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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005941

JUN 16 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dimethoate: Review of a Mutagenicity Study
EPA Reg. No. 241-75.* Record No. 145957.
Accession No. 256594.

TO: William H. Miller, PM #16
Registration Division (TS-767)

FROM: Karen L. Hamernik, Ph.D., Pharmacologist KLH for KM 6/16/87
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769) ABK 6/16/87

Tox Chem File No. 358

The mutagenicity study listed below, was reviewed by Dynamac and was then secondarily reviewed by Irving Mauer, Ph.D., Toxicology Branch geneticist. The final version of the study review is attached.

Mutagenicity Testing of Dimethoate (AC 12,880) in the in vitro (CHO/HGPRT Mutation Assay), American Cyanamid Co., Princeton, NJ, 1/30/87. Study No. 0423.

Comment: The study was found to be acceptable provided that the sponsor explain why the test material, technical Dimethoate, was described as a grayish white solid with a purity of 97.3% when it was from the same batch (611A) as the technical material used in two other mutagenicity studies (In vivo Bone Marrow Cytogenetics Rat Metaphase Analysis, Pharmakon Report No. PH 315-AC-001-84, 8/29/85, Accession No. 259921 and Dominant Lethal Study with Dimethoate Technical in the Mouse, RCC Report No. 039003, 7/24/85, Accession No. 259921). In the later two studies, the test material was described as a white crystalline solid with a purity of 96.89%.

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EPA: 68-02-4225
DYNAMAC No. 262-B
March 19, 1986

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DATA EVALUATION RECORD

DIMETHOATE

Mutagenicity--CHO/HGPRT Forward Gene Mutation Assay

STUDY IDENTIFICATION: Allen, J. S. and Johnson, E. Mutagenicity testing of dimethoate (AC 12,880) in the in vitro CHO/HGPRT mutation assay. (Unpublished study No. 0423 prepared and submitted by American Cyanamid Co., Princeton, NJ; dated January 30, 1985.) Accession No. 256594.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-19-87

1. CHEMICAL: Dimethoate: phosphorodithioic acid, O,O-dimethyl-S-methyl-carbamoylmethyl ester.

2. TEST MATERIAL: Dimethoate (AC 12,880), batch No. 611A, was described as a crystalline, grayish white solid with a purity of 97.3%.

3. STUDY/ACTION TYPE: Mutagenicity--CHO/HGPRT forward gene mutation assay.

4. STUDY IDENTIFICATION: Allen, J. S. and Johnson, E. Mutagenicity testing of dimethoate (AC 12,880) in the in vitro CHO/HGPRT mutation assay. (Unpublished study No. 0423 prepared and submitted by American Cyanamid Co., Princeton, NJ; dated January 30, 1985.) Accession No. 256594.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
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Signature: Nancy E. McCarroll
Date: 3-19-87

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6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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Signature: I. Cecil Felkner
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Karen Hamernik, Ph.D.
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Signature: KBK for KH
Date: 6/16/87

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 6/16/87

7. CONCLUSIONS:

A. Under the conditions of the CHO forward mutation assay, the mutagenic potential of dimethoate cannot be fully assessed. However, in the absence of S9 activation significant but not dose-related increases in mutation frequencies (MFs) were observed at 2,700 and 3,500 µg/mL; a significant increase in MF was also seen at 2,700 µg/mL under S9-activated conditions. Although not significant, increases in mutant colonies were also noted at various test concentrations with and without S9 activation at both short (72-96 hours) and prolonged (160-200 hours) expression intervals.

B. The study is acceptable, although, the biological significance of the increased MFs at the two highest test doses has not been resolved.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dimethoate, batch No. 611A, was described as a crystalline, grayish white solid with a purity of 97.3%; the structural formula was provided. The test material was dissolved in dimethylsulfoxide.

2. Test System: The Chinese hamster ovary (CHO) cell line (CHO-K_1-BH_4) was obtained from J.P. O'Neill, Univ. of Vermont, and was maintained as a frozen stock. Cells obtained from the frozen stock were maintained as monolayers in Ham's F-12 supplemented with 10% heat-inactivated fetal bovine sera and 2 mM L-glutamine.

3. Metabolic Activation: The S9 fraction used for metabolic activation was prepared from Aroclor 1254-induced male Sprague-Dawley rat liver.

4. Preliminary Cytotoxicity Assay: Duplicate monolayers of prepared cells ($5x10^5$ cells/25-cm^2 flask) were exposed up to the cytotoxic and solubility limits of the test material, in both the presence and absence of S9 activation. After a 5-hour exposure period, cultures were washed, refed, and incubated overnight to allow recovery. Recovered cells were

[1]Only items appropriate to this DER have been included.

trypsinized, suspended in supplemented Ham's F-12 medium, and counted with a Coulter Counter to determine cell numbers. Cell suspensions were appropriately diluted, plated at a density of 200-500 cells, and incubated 8-10 days. Colonies were stained and counted, and cloning efficiencies were determined; doses that yielded 10, 50, and 100% survival were used to determine a dose-response range for the mutagenicity assay.

5. Mutagenicity Assay:

Triplicate cultures, seeded at $5x10^5$ cells, were prepared and treated with at least three doses of the test material, solvent, or positive control with or without S9 activation.

After the appropriate expression interval (72-96 or 160-200 hours), relative cloning efficiencies (CEs) were determined as described in the cytotoxicity assay. The short expression period (72-96 hours) was included in this study at the request of Italian regulatory officials. For mutant selection the remaining cells from each treatment were plated at a density of $2x10^5$/100-mm plate (five plates) in medium containing 10 µM 6-thioguanine. After 7-10 days of incubation, the cells were stained and the number of surviving cells and mutant clones were counted; CEs and MFs were calculated.

6. Evaluation Criteria: The assay was evaluated in accordance with the criteria of Hsie et al[2].

7. MFs were transformed with the power transformation, $Y = (\text{mutation frequency} + 1)^{0.15}$, and analyzed by ANOVA and Student's t test.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: Five cytotoxicity assays were conducted with the test material. The reported results were as follows:

1. Cytotoxicity Assay 1: Seven doses of the test material (1, 10, 100, 250, 500, 1,000, and 5,000 µg/mL) were assayed with and without S9 activation. At the highest test dose, either with or without S9 activation, no cells survived.

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[2] Hsie, A.W., Casciano, D.A., Couch, D.B., Karhn, D.F., O'Neill, J.P., and Whitefield, B.L. The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of Chemicals. A report of the Gene-Tox program. Mutat. Res. 86(1981): 193-214.

Following exposure to 1,000 µg/mL (+S9), 83% cell survival was reported; in the absence of S9, survival was 101%. Since a dose curve of 10, 50, and 100% could not be deduced from these results and the CE for control cultures was low (31%, +S9; 50%, -S9) the assay was repeated.

2. Cytotoxicity Assay 2: Identical doses with or without S9 activation were tested in the second assay. Due to unspecified technical problems, the S9-activated phase of testing was aborted. Results without S9 activation were comparable to the earlier findings, indicating that 5,000 µg/mL of test material was excessively cytotoxic and that lower doses were not appreciably cytotoxic. However, the CE for the solvent control was low (41.2%).

3. Cytotoxicity Assay 3: The assay was repeated with the same test compound doses. The CEs for control cultures were within an acceptable range (≥50%).[3] In the absence of S9, 2.5 and 102.7% survival occurred at 5,000 and 1,000 µg/mL, respectively. In the presence of S9, cytotoxicity was more severe, doses ranging from 250 to 5,000 µg/mL yielded survival rates ranging from 55.2 to 0.6%. Below the 250-µg/mL, S9-activated dose, cytotoxicity was marginal. Based on these results a fourth cytotoxicity assay was undertaken.

4. Cytotoxicity Assay 4: A narrower range of doses was examined (500, 750, 1,000, 1,500, 2,000, and 3,000 µg/mL/-S9; 750, 1,500, and 3,000 µg/mL/+S9). Although cytotoxicity was achieved at 3,000 µg/mL (29.7% survival, -S9; 32.7% survival, +S9), the shape of the survival curve precluded the selection of appropriate doses.

5. Cytotoxicity Assay 5: For the fifth attempt, cells were exposed to nine S9-activated and nonactivated doses ranging from 50 to 3,000 µg/mL. A more defined dose response curve, which resulted in approximately ≥20% survival at 3000 µ/mL either with or without S9 activation, permitted selection of doses approximating 10, 50 and 100% survival.

B. Mutation Assays: Based on the combined results of the five cytotoxicity assay, the doses selected for the mutational studies were 1,000, 1,500, 2,000, 2,700, and 3,500 µg/mL with and without S9 activation. Cells surviving treatment with the appropriate doses

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[3] O'Neill, J. P., Brimer, P. A., Machanoff, R., Hirsch, G. P., and Hsie, A. W. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Development and definition of the system. Mutat. Res. 45(1977): 91-101.

of the test material, solvent, or positive controls, ethylmethanesulfonate (EMS; 200 µg/mL/-S9) or 7,12-dimethylbenz(a)anthracene (DMBA; 5 µg/mL/+S9), were plated at 24 hours posttreatment and at the 72- to 96-hour and 160- to 200-hour selection intervals.

1. 72- to 96-Hour Selection: Two nonactivated and one S9-activated assays were conducted at a 72-to 96-hour selection time.

   a. Nonactivated Assay: In the absence of S9 activation, insufficient cells were available to plate five mutant selection replicates per culture. To overcome the limitations posed by the short recovery interval, the number of treated cultures per dose was increased to 15, and cultures were pooled to yield three independent cultures per treatment level containing $1x10^6$ cells. The authors stated, however, that most of the pooled replicates had $< 10^6$ cells. The total number of surviving cells was corrected to reflect reduced cell numbers. No 6-thioguanine-resistant clones (6-TG^r) were recovered from control or treated cultures, and the average CE in the solvent control was low (27.5%); the nonactivated assay was repeated.

   In the repeat nonactivated assay, mutant colonies were observed at all doses, and the CEs were higher than the previous study.

   The authors calculated MFs for cultures with no mutant clones and presented them as values "less than" what the MF would have been if one mutant clone was scored relative to the surviving population. Since an absolute MF could not be assigned to these cultures, the MFs presented in all the tables do not include these values. As shown in Table 1, test dose MFs were lower or comparable to the solvent control. However, the positive control cultures did not respond to EMS.

   b. S9-Activated Assay: In the S9-activated, 72- to 96-hour selection, an MF of 6.9 was calculated at 3,500 µg/mL as compared to 2.2 for the solvent control. This increase was neither dose related nor statistically significant; however, no mutant clones were recovered for the positive control DMBA.

   The lack of mutagenic induction by both the nonactivated and S9-activated positive controls was not an unexpected result and is in agreement with O'Neill's[4] conclusion

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[4] O'Neill, J. P. and Hsie, A. W. Phenotypic expression time of mutagen induced 6-thioguanine resistance in Chinese hamster ovary cells (CHO/HGPRT system). Mutat. Res. 54(1979): 109-118.

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TABLE 1. Representative Results of the CHO Forward Gene Mutation Assay with Dimethoate (72- to 96-Hour Selection)

Substance	Dose (µg/mL)	S9 Activation	Average Relative % Survival[a]	% Cloning Efficiency[b]	Mean Mutation Frequency x 10^{-6} ± S.D.[c]	Fold Increase[d]
Solvent Control						
Dimethylsulfoxide		-	27.5(100)	54.5	-	-
		-[e]	95.5(100)	86.5	7.5 ± 1.0	-
		+	97.0(100)	68.0	2.2 ± 2.8	-
Positive Control						
Ethylmethane sulfonate	200	-	36.4	36.6	-	-
	200	-[e]	73.8	50.7	3.1 ± 2.7	> 1
7, 12-Dimethylbenz-anthracene	5	+	23.2	32.0	-	-
Test Material						
Dimethoate	3,500[f]	-[e]	41.8	19.5	-	-
	3,500[f]	-[e]	50.8	43.8[g]	1.3 ± 2.3	> 1
	1,000	+	78.9	55.3	3.8 ± 2.4	1.7
	1,500	+	69.6	49.0	2.6 ± 2.9	> 1
	2,000	+	52.1	45.0	2.3 ± 3.9	> 1
	2,700	+	46.4	32.3	1.2 ± 2.1	> 1
	3,500	+	31.4	22.6	6.9 ±12.0	3.1

[a] $\frac{\text{Average No. of Clones/Dish/Dose Level}}{\text{Average No. of Clones/Dish/Solvent Control}}$ X 100; derived from cloning efficiencies obtained 24 hours after treatment.

[b] $\frac{\text{Average No. of Cells on Nonselective Plates/Dose Level}}{\text{Average No. of Cells on Nonselective Plates/Solvent Control}}$ X 100; derived from cloning efficiencies obtained at selection interval.

[c] Means and standard deviations as calculated by our reviewers.

[d] Fold Increase = $\frac{\text{Mean Mutation Frequency of Test Dose}}{\text{Mean Mutation Frequency of Solvent Control}}$; calculated by our reviewers.

[e] Repeat test.

[f] Highest nonactivated dose test; mutation frequencies of lower doses (1,000, 1,500, 2,000, and 2,700 µg/mL in initial test and 1,000 and 2,700 µg/mL in repeat test) ≤ than mutation frequency of solvent control.

[g] Corrected to exclude contaminated cultures.

that an early selective time does not provide the optimum conditions for recovery of induced mutants.

Representative data for the 72- to 96-hour selection are shown in Table 1.

2. 160- to 200-Hour Selection

a. Nonactivated: Two nonactivated, 160- to 200-hour selection assays were performed. In the initial assay 6-TG^r clones were recovered for all treatment conditions. Erratic MFs were recorded for the test group; 2.1×10^{-6} for the mid dose (2,000 µg/mL), 33.4×10^{-6} for the lowest dose (1,000 µg/mL), and 21.8×10^{-6} for the highest dose (3,500 µ/mL). The increases at 1,000 and 3,500 µg/mL were not significant. The authors stated that due to the low relative survival for the solvent control (27.5%), the assay was repeated. The repeat assay was conducted with 1,000, 2,700 and 3,500 µg/mL. Increased MFs were again observed in a nonlinear manner (7.9, 17.6, and 9.6×10^{-6} at 1,000, 2,700 and 3,500 µg/mL of test material, respectively). The reported increases at 2,700 and 3,500 µg/mL of test material were, however, significantly higher than the control ($p < 0.5$).

Representative data from all dose levels in the initial and repeat 160- to 200-hour selection, nonactivated assay are shown in Table 2.

b. Activated: A dose-related decrease in cell survival accompanied exposure of CHO cells to increasing S9-activated concentrations of the test material. As previously observed in the nonactivated assay, nondose responsive, but increased MFs were calculated from the data. MFs at 1,000, 1,500, 2,000, 2,700, and 3,500 µg/mL were 7.7, 0.6, 3.5, 16.0 and 12.5×10^{-6}, respectively. At 2700 µg/mL the effect was significant ($p < 0.05$). Representative results from all S9-activated doses are presented in Table 3.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors stated, "It was concluded, therefore, that no biological significance exists, and that no treatment level of dimethoate was mutagenic."

B. A quality assurance statement was signed and dated January 11, 1985.

TABLE 2. Representative Results of the CHO Forward Gene Mutation Assay with Dimethoate (160- to 200-Hour Selection) Without S9

Substance	Dose (µg/mL)	S9 Activation	Average Relative % Survival[a]	% Cloning Efficiency[b]	Mean Mutation Frequency x 10^{-6} ± S.D.[c]	Fold Increase[d]
Solvent Control						
Dimethylsulfoxide		-	27.5(100)	24.8[e]	8.5 ± 12.0	-
		-[f]	95.5(100)	67.5	2.5 ± 4.3	-
Positive Control						
Ethylmethanesulfonate	200	-	36.4	25.7	258.1 ±158.4*	30.4
	200	-[f]	73.8	57.2	212.3 ± 40.4*	84.9
Test Material						
Dimethoate	1,000	-	89.1	25.5[e]	33.4 ± 47.2	3.9
	1,500	-	85.5	29.0	18.2 ± 6.1	2.1
	2,000	-	78.2	31.3	2.1 ± 1.9	> 1
	2,700	-	69.0	30.1	7.2 ± 12.5	> 1
	3,500	-	41.8	24.3	21.8 ± 19.9	2.6
	1,000	-[f]	85.9	63.2	7.9 ± 7.4	3.2
	2,700	-[f]	71.2	59.5	17.6 ± 7.8*	7.0
	3,500	-[f]	50.8	58.8	9.6 ± 4.2*	3.8

[a] $\frac{\text{Average No. of Clones/Dish/Dose Level}}{\text{Average No. of Clones/Dish/Solvent Control}}$ X 100; derived from cloning efficiencies obtained 24 hours after treatment.

[b] $\frac{\text{Average No. of Cells on Nonselective Plates/Dose Level}}{\text{Average No. of Cells on Nonselective Plates/Solvent Control}}$ X 100; derived from cloning efficiencies obtained at selection interval.

[c] Means and standard deviations as calculated by our reviewers.

[d] Fold Increase = $\frac{\text{Mean Mutation Frequency of Test Dose}}{\text{Mean Mutation Frequency of Solvent Control}}$; calculated by our reviewers.

[e] Corrected to exclude contaminated cultures.

[f] Repeat test.

*Significantly higher than control value ($p < 0.05$) by ANOVA.

TABLE 3. Representative Results of the CHO Forward Gene Mutation Assay with Dimethoate (160- to 200-Hour Selection) With S9 Activation

Substance	Dose (µg/mL)	S9 Activation	Average Relative % Survival[a]	% Cloning Efficiency[b]	Mean Mutation Frequency x 10^{-6} ± S.D.[c]	Fold Increase[d]
Solvent Control						
Dimethylsulfoxide		+	97.0(100)	68.0	3.7 ± 5.2	-
Positive Control						
7, 12-Dimethylbenz-anthracene	5	+	23.2	33.7[e]	174.3 ± 20.2*	47.1
Test Material						
Dimethoate	1,000	+	78.9	61.3	7.7 ± 3.6	2.1
	1,500	+	69.6	51.7[e]	0.6 ± 1.0	< 1
	2,000	+	52.1	45.3	3.6 ± 2.9	< 1
	2,700	+	46.4	42.7	16.0 ± 10.6*	4.3
	3,500	+	31.4	38.0	12.5 ± 10.8	3.4

[a] $\frac{\text{Average No. of Clones/Dish/Dose Level}}{\text{Average No. of Clones/Dish/Solvent Control}}$ X 100; derived from cloning efficiencies obtained 24 hours after treatment.

[b] $\frac{\text{Average No. of Cells on Nonselective Plates/Dose Level}}{\text{Average No. of Cells on Nonselective Plates/Solvent Control}}$ X 100; derived from cloning efficiencies obtained at selection interval.

[c] Means and standard deviations as calculated by our reviewers.

[d] Fold Increase = $\frac{\text{Mean Mutation Frequency of Test Group}}{\text{Mean Mutation Frequency of Solvent Control}}$; calculated by our reviewers.

[e] Corrected to exclude contaminated cultures.

*Significantly higher than solvent control value ($p < 0.05$) by ANOVA.

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14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study authors undertook an elaborate series of experiments to evaluate the mutagenic potential of dimethoate in the CHO assay. Five cytotoxicity tests were performed to ensure that a definitive dose range was assayed in the mutation tests. The overall results of the cytotoxic tests suggested to us that both the physical properties of the test material and the inherent technical difficulties associated with the CHO/HGPRT assay accounted for the unusually high number of repeated cytotoxicity assays. Similarly, technical difficulties (low survival or low CE for solvent control cultures) necessitated repeat tests for the nonactivated mutation assays.

We assess, however, that the study was conducted properly, and the authors interpreted the data in accordance with accepted criteria[5] (concentration-related increase over at least 3 concentrations). Although a statistically significant dose-related effect was not observed in either the initial or repeat studies, statistically significant positive responses were observed at 2,700 µg/mL with or without S9 activation. Increased, but not reproducibly significant, MFs were also noted at 3,500 µg/mL in the activated and nonactivated assays.

As shown in Table 1, at the 72- and 96-hour selection, 3,500 µg/mL (+S9) induced a 3.1-fold (nonsignificant) increase in MF. In the first nonactivated, 160- to 200-hour selection assay (Table 2) 3,500 µg/mL induced a 2.6-fold increase in MF. When the nonactivated, prolonged selection assay was repeated at doses of 1,000, 2,700, and 3,500 µg/mL, dimethoate induced MFs that were 3.2-, 7.0-, and 3.8-fold higher than the solvent control, respectively. The increases at 2,700 and 3,500 µg/mL were significant.

Results from the S9-activated, 160- to 200-hour selection presented in Table 3 showed that 2,700 and 3,500 µg/mL of test material induced 4.3- and 3.4-fold increases in the MF. The result at 2,700 µg/mL was statistically significant.

The lack of reproducible data free from wide variations in these experiments has confused the interpretation of this series of studies. These erratic results were not necessarily indicative of poor laboratory performance, but were probably due to inherent technical problems with the CHO/HGPRT assay. It is of note that at the doses where statistical significance was achieved, the MF's did not exceed either the published range of spontaneous mutation[6] (0-20 x 10^{-6}) or the historical background rate reported by the performing laboratory (0-37 x 10^{-6}).

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[5] Hsie, A.W. et al. Mutat. Res. 86(1981): 193-214.

[6] Ibid.

Based on these observations, we conclude that the compound-related increases in MF were equivocal because of the inherent technical problems with the CHO/HGPRT assay and also because the increased MFs did not exceed normal background rates of mutation.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7-16.

APPENDIX A

Materials and Methods

Dimethoate toxicology review

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Pages 15 through 24 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

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