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Review and Audit of IBT No. 663-03222, Chronic Vapor
Inhalation Toxicity Study with Vinyl Chloride (Ethylene
Derived) in Albino Rats, Albino Mice and Golden Hamsters
for the Manufacturing Chemists Association

The review and audit of this study was conducted at IBT's offices in Northbrook, IL, April 14 to April 18, 1980. The review and audit on this study concentrated on the conduct and monitoring of the exposures. However, attention was also devoted to the source of the animals and body weight data as they affected fate and mortality. In addition, the review and audit included inspection of hand written observation books reflecting exposure concentrations, as well as gas chromatographic charts where they were available, hand written and computer body weight printouts, hand written mortality logs, hand written observations and correspondence.

CORRESPONDENCE, PROTOCOL AND TIMETABLE

The timetable indicates the exposures were to be initiated on 9/10/73 for the T-I, T-II and T-III groups and on 10/1/73 for the T-IV groups. The termination of the exposures for the first three groups was to be at 9/8/74 and for the T-IV group at 9/27/74. The animals were then to be held for a year prior to terminal sacrifice. A letter dated June 15,

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1973 from Dr. Torkelson of Dow reflects a site visit was made by the MCA Task Force, visiting the Decatur research labs of IBT where the study was conducted. Questions were raised about the adequacy of equipment, the exposures maintained, the exposure concentrations, the question of contamination and record keeping. A major concern was raised about the adequacy of the analyses and record keeping. Hand written notes alongside the margin of this letter indicate that certain corrective actions were taken at IBT. I was unable to tell whether there were any other documented site visits. In addition, a progress report issued October 13, 1973 after about a month of exposure, reflects adjustments that were made to compensate for the issues that were raised during the MCA site visit.

ANIMALS, HOUSING AND HUSBANDRY

The animals for this study were supplied by the Charles River Breeding Laboratories. Unfortunately, we are not able to identify specific animal receipts with this project. However, we were able to identify from September invoices receipts of hamsters as replacements without charge for animals that were dead on arrival in the August shipment. We were able to identify an additional receipt of hamsters in early November, 1973, two months after the study started, which indicated 130 males and 130 females were received at that time.

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Inspection of the body weights early in the study indicates despite the report not reflecting the mortalities that there was a rather high mortality in the hamsters early in the study. At four and five months there were extensive replacements of the hamster groups.

The animals were ear punched and the toes removed so the animals could be individually identified. This was to allow for identification for individual body weight determination and identification of when the animals died, because the animals were group housed in groups of 3-5 in stainless steel cages. Inspection of some of the hand written notes shows the layout of the rats in the rack and indicates that extra animals were to be spaced evenly throughout the rack. In addition, another hand written sheet shows the arrangement of the gang cages in the chambers. There were 18 gang cages, 8 rats each, to equal 144 rats. However, the protocol only calls for 100 rats at each level. This procedure allows for ample opportunities for replacement and substitution.

Despite the fact that the animals were identified by ear punching and toe removal, most of the time the animals were not weighed individually but rather by the cage, e.g., 3-5 animals. Numerous hand written notes showing the arrangement of some of the animals in the racks reflect the animals escaped or were cannibalized, along with mortality dates for the remaining individual animals.

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VINYL CHLORIDE, SOURCE, IDENTITY

Vinyl chloride (ethylene based) appears to have been supplied as, e.g., containers in excess of 500 pounds, by PPG Industries, Industrial Chemical Division, Lake Charles, LA. A letter from W. B. Braybill of PPG to Dr. John Goode of Bio-Test in Decatur indicates that one cylinder would be required per month. Heel sample analyses from the Bio-Test residues were also performed by PPG at the Lake Charles, LA operation and those that were available appeared to be in good agreement with the new sample analyses supplied to Bio-Test. Unfortunately, there was no inventory log at IBT for vinyl chloride. PPG personnel should be asked for accountability of the vinyl chloride sent to IBT. In addition, they would also have records of the returns for heel analyses.

GENERATION OF VINYL CHLORIDE VAPORS

The vinyl chloride supply tanks were kept in an explosion room. The material was piped to the test chambers through thin-walled stainless steel tubing. Before the thin-walled tubing delivered the vinyl chloride to the chambers, it was connected to a micrometering valve and a glass rotometer to control and monitor the supply of vapor to each chamber. Vapor-air mixture entered the top of the inhalation chamber, circulated through the chamber and was exhausted at the bottom. Unfortunately, we were unable to find a daily log for

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the weighing or measuring of the vinyl chloride that was dispensed during each daily run.

CHAMBER MONITORING

There are records of standardizations being conducted in September of 1973, covering the range from 20 to 100 ppm standardization and from 50 to 400 ppm for the 200 ppm standardization and from 400-4000 ppm for the 2500 ppm level. Standardizations were performed at approximately weekly intervals prior to that point where the automatic sampling system was hooked up to the on-line gas chromatograph recorder. The initial samples from the chambers for the first part of the study (six weeks) were obtained by use of grab samples which were then analyzed by the gas chromatograph. We were unable to find any record of daily sample analyses being taken in the research books for the month of October, 1973, the second month of the study, other than ranges and mean concentrations. There also was no evidence of chamber temperature or humidity being recorded.

General Observations

The following observations were obtained by inspection of research log books which present means and ranges of exposure concentrations, time interval sampling for the gas chromatographic concentrations and charts, as well as the hand written observations in the research log books.

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1. Most of the time the chambers were not on for six hours.
2. Most of the time the chambers were not even monitored for five hours, in some instances they were only monitored for one to two hours.
3. In some instances there was selected data for the daily exposure concentration, e.g., values on the low side may have been eliminated or values on the high side may have been eliminated.
4. Periodically, there were mechanical problems associated with the rotometer, e.g., broken or clogged or unclean lines.
5. Some days there was no exposure because of equipment failure or TV filming or only a one-hour exposure was conducted.
6. There was evidence that the chambers did not have an adequate clearance time, since measurements made 45 minutes after termination of the exposure still showed rather high levels of vinyl chloride present.
7. There was evidence of contamination in the solvents room and the pilot plant which were in close proximity to the exposure room.
8. Some days there was no analytical data recorded for the exposure concentrations.
9. In some cases, the research log book showed means and ranges for exposure concentrations, with no raw supportive

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data shown for periodic sampling with the gas chromatograph.

10. In some instances, the 50 ppm nominal concentration may have reached a concentration as high as 100 ppm and, in one instance, as high as 400 ppm which is not reflected in the means or ranges presented in the interim report.
11. In some instances, the control chamber indicated levels of vinyl chloride in a range of 5-11.5 ppm.
12. In September of 1974, near the end of the year of exposure, there was some research book data reflecting exposure concentrations but no supportive gas chromatographic data.
13. In some cases there is no data available for one or two levels out of the four exposure chambers being run.
14. There were periodic problems with the gas chromatograph itself, either with the Ignitor, Detector or the Recorder.
15. In some instances there were means and ranges presented in the research book but no interval data were available.
16. In some instances, the chamber may have been on for four or five hours yet gas chromatographic readings were only taken for two hours.
17. There were a number of instances where the data presented in the research book do not agree with the data shown on the gas chromatographic chart.

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Specific Exposure Observations

The following notations were associated with the specific exposures and may also be used together with the inhalation chamber concentrations presented in Table I. The exposure concentrations presented in Table I are those presented by IBT and one can see with the use of these specific notations that the values presented in Table I are not an accurate reflection, in many instances, of the exposure concentrations or are based on minimal supportive analytical data.

<u>Exposure Number</u>	<u>Comment</u>
5	The values for the T-I mean and the range for the raw data in the report do not agree.
20 & 35	In the daily intervals, there were problems with sampling and the curve does not appear to be of value.
53	200 ppm equaled 400 ppm.
56	The gas chromatographic charts are no good and the research book concentration is not shown or recorded.
80	The gas chromatographic chart agrees with the data in the research book.
83	There are no gas chromatographic charts but there is data in the research book.

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<u>Exposure Number</u>	<u>Comment</u>
85	There are no T-I or T-II data on the chart or in the book. T-III data is present in the research book but not on the gas chromatographic chart.
86	The gas chromatographic chart and the data in the research book agree but the exposure concentrations are on the low side.
108-110	The gas chromatographic charts do not match the data in the log book. Observations were noted that some of the data was off the GC chart and there were some operator errors and missing data.
134&135	There were no GC charts to go with the data presented in the research books. No charts available for the exposures 110-136.
141	A line was drawn through the first hour values and apparently discarded.
144	There are gas chromatographic charts and research book data available but another notebook says that exposures were not run. In addition, the control line shows contamination.
145	A notation was made that some of the readings were off the chart -- wide excursions on both the low and high sides. In addition, the controls were contaminated.

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<u>Exposure Number</u>	<u>Comments</u>
146	Some of the data from the charts has been discarded.
150	The T-II value at the first sampling interval does not agree with the data presented in the research book. The negative control line also shows contamination. The T-III value is on the high side and reaches 3420.
152	A broken rotometer on line T-I. The values for the T-III line indicate they are not working and they have been deleted. The control background is high. Selected data has been used for the tabulated data presented.
158	Analytical samples are present for four hours. There are missing data when one compares the GC charts versus that present in the research book.
159	The chambers were only on for two hours. There are no exposure concentrations for T-II, T-III and T-IV at the first sampling. There are incomplete values for the second sample at two hours.
165	The chamber was operated for 4 1/2 hours. Zero time was taken ten minutes after the start of the exposure, at which time the gas chromatographic readings were taken. Environmental samples on the

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<u>Exposure Number</u>	<u>Comments</u>
165 (cont'd)	roof appeared high. Hood efficiency, where the syringes were cleaned was poor. Vinyl chloride was still detectable in the T-IV chamber 45 minutes after the exposure was terminated. The solvent room and the pilot plant both showed vinyl chloride contamination.
166	The first reading on the gas chromatographic chart is 3:55 pm. The readings continue until 4:40 pm at sharply lower concentration. The chamber was turned on at 10 o'clock in the morning.
169	Sampling was only conducted for 4 hours. The T-III value was 3710 ppm. For the T-I and T-II lines, there was no sample taken for the second sampling interval or the fourth interval.
180	Basically no analytical data on exposure concentration.
196	Exposures were sampled for five hours. Selected data in the daily range and no noon samples. The control line was contaminated.
197	The chambers ran for five hours and GC data are available for three.

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<u>Exposure Number</u>	<u>Comment</u>
201	A seven-hour exposure is indicated as having been run. The sampling for GC analysis was run for somewhat less than five hours. Selected data has been taken at the initial reading and then sporadic data is missing throughout the run.
206	The chambers were not monitored and no data are available.
210	No sampling. Exposures were only on for one hour with a notation of TV filming.
218	There was one reading for each line for the first two hours, obtained manually. Half of the samplings were done on automatic, but two, rather than three, samples were obtained.
224	Notation is made of CBS filming tomorrow; exposures were run for 4 1/2 hours. The line control had a reading of 6. The GC chart shows no agreement with the research book, which is sparse. However, it does show T-III range from 3-320 ppm. The T-I 50 ppm range equals 445 ppm.
225	No gas chromatographic charts to support the minimal data in the research book. The chambers were turned on at 9:00 am and were off at 11:40 am. GC readings were taken only at 10:00 and 11:00 am.

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<u>Exposure Number</u>	<u>Comment</u>
228	For T-I and T-II, there are missing data. For T-III, there are no values for the first and second samplings.
231	One-third of the values which were on the low side were deleted from the calculation of the averages, one of which was off the graph. Selected data is shown. The control shows a peak height of 7.5, indicating some contamination.
263&237	The gas chromatograph was not working; no data but means have been reported for one value at one interval.
238&239	The research book shows a mean and ranges but no supportive data were available.
243	The line control shows a peak height of 8. One-third of the data from the GC charts were not used in the calculations or averages in the research book.
244	Samples are only shown for three hours.
251	The GC chart data is sparse. The line control is shown as 6.5. The 50 ppm equaled 400 ppm and a notation of major problems.
255	Air recirculated was causing high concentrations in the control chamber. The chambers were turned

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<u>Exposure Number</u>	<u>Comment</u>
255 (cont'd)	off. The control chamber had a level equal to 3.4 ppm of vinyl chloride. A notation of definite leakage into the room was recorded. Selected data only for two hours of the exposure was used.
257	The T-IV chambers were turned on two hours after the rest of the chambers. The line control shows vinyl chloride contamination. One of the 50 ppm values is equal to at least 100 ppm. In addition, one-third of the values were unavailable and selected data were used.
258	The first exposure concentration readings were taken at 1:15 pm and also at 2:15, despite the fact the chambers were turned on at 11:30 in the morning. Most of the data were incomplete.

This appeared to be the last exposure where gas chromatographic chart data were available. The research book has data for exposures 259, 260, 261, 262, but there are no supportive gas chromatographic data.

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BODY WEIGHTS

Body weights were available, either as hand written calculations or as computer printouts. Unfortunately, many of the computer printout body weight tabulations reflected the animals being weighed on a gang-cage basis. Therefore, it becomes more difficult to determine substitutions. A tabulation beginning with month 5 and proceeding through month 13 is shown below for the body weight data. This compares the data presented in the interim IBT reports versus that obtained when one inspects the computer printout itself. These data have only been examined for the T-III group.

MEAN BODY WEIGHTS FROM PRINTOUTS
VINYL CHLORIDE RATS, T-III - 2,000 PPM

<u>Month</u>		<u>Male</u>	<u>Female</u>	<u>Month</u>		<u>Male</u>	<u>Female</u>
5	Report	549	321	9	Report	681	359
	Printout	550.5	312		Printout	627	359
6	Report	567	329	10	Report	663	356
	Printout	567.3	339		Printout	629	356
7	Report	565	346	11	Report	696	374
	Printout*	714	436		Printout	680	374
		691		12	Report	736	378
		702			Printout	718	378
		606		13	Report	740	420
8	Report	585	359		Printout	729	420
	Printout**	581	359				

*All values shown on printout

**Subtracted .329 grams from
printout mean

Inspection of these data indicates that during the seventh month there is a major difference between the body weights in the printouts and those presented in the final report, suggesting replacements.

MORTALITY AND FATE

Hand written sheets reflecting tabulations of mortalities are available. These sheets bear various dates at periodic intervals. Additional tabulation reflects animals that are missing or cannibalized, based upon the gang housing. However, there are numerous errors present and inconsistencies in these tabulations. A chart of missing rats, prepared near the end of the third month of the study, shows numerous animals not accounted for in Dr. Shah's tabulation. These differences are as follows:

Untreated control male rats - 2 animals missing; none shown on Dr. Shah's tabulation.

Untreated control females - 3 missing; none shown on Dr. Shah's tabulation.

T-I male rats - 7 missing; 1 shown on Dr. Shah's tabulation.

T-II male rats - 6 missing; none shown on Dr. Shah's tabulation.

T-III male rats - 4 missing; none shown on Dr. Shah's tabulation.

T-I female rats - 6 missing; none shown on Dr. Shah's tabulation.

T-II female rats - 2 missing; none shown on Dr. Shah's tabulation.

T-III female rats - 4-5 missing; none shown on Dr. Shah's tabulation.

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There are numerous evidences of errors in the animal numbers or the animals being missexed, e.g., animal 751 female should be 638 male in the T-III group; animal 787 female should be male 691 in the T-III group; animal 788 female should be 692 male in the T-III group; animal 689, T-III male rat should be 686; 605, T-III female rat, is a male. Autopsy sheets that reflect extra animals were available.

A mortality tabulation prepared from the animals available in each group, as determined by the body weight printouts, beginning with month five and continuing through month 13 is shown in the accompanying Table. You will note a substantial difference in the numbers available as tabulated between the printout and that presented in the report for the male T-III group, month 7, 12 and 13. Similarly substantial differences in the females in months 12 and 13, as well, also suggests substitution of the animals was occurring.

Close attention was paid to determining the specific fate of the five animals examined in detail by Dr. Bussey in the T-III male group, as reported to have brain tumors. Inspection of the hand written post mortem tabulations by IBT staff, as opposed to the data presented on the autopsy sheets and the mortality data presented in the final report indicates substantial discrepancies.

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Animal #641, T-III male, was found dead at 369 days, according to the report. Hand written P.M. tabulation sheet shows the animal to be dead by 8/6/74, which is about 330 days into the study. The necropsy sheet shows the animal's date of death as 9/3/74 (359 days). These differences have not been explained.

In the case of animal #649, the necropsy sheet and the report shows that the animal died at 402 days. The sheet presenting post mortem tabulations, dated 9/11/74, however, shows the animal as being dead. This date would be approximately one year into the study.

Animal #664 was reported as having died at 373 days. This animal is shown as being dead 7/17/74 on the necropsy sheet and as being dead on a hand written tabulation on 8/9/74. Therefore, the 373 day mortality presented in the report is incorrect and was probably 311 days.

Animal #672, shown in the report as having died at 310 days, is also shown on a post mortem mortality tabulation dated 7/26/74 as being dead by that time. A necropsy date of 7/16/74 for this animal is noted. Therefore, the fate of this animal appears to be correct.

In the case of animal #667, the report indicates days on test as 383 but the necropsy sheet is dated 6/27/74, reflecting less than a year on test. A post mortem log dated 6/26/74 also shows the animal as being dead. Therefore, the date presented as days on test in the report is in error.

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CONCLUSIONS

An intensive audit of certain aspects of IBT No. 633-03222, chronic vapor inhalation toxicity study with vinyl chloride, devoted to inspection of exposure concentrations and pertinent body weight and mortality logs indicates the following:

- (a) There are numerous evidences of replacement of animals in this study. There appears to be an exceptionally high replacement of the hamsters. Also, because of the differences in body weights and mortalities noted in the T-III rats, it is possible that there was major substitution of the animals between the sixth and seventh months of the study and perhaps at 12 and 13 months. There are evidences of extra animals being available for the various species in the study. The accountability for the animals and the mortalities show substantial discrepancies.
- (b) The monitoring of the exposure concentrations and the conduct of the exposures shows poor technique and selection of data in many instances but does seem to indicate that a three-level study was conducted, even though the swings in exposure concentrations periodically may have been $\pm$ 50-100%. The instances of actual overlap between exposure concentrations at the T-I level and the T-II level were relatively infrequent and there do not appear

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to be any real instances of overlap between the T-II and T-III levels.

- (c) The animals reported to have brain tumors indicate discrepancies as to their actual dates of death versus those reported in interim reports and in some cases on the necropsy sheets.

In summary, the study was conducted in an extremely sloppy fashion and one would have to look at any of the results more from a qualitative standpoint than a quantitative standpoint.


Bob West, Ph.D.

Date: May 10, 1980

BW/am

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TABLE I

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Toxicity Study - Albino Rats, Albino Mice, and Golden Hamsters

Chamber Concentration Data

Day Number	Exposure Number	Chamber Concentration (ppm)							
		TE-I		TE-II		TE-III		TE-IV**	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	1	39	45	43	130	180	173	1820	2610
4	4	45	54	51	24	162	87	1190	3600
5	5	50	56	52	182	237	218	1160	3100
16	12	42	63	60	54	266	156	2400	3100
29	21	48	66	58	192	248	219	1090	2490
39	29	42	55	43	188	216	209	1900	3010*
56	48	48	114	84	172	452	298	2240	4000
73	53	42	58	49	178	211	202	2380	2700
80	58	45	60	54	220	364	243	2020	2760
99	71	29	31	30	258	310	277	2740	3940
120	86	45	46	45	168	232	199	1960	2960
130	94	42	63	53	180	228	201	2680	3100
150	108	35	58	49	90	288	200	1780	2920
151	109	32	62	44	150	234	193	1280	2800
152	110	41.5	110	58.9	170	292	198	2300	2800
171	123	35	55	48	186	210	197	2320	3020
186	134	47	53	49.6	184	224	209	2040	2680
187	135	46.5	55.5	50.7	180	262	194	2180	3400
197	141	40	55	45.1	178	216	196	2400	2700
200	144	38.5	56	49	178	310	223	2340	3120
201	145	37.5	55.5	48	174	212	197	2340	2740
208	150	48	54.5	51.4	170	242	200	2260	3420
212	152	36	64.5	48.1	154	282	194	2160	3200
220	158	36.5	52.5	45	138	250	192	1440	2600
221	159	44.5	55	49.2	130	138	134	2460	2620
229	165	46.5	46	49.4	190	216	200	2420	2800
232	166	47	71	52.3	184	228	201	2100	2820
235	169	44	51	47.6	196	215	207	2440	3710

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TABLE I continued

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Toxicity Study - Albino Rats, Albino Mice and Golden Hamsters

Chamber Concentration Data

Day Number	Exposure Number	Chamber Concentration (ppm)								
		TE-I		Mean	TE-II		Mean	TE-III		Mean
		Range			Range			Range		
248	178	47.5	52.5	51.2	186	222	203	2420	2700	2573
274	196	44.5	51	47.9	188	216	202	2180	2580	2377
275	197	38	56	49.5	174	242	198	2160	2640	2386
281	201	40	56	47.5	168	232	204	2360	2740	2515
304	218	45.5	50.5	48.2	202	226	218	2140	2460	2288
312	224	39	46	42.8	148	172	160	1800	2040	1920
315	225	37	52.5	44.2	146	196	174	1800	2440	2073
316	226	46	55	54	182	220	208	2080	2500	2473
318	228	46.5	52.0	49.2	170	280	192	1960	2380	2022
323	231	49.4	62	54.9	176	250	208	2160	2840	2475
332	238	56	56	56	206	208	208	2260	2380	2300
333	239	47.5	55	51.3	185	210	202	2160	2600	2556
339	243	40	60	48.9	184	234	203	2380	2960	2607
345	247	49.5	51	50	190	210	203	2140	2620	2525
351	251	35	45.5	39.5	180	246	228	2640	2900	2783
359	257	46	71	56	202	208	206	2420	2560	2487
360	248	44.5	59.5	50.7	170	216	202	2360	2840	2602

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*Previously reported by IBT as 1970-2770, with a mean of 2474

**Only gross inspections were made of the TE-IV concentrations but the same problems existed with this concentration as with the others.

TABLE II
IBT No. 663-03222
VINYL CHLORIDE RATS - SURVIVAL
T-III - 2,000 ppm

<u>Month</u>		<u>Male</u>	<u>Female</u>
5	Report	97	96
	Printout	96	95
6	Report	95	96
	Printout	96	94
7	Report	94	95
	Printout*	89 or 92	92
8	Report	90	92
	Printout	89	90
9	Report	84	85
	Printout	82	81
10	Report	76	72
	Printout	74	68
11	Report	66	48
	Printout	62	46
12	Report	42	34
	Printout	37	15(?)
13	Report	27	17
	Printout**	23	5(?)

* None of #'s or weights agree with report

** All over the lot, 5 body weights shown

CONFIDENTIAL
Subject to the provisions of the
Atomic Energy Act of 1954
14th J. 1954
California 1954

CMA 011043