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

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JUN - 1 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Chlorpyrifos: Tissue Distribution and Metabolism of Orally Administered ^{14}C-Labeled Chlorpyrifos in Fischer 344 Rats - EPA Accession No. 404589-01; Toxicology Branch Project No. 8-0458; Caswell No. 219AA.

FROM: Alan C. Levy, Ph.D.
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TO: Dennis Edwards (PM 12)
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THRU: Quang Q. Bui, Ph.D., D.A.B.T.
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and

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6/1/88

Registrant: Dow Chemical Company

Action: Review a tissue distribution and metabolism study in Fischer 344 rats administered ^{14}C-labeled Chlorpyrifos.

Recommendations:

This study is accepted as Core Minimum Data.

The majority of the radioactivity was recovered in the urine (>84%) and feces (>5%) within 72 hours. Less than 0.2% of the radioactivity retained in tissues and carcass. No unchanged Chlorpyrifos was found in the urine and the main urinary metabolites were identified as 3,5,6-TCP and conjugates (glucuronide and possibly sulfate) of 3,5,6-TCP.

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Review Section V/HED (TS-769C)

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Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V

I. Study Type: Metabolism Study
(Guideline § 85-1)

Study Title: Chlorpyrifos: Tissue Distribution and Metabolism of Orally Administered ^{14}C-Labeled Chlorpyrifos in Fischer 344 Rats

EPA Identification Numbers:

EPA Identification: 464-404, 1H5295, 3H5396, 3F2884
EPA Accession: 404589-01
EPA Record: 211662, 211663, 211664, 211665
Caswell: 219AA

Sponsor: The Dow Chemical Company
Midland, MI 48674

Testing Laboratory: Mammalian and Environmental Toxicology Research Laboratory
Health and Environmental Sciences
The Dow Chemical Company
Midland, MI 48674

Study Number: K-044793-(76)

Study Date: December 23, 1987

Study Authors: R. J. Nolan, Ph.D., D.A.B.T., M. D. Dryzga, B.S., B. D. Landenberger, M.S. and P. E. Kastl, B.S.

Test Material:

Name: ^{14}C-labeled Chlorpyrifos
Inventory No.: 541
Labeling: 2 and 6 positions of the pyridine ring
Specific Activity: 15.78 (45 uCi/mg)
Radiochemical Purity: >99%

Analytical Grade Material: Chlorpyrifos
Lot No.: AGR 200341
Purity: 99.9%

II. Materials and Methods

Test Animals - Male and female CDF Fischer 344 rats were obtained from Charles River Breeding Laboratories (Kingston, NY). The males weighed 173-250 gm and the females, 128-170 gm when given the ^{14}C-labeled Chlorpyrifos. [The age of the animals was not given in the report.] They were acclimated for at least 7 days prior to the start of the study. Rats given ^{14}C-labeled Chlorpyrifos were selected by computer randomization and were transferred from stainless steel cages

(2/cage) to individual all-glass metabolism cages three days prior to being given the ^{14}C material. Food was removed 14-18 hours prior to administration of ^{14}C-labeled Chlorpyrifos and was returned about 4 hours post-dosing.

Dose Solutions Containing ^{14}C-Labeled Chlorpyrifos - Doses of 0.5 and 25.0 mg/kg were administered in the study. The 0.5 mg/kg dose solution was prepared by dissolving sufficient ^{14}C-labeled Chlorpyrifos in USP corn oil (Eastman Kodak) to produce a solution containing approximately 0.27 mg of Chlorpyrifos and 12 uCi/gm of corn oil. The 25 mg/kg dose solution was prepared by dissolving sufficient analytical grade and ^{14}C-labeled Chlorpyrifos in corn oil to produce a solution containing approximately 13.6 mg Chlorpyrifos and 12 uCi/gm of corn oil. Solutions were administered by gavage at a rate of 2 ml/kg using a syringe and stainless steel feeding needle. The quantity of solution actually administered was determined by weighing the syringe prior to and following administration of the dose.

Dose Solution Containing No Radiolabeled Chlorpyrifos - The dose solution administered for 15 consecutive days (0.5 mg/kg) was prepared by dissolving sufficient analytical grade Chlorpyrifos in corn oil to produce a solution containing about 0.27 mg Chlorpyrifos/gm of corn oil. HPLC analysis showed 0.313 mg Chlorpyrifos/gm. The report stated stability for two months at room temperature. The solution was administered by gavage at a rate of 2 ml/kg based on the rat's body weight taken at 3-4 day intervals.

Specimen Collection - Urine was collected from males and females in dry-ice chilled containers that were changed at 12-hour intervals for the first 72 hours, as well as from females at 24-hour intervals between 72 and 144 hours post-dosing. Feces were collected at 24-hour intervals for 72 hours from males and for 144 hours from females. After the 25 mg/kg dose, the air was drawn through the cage (about 500 ml/min), through charcoal to trap volatile organics and then through a mixture of monoethanolamine and 1-methoxy-2-propanol to trap expired $^{14}CO_2$. The charcoal and $^{14}CO_2$ traps were changed at 6, 12 and 24 hours post-dose. No trapping was done after the single or multiple 0.5 mg/kg doses as radioactivity was not found following the 25 mg/kg dose. Males and females were anesthetized with CO_2 and exsanguinated 72 and 144 hours, respectively, after administration of ^{14}C-labeled Chlorpyrifos. Males were terminated earlier than females as they excreted the 25 mg/kg dose faster than females (termination when >90% of the dose was found in excreta). At termination, brain, gonads, heart, kidneys, liver, lungs and spleen as well as portions of the blood, bone, perirenal fat, skeletal muscle and skin plus the carcass were collected and prepared for ^{14}C-analysis.

^{14}C-Analysis - The following were analyzed for radioactivity: weighed aliquots of the dose solution, eluent from the HPLC column, urine and an aliquot from the contents of the charcoal and $^{14}CO_2$ traps. Weighed aliquots of homogenates (33-50%) from feces, brain, testes, kidneys, liver, lungs and carcass as well as aliquots of blood, heart, ovaries, bone, perirenal fat, skeletal muscle, spleen and skin were

oxidized and the $^{14}CO_2$ released was analyzed for radioactivity. Counts per minute (CPM) were corrected for quench and background to obtain disintegrations per minute (DPM). Sealed standards were counted with samples, and samples with net counting rates <1.5 times background were considered to have insufficient radioactivity to reliably quantify.

Analysis of High Performance Liquid Chromatography (HPLC) - Individual and composite urine specimens from rats given ^{14}C Chlorpyrifos were analyzed by HPLC with separation of the metabolites achieved by reverse phase HPLC. Retention times and peak shapes for Chlorpyrifos and metabolites were affected by multiple injections of undiluted urine, and therefore, the performance of the HPLC system was evaluated daily by determining retention times for analytical grade Chlorpyrifos and 3,5,6-TCP (AGR-65077). Selected urine samples from the multiple dose group were subjected to acid hydrolysis prior to HPLC analysis.

Isolation of Urinary Metabolites - The three radioactive fractions were collected separately and extracted with diethyl ether or ethyl acetate, or lyophilized. The extracted fractions were blown to dryness before analysis and the lyophilized fractions reconstituted in methanol, acetone or ethyl acetate.

Mass Spectrometry Analysis of Urinary Metabolites - Urine specimens from the 25 mg/kg dose rats were used for metabolite identification. Identification of the first and third fractions to elute from the HPLC system was by direct exposure probe (DEP) negative ion chemical ionization mass spectrometry. The second fraction was analyzed by thermospray negative ion mass spectrometry as well as by positive and negative ion fast atom bombardment mass spectrometry.

Statistical Analysis - If tissues in a group did not contain sufficient radioactivity to quantify, the mean was calculated using half the limit of quantitation for those tissues which did not contain sufficient radioactivity to quantify. If the resulting mean was less than the limit of quantitation, the mean concentration was reported as being below the limit of quantitation. [Detailed descriptions regarding calculations of slopes and half-lives were included.]

A Quality Assurance statement was included.

A copy of the Materials and Methods section from the report is appended. This reviewer assumes that female rats are "nulliparous and nonpregnant" [Guideline §85-1, (2), iii, (B)]. An intravenous route was not used. No other comments regarding this section.

III. Results

Dose Solutions and Administered Doses - Table 1 indicates the targeted and actual concentrations of Chlorpyrifos in dose solutions. Actual radioactivity and mg/gm were 114-149% of targeted concentration.

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Table 1

CONCENTRATIONS OF RADIOACTIVITY AND CHLORPYRIFOS IN DOSE SOLUTIONS

	Targeted Concentrations		Actual Concentrations	
	uCi/g	mg/g	uCi/g	mg/g
Single Dose				
25 mg/kg	12	13.6	15.5 (129%)	15.53 (114%)
0.5 mg/kg	12	0.27	15.5 (129%)	0.322 (119%)
Multiple 0.5 mg/kg Dose				
Non ^{14}C-Labeled	--	0.27	--	0.313 (116%)
^{14}C-Labeled	12	0.27	17.9 (149%)	0.403 (149%)

Targeted concentrations are taken from the protocol and have been converted to a per gram basis using 0.92 as the specific gravity for corn oil (Merck Index, 8th Edition, 1968). Actual concentrations were determined by liquid scintillation and HPLC analysis, respectively. Numbers in parenthesis represent the percent of target concentrations.
These data extracted from Table 1, page 22 of the report.

The amounts of Chlorpyrifos and radioactivity administered to each group of 5 rats/sex are presented in Table 2.

Table 2

AVERAGE AMOUNT OF RADIOACTIVITY AND CHLORPYRIFOS ADMINISTERED TO RATS

	Sex	Wt (g)	Dose Solution Concentration DPM/g	Dose Solution Concentration ug/g	Dose Solution Admin. (g)	Administered Dose uCi	Administered Dose mg	Administered Dose mg/kg Body Wt
Single 25 mg/kg	M	183	34436725	15.53	0.3388	5.26	5.262	28.8
	F	130	34436725	15.53	0.2394	3.71	3.718	28.5
Single 0.5 mg/kg	M	206	34405517	0.322	0.3726	5.77	0.129	0.625
	F	135	34405517	0.322	0.2432	3.77	0.084	0.622
Multiple 0.5 mg/kg	M	223	39746030	0.403	0.4162	7.45	0.166	0.747
	F	136	39746030	0.403	0.2625	4.70	0.105	0.774

Admin. = Administered
Values represent the mean for 5 animals.
Data extracted from Table 2, page 23 of the report.

Distribution of Recovered Radioactivity - The group mean percent of recovered radioactivity after 72 hours in males and 144 hours in females is presented in Table 3 (a photcopy of the table on page 24 of the report). Total percent recovery, regardless of dose (25, single 0.5 or multiple 0.5 mg/kg), was between 96.88 and 98.46. The principal route of excretion was by urine (83.9-91.7%), with feces containing 5.75-11.42% and cage wash 0.54-1.98%. CO_2 and charcoal traps contained <0.01% (insufficient to quantify after 25 mg/kg and not collected after single or multiple 0.5 mg/kg dose). Tissues and carcass had 0.19-0.20% after 25 mg/kg and <0.01% after single or multiple 0.5 mg/kg doses. Animals which received multiple 0.5 mg/kg doses excreted a larger percent of the dose in the urine (less in feces) than those given a single 0.5 mg/kg dose. There did not appear to be any differences between 25 and single dose 0.5 mg/kg recovery, or between males and females.

The only tissue concentrations of radioactivity sufficient to quantify (% of dose/gm of tissue wet weight) were as follows (group means): males (after 72 hours) = liver after 25 mg/kg (0.0059%) and perirenal fat after 25 mg/kg (0.0559%), after single 0.5 mg/kg (0.0121%) and after multiple 0.5 mg/kg (0.0135%); females (after 144 hours) = ovaries (0.0362%) and perirenal fat (0.1393%) after 25 mg/kg. [Data were presented on page 25 of the report.]

Excretion of Radioactivity in the Urine and Feces - The group mean percent of radioactivity present in the urine is presented in Table 4 (photocopied from page 26 of the report). During the first three days following the single and multiple 0.5 mg/kg dose, rats of both sexes eliminated the radioactivity with an average half-life of 8-9 hours. Half-lives after the 25 mg/kg dose were 12.4 hours for males and 23.2 hours for females (about twice as long as for males). About 1.3% of the 0.5 mg/kg dose and about 4.7% of the 25 mg/kg dose were found in the urine of females on days 4-6 post-dosing. As the males eliminated >90% of the 25 mg/kg dose (urine and feces) within 3 days, the decision was made to sacrifice this sex 72 hours post-dose.

Fecal radioactivity results are shown in Table 5 (photocopied from page 27 of the report). As noted regarding urine, most fecal radioactivity was present during the first 24 hours post-dosing, and the 0.5 mg/kg dose was eliminated more rapidly than the 25 mg/kg dose.

Separation and Identification of Urinary Metabolites - An average of over 95% of the radioactivity applied to the HPLC column was recovered in the column eluent (data not shown in the report). HPLC analysis revealed three peaks (labeled A, B and C), all of which eluted ahead of Chlorpyrifos. More than 90% of the radioactivity appeared in peaks B and C. Less than 6% radioactivity was noted in peak A, with none eluted between 28 and 31 minutes as Chlorpyrifos. All radioactivity in urine that was subjected to acid hydrolysis eluted from the HPLC column at peak C which had the same retention time as an authentic standard of 3,5,6-TCP (3,5,6-trichloro-2-pyridinol). Peak C (similar to 3,5,6-TCP) was extracted, blown to near dryness and analyzed by negative ion chemical ionization mass spectrometry using the direct exposure probe. The mass spectra contained a M-1 quasi-molecular ion with a mass/charge ratio (m/z) at 196. Fragment ions were observed at 162, 161, 125 and 90. The ions exhibited the chlorine isotope pattern and are consistent with those observed for 3,5,6-TCP.

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Table 3

Distribution of Radioactivity Recovered 72 hr (Male) or 144 hr (Female) After Fischer 344 Rats Were Given Oral Doses of 25 or 0.5 mg ^{14}C-Chlorpyrifos/kg of Body Weight

	Percent of Dose		
		Single	Multiple
	25 mg/kg	0.5 mg/kg	0.5 mg/kg
MALES			
Urine	88.73±2.53	85.23±3.40	91.71±4.74
Feces	7.49±3.48	9.76±2.34	5.75±3.99
Cage Wash	1.98±0.76	1.89±0.33	1.00±0.59
CO_2 Trap[a]	<0.01	--	--
Charcoal Trap[a]	<0.01	--	--
Tissues & Carcass	0.20±0.16	<0.01	<0.01
TOTAL	98.36±1.93	96.88±1.25	98.46±0.99
FEMALES			
Urine	87.99±10.53	83.94±13.04	90.65±2.62
Feces	8.35±4.65	11.42±3.12	5.59±2.49
Cage Wash	0.54±0.28	1.83±1.24	0.75±0.19
CO_2 Trap[a]	<0.01	--	--
Charcoal Trap[a]	<0.01	--	--
Tissues & Carcass	0.19±0.13	<0.01	<0.01
TOTAL	97.05±8.28	97.19±13.33	96.99±1.34

Values represent the mean ± 1 standard deviation for 5 animals.

[a]CO_2 and charcoal traps contained insufficient radioactivity to quantify following the 25 mg/kg dose and were not collected following the single or multiple 0.5 mg/kg dose.

Table 4

Radioactivity Excreted in the Urine During the Indicated Intervals by Male and Female Fischer 344 Rats Given Oral Doses of 25 or 0.5 mg ^{14}C-Chlorpyrifos/kg of Body Weight

Collection Interval (Hr Post-Dosing)	Percent of Dose		
	25 mg/kg	Single 0.5 mg/kg	Multiple 0.5 mg/kg
MALES			
0 - 12	52.84 ± 3.89	54.20 ± 8.19	59.21 ± 15.36
12 - 24	20.26 ± 4.75	23.42 ± 9.16	25.21 ± 12.47
24 - 36	6.95 ± 1.03	4.91 ± 1.26	4.27 ± 0.88
36 - 48	4.25 ± 1.91	1.58 ± 0.37	1.47 ± 0.45
48 - 60	2.00 ± 1.49	0.70 ± 0.34	0.51 ± 0.12
60 - 72	2.43 ± 0.63	0.42 ± 0.14	1.04 ± 0.40
TOTAL	88.73 ± 2.53	85.23 ± 3.40	91.71 ± 4.74
FEMALES			
0 - 12	26.74 ± 6.61	50.79 ± 6.53	51.46 ± 16.23
12 - 24	21.06 ± 5.41	24.95 ± 8.35	28.67 ± 10.65
24 - 36	13.42 ± 3.80	3.50 ± 1.42	6.23 ± 4.39
36 - 48	10.97 ± 6.79	2.35 ± 1.28	1.93 ± 0.71
48 - 60	5.48 ± 4.26	0.60 ± 0.15	0.62 ± 0.27
60 - 72	5.65 ± 1.54	0.52 ± 0.26	0.46 ± 0.10
72 - 96	2.65 ± 0.32	0.50 ± 0.21	0.46 ± 0.14
96 - 120	1.29 ± 0.29	0.45 ± 0.20	0.36 ± 0.07
120 - 144	0.73 ± 0.07	0.28 ± 0.17	0.47 ± 0.16
TOTAL	87.99 ± 10.53	83.94 ± 13.05	90.65 ± 2.62

Values represent the mean ± 1 standard deviation for five rats.

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Table 5

Radioactivity Excreted in the Feces During the Indicated Intervals by Male and Female Fischer 344 Rats Given Oral Doses of 25 or 0.5 mg ^{14}C-Chlorpyrifos/kg of Body Weight

Collection Interval (Hr Post-Dosing)	Percent of Dose		
	25 mg/kg	Single 0.5 mg/kg	Multiple 0.5 mg/kg
MALES			
0 - 24	5.81 ± 2.94	9.23 ± 2.28	5.04 ± 3.97
24 - 48	1.02 ± 0.44	0.42 ± 0.06	0.59 ± 0.21
48 - 72	0.66 ± 0.35	0.10 ± 0.01	0.12 ± 0.05
TOTAL	7.49 ± 3.48	9.76 ± 2.34	5.75 ± 3.99
FEMALES			
0 - 24	5.28 ± 2.93	10.11 ± 2.70	4.65 ± 2.42
24 - 48	1.57 ± 0.67	1.18 ± 1.79	0.55 ± 0.38
48 - 72	0.58 ± 0.35	0.11 ± 0.10	0.19 ± 0.13
72 - 96	0.71 ± 1.06	<0.03[a]	0.08 ± 0.07
96 - 120	0.11 ± 0.06	<0.03	0.07 ± 0.04
120 - 144	0.11 ± 0.08	<0.03	0.04 ± 0.01
TOTAL	8.35 ± 4.65	11.42 ± 3.12	5.59 ± 2.49

Values represent the mean ± 1 standard deivation for five rats.

[a]Specimen contained insufficient radioactivity to quantify; i.e., net DPM were less than 1.5 times average background.

Peak B was dried, reconstituted and analyzed by thermospray negative ion mass spectrometry and positive/negative ion FAB/MS. The thermospray mass spectra is dominated by a fragment ion cluster at m/z 196/198/200 which indicated a trichloropyridinol moiety. A weak (1% relative abundance) M-1 quasi-molecular ion cluster (3 chlorines) is present at m/z 372. The above is what would be expected for a glucuronide conjugate of 3,5,6-TCP (molecular weight of 373). The molecular weight was confirmed in the positive and negative ion FAB/MS (374 for positive and 372 for negative ion spectra).

Peak A was dried, reconstituted and analyzed by negative ion chemical ionization mass spectrometry using the direct exposure probe. There was a weak (2% relative abundance) M-1 quasi-molecualr ion at m/z 276. A fragment ion cluster was observed at m/z 197 (suggestive of 3 chlorine atoms). This peak was tentatively identified as a sulfate conjugate of 3,5,6-TCP (molecular weight of 277).

IV. Discussion

Chlorpyrifos was rapidly absorbed as evidenced by more than half of the 0.5 mg/kg doses being excreted in the urine within 12 hours. Based upon the amount of the dose found in the urine, it was concluded that at least 84% was absorbed, although the half-life for absorption could not be calculated from the data.

As more than 84% of the radioactivity was found in the urine and none seemed to be Chlorpyrifos, it appeared that Chlorpyrifos, orally administered to the rat, was rapidly metabolized and eliminated. Based upon the first 3 days of radioactive excretion, the half-life following 0.5 mg/kg was calculated to be 8-9 hours. A relatively short half-life and rapid excretion are further supported by the fact that less than 0.2% of the radioactivity remained in tissues and carcass at the end of the study (72 and 144 hours for males and females, respectively). The authors therefore concluded that Chlorpyrifos and its metabolites were unlikely to accumulate in the rat after repeated administration.

Not only was the 0.5 mg/kg dose excreted more rapidly than the 25 mg/kg dose, but a smaller fraction of the lower dose was found in the tissues and carcass. It was postulated by the authors that the slower excretion rate after the 25 mg/kg dose may have been due to saturation or inhibition of enzymes involved in Chlorpyrifos metabolism, to depletion of co-substrates in conjugation of 3,5,6-TCP and/or to saturation of the renal excretory process. It was considered possible that the difference in excretion rates may have been due to differences in absorption rates.

Other investigators also found that male rats excreted about 90% of a single 5 mg/kg oral dose of Chlorpyrifos in the urine (Bakke, et al, 1976, J. Environ. Sci. Health, B11:225-230; Smith, et al, 1967, J. Agr. Food Chem, 15:132-138). In agreement with data from this report, Bakke identified the principal urinary metabolites to be 3,5,6-TCP (12%) and the glucuronide conjugate (80%) of 3,5,6-TCP (also no unchanged ^{14}C-Chlorpyrifos in the urine). In addition, Bakke found about 4% of an unspecified glycoside of 3,5,6-TCP. The authors of this report did not find this metabolite, but did observe one tentatively identified as the sulfate conjugate of 3,5,6-TCP. Smith reported urinary radioactivity

as: 3,5,6-TCP phosphate, 75-80%; 3,5,6-TCP, 15-20%; and a small amount of unchanged Chlorpyrifos. (The authors of this report consider the identity of the metabolites to be uncertain because structures were assigned based only upon paper chromatography results).

The report proposes the metabolism of Chlorpyrifos to be as follows: Chlorpyrifos to 3,5,6-TCP; 3,5,6-TCP excreted as such or conjugated with glucuronic acid or sulfate prior to excretion. Sultalos and Murphy (1983, Fund. Appl. Toxicol.,3:16-21) demonstrated that the formation of 3,5,6-TCP could be catalyzed by hepatic mixed function oxidases and involved more than one pathway (cleavage of diethyl phosphorothioate group to form 3,5,6-TCP directly; oxidative desulfuration to form the oxon of Chlorpyrifos which undergoes hydrolysis to yield 3,5,6-TCP). Based on relative rates for formation and hydrolysis, Sultatos and Murphy indicated little or none of the oxon would be expected to escape the liver or reach the urine.

V. Recommendation

This study is accepted as Core Minimum Data.

The majority of the radioactivity was recovered in the urine (>84%) and feces (>5%) within 72 hours. Less than 0.2% of the radioactivity remained in tissues and carcass. No unchanged Chlorpyrifos was found in the urine and the main urinary metabolites were identified as 3,5,6-TCP and conjugates (glucuronide and possibly sulfate) of 3,5,6-TCP.

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Pages 12 through 18 are not included.

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