and did not reach the level of effect seen in CHL cells. This could indicate that CHL cells have a higher metabolic capacity than CHO cells for certain aromatic amines.

In 3 cases (Nos. 1, 7, 22), the test chemical was positive in CHO cells with S9 mix and negative in CHL cells with S9 mix. For chemicals No. 7 and No. 22, the doses used in CHL cells were lower than those used in CHO cells. This may be the reason for the difference in results. A 2-h treatment in the CHL system might allow the use of higher doses and may reveal activity in the presence of S9 mix. However, because these 2 chemicals were positive in both test systems in the absence of S9, the differences in results with S9 mix might be considered relatively unimportant.

Chemical No. 1 (2,3,4-trichlorophenol) wa tive only in CHO cells and only with S9 mix. effect was dose-related and reproducible and resents the single case in which a clear pos result was observed only in CHO cells. The c used in the 2 systems were similar and, in fact 2 highest doses used in CHL cells were some higher than those used in CHO cells. In this the composition of the S9 mix, its concentrati the medium or the length of treatment of cells may not have been appropriate to eli response.

In chemical No. 1, the positive result wa tained in the CHO system using a harvest tin 18 h. On the other hand, 7 test chemicals (N 9, 17, 21, 23, 24, 25), in which the CHL sy had positive or weakly positive results wit mix, showed negative results in the CHO sy using a harvesting time of only about 10 h. T findings suggest that the differences in ha times contribute to the different results bet the 2 test systems.

Among the test chemicals showing qualita similar results in both test systems, some c icals showed quantitative differences in the re For example, in the absence of S9 mix, che No. 19 (4,4'-dimethoxydiphenylamine) was w

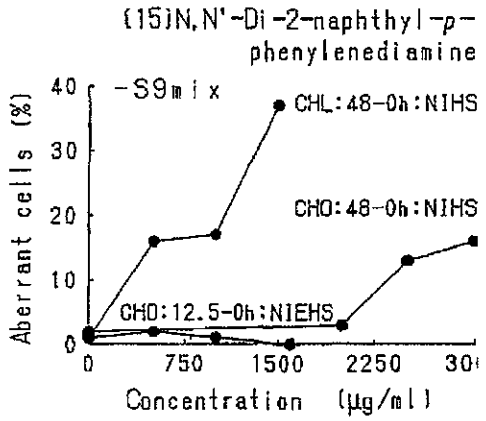

Fig. 4. Results of chromosomal aberration tests on *N,N* naphthyl-*p*-phenylenediamine (No. 15) in the absence mix. CHL cells were exposed for 48 h before harvestir formed at NIHS). CHO cells were exposed for 12.5 h harvesting (performed at EHRT). An additional test w ried out at NIHS using CHO cells (obtained from U.S.A.) according to the CHL protocol (48-h exposure

TABLE 4

COMPARISON [a] OF THE RESULTS BETWEEN THE CHL AND CHO TEST SYSTEMS

	CHL	+	+	+w	±	−	Sub-	+	+w	+w	±	±	−	Sub-	Total
	CHO	+	+w	+	−	−	total	−	−	±	+	+w	+	total	
						24 25									
−S9	Chemi-	22				20 21									
mix	cal	9				16 17		15							
	No.	8	19		23	4 6		14							
		7	11	12	13	1 3		10	18	0	2	5			
	No. of chemicals	4	2	1	2	10	19 (76%)	3	1	0	1	1	0	6 (24%)	25
						20									
+S9	Chemi-				18	16		25							
mix	cal			11	15	14		23	24				22		
	No.	6		5	10	13		21	17				7		
		2		4	3	12		9	8	19			1		
	No. of chemicals	2	0	3	4	5	14 (56%)	4	3	1	0	0	3	11 (44%)	25

[a] For overall comparison, +w is classified as a positive response, and ± as a negative response.