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June 20, 1972

ASTM D-27.07.19

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R. H. MUNCH

Enclosed is the first ASTM draft of Monsanto's method for measuring PCBs in the environment.

Please indicate any changes that you believe are needed.

I just received these copies today. Unfortunately our ASTM meeting is next Monday in California which gives us little time to comment. Perhaps we will have to prepare for the next meeting.

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Enclosure

DSW 362882

STANDARD METHOD FOR ANALYSIS OF ENVIRONMENTAL MATERIALS FOR POLYCHLORINATED BIPHENYLS

1. Scope

1.1 These methods cover the determination of the amount and type of polychlorinated biphenyls (PCB's) in environmental materials, specifically air, water and soils and sediments. Absolute confirmation of PCB structures is not obtained with these methods. Structure proof can be obtained using additional techniques such as mass spectrometry.

2. Significance

2.1 Since it is possible for minute quantities of PCB's to find their way into the environment, it is desirable to have a method to identify and measure the quantity of such trace amounts.

3. Sampling

3.1 Water Sampling

3.1.1 Environmental sampling of natural bodies of water and effluents are covered in many methods under the jurisdiction of Committee D-19. Any of the methods of obtaining a representative sample are acceptable for this method as long as the sample is at least 250 ml. See Method D-510, ASTM Part 23.

3.2 Sediment and Soil Sampling

3.2.1 A sediment or soil sample of at least 1000 grams should be taken according to ASTM Method D-1587, ASTM Part 11.

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3. Sampling (Cont'd)

3.3 Air Sampling

3.3.1 Environmental sampling of ambient air or source sampling is under the jurisdiction of ASTM Committee D-22. There are various methods of sampling from stacks or ambient air available. ~~as to the intake nozzle and gas volume measurements.~~ See Method D-1605, ASTM Part 23.

3.3.2 For the measurements of Airborne PCB's the sampled air is drawn through one or more fritted bubblers or impingers in cylinders filled with toluene. The PCB's are absorbed in the toluene.

4. Principle

4.1 The PCB's present in environmental samples are isolated from the bulk of the sample matrix by solvent extraction. Interfering matrix components and endogenous residues are then removed from the sample by a suitable combination of clean-up procedures and the amount and type of PCB's present determined by the election capture gas chromatography (EC/GC).

5. Reagents

5.1 Hexane - Nanograde.

5.2 N,N-Dimethyl Formamide - Certified. (Saturated in a separatory funnel with hexane.)

5.3 Ethyl Ether - Anhydrous, AR grade.

5. Reagents (Cont'd)

5.4 Acetonitrile - Nanograde.

5.5 Sodium Sulfate - Anhydrous, granular: AR grade. Heat 400°C for one hour prior to use.

5.6 Alumina Adsorption - (For chromatographic analysis) 800/200 mesh. Heat at 400°C for a minimum period of 4 hours and deactivate with 5% (w/w) distilled water.

Alumina column preparation: Fill a chromatographic column with hexane up to the point where the reservoir joins the column and push a glass wool plug to the bottom with a glass rod. In a 50 ml beaker measure 35 ml of deactivated alumina (30g), and pour this slowly into the column. Tap or vibrate the column to settle the alumina and top the alumina with 2-3 cm of anhydrous sodium sulfate. Wash the column with 50-100 ml of hexane prior to the addition of the sample.

5.7 Distilled Water - Extracted with hexane to remove hexane soluble electron capturing impurities.

5.8 Sulfuric Acid - Analytical Reagent Grade, SG = 1.84.

5.9 Potassium Oxalate - Analytical Reagent Grade.

5.10 Potassium Hydroxide - Analytical Reagent Grade.

5.11 Ethanol - Formula 2B.

5.12 2.5% (w/v) Alcoholic Potassium Hydroxide - Dissolve 12.5 grams of AR grade KOH in 500 ml of ethanol.

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5. Reagents (Cont'd)

5.13 5% (w/v) Aqueous Potassium Oxalate - Dissolve 5 grams of AR grade potassium oxalate in 100 ml of distilled water.

5.14 Toluene - Nanograde.

5.15 9/1 (v/v) Sulfuric Acid-Water - Carefully add 270 ml of AR grade sulfuric acid to 30 ml of distilled water in a 500 ml iced beaker.

5.16 PCB Standards - Production Grade - Aroclor 1221, Aroclor 1242, Aroclor 1248, Aroclor 1254 and Aroclor 1260.

6. Apparatus

6.1 Separatory funnels equipped with ground glass stoppers and Teflon stopcocks: 125, 250, 500, 1000 and 2000 ml capacities.

6.2 Kunderna-Danish Evaporative Concentrators, 500 ml capacity equipped with 3-ball Snyder columns and graduated 5 ml capacity vials.

6.3 Chromatographic columns, glass, 10" x 20 mm (OD) with a 5" x 50 mm (OD) reservoir at the top, equipped with Teflon stopcocks.

6.4 Sintered glass filter funnels, 600 ml capacity, 90M.

6.5 Flat bottomed boiling flasks, 125 ml capacity.

6.6 Liebig Condenser, 200 mm in length.

6.7 Hot plates.

6.8 Water bath.

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6. Apparatus

6.9 Reciprocating variable speed shaker.

6.10 10 Hl Syringes.

6.11 32 oz. all glass mortars and pestles.

6.12 8 x 12 x 2" (2-1/2 qt.) Pyrex baking dishes.

6.13 U.S. Standard Sieve, No. 30.

6.14 Grinding Mills and Grinding Unit.

6.15 Usual laboratory glassware.

6.16 Gas Scrubbing Bottled, High Form, ground glass joint, fritted coarse discs, 250 ml capacity.

6.17 Dry Test Meter (0.1 ft^3/revolution).

6.18 Laboratory Vacuum Pump.

6.19 Rotatory Vacuum Evaporator.

7. Sample Preparation and Extraction

7.1 Water

7.1.1 Where possible, the entire water sample, including the container in which it was collected, is extracted with hexane. With larger samples, where this is not physically possible, the containers are simply agitated and a 250 ml portion used for analysis.

7.1.2 Extraction of water samples - after agitating, transfer the entire aqueous sample or a 250 ml aliquot into a graduated glass cylinder. Record the volume of the sample and quantitatively transfer it to a separatory funnel with distilled water.

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7. Sample Preparation and Extraction (Cont'd)

7.1 Water (Cont'd)

7.1.3 Rinse the sample container and graduated cylinder with two 50 ml portions of hexane and add each to the separatory funnel.

7.1.4 Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

7.1.5 Extract the water sample a second time with a 50 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous layer to the original separatory funnel.

7.1.6 Repeat the extraction with a third 50 ml portion of hexane. Discard the aqueous layer and combine the hexane extracts.

7.1.7 Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

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7. Sample Preparation and Extraction (Cont'd)

7.2 Sediment and Soil

7.2.1 Any excess water is decanted and the entire sediment or soil sample transferred to a glass baking dish to air dry at room temperature. The dried material is transferred from the dish into a mortar and pestle and ground. The ground sediment is sieved, remixed, and a 250g portion taken for analysis.

7.2.2 Weigh 250g (to the nearest 0.01g) into a 16 oz. narrow neck screw cap (aluminum foil liner) glass bottle.

7.2.3 Moisten the soil with water (10 ml) and add 150 ml of acetonitrile. Cap the bottle tightly, and mechanically shake for a minimum period of 1 hour.

7.2.4 Quantitatively transfer the acetonitrile extract into a 600 ml sintered glass filter funnel containing a 1/4" layer of anhydrous sodium sulfate. Collect the filtrate in a 600 ml beaker (vacuum filtration may be necessary).

7.2.5 After the acetonitrile has completely drained into the beaker, wash the bottle twice with 50 ml portions of acetonitrile, adding each wash to the funnel after the previous has completely percolated through the sediment.

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7. Sample Preparation and Extraction (Cont'd)

7.2 Sediment and Soil (Cont'd)

7.2.6 Quantitatively transfer the extract to a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the solvent volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED INTO A HOOD.)

7.2.7 Carefully evaporate the acetonitrile extracts to dryness with the aid of a gentle stream of dry nitrogen. Re-dissolve the residue in maximum of 5 ml of nanograde hexane.

7.3 Airborne PCB's

7.3.1 After scrubbing the desired amount of air, record the metered volume, pressure, and temperature.

7.3.2 Quantitatively transfer the scrubbing solvent to a round bottom flask and reduce the volume to approximately 2 ml by rotary vacuum evaporation.

7.3.3 Quantitatively transfer the concentrate to a 30 ml beaker with the aid of several small portions of toluene.

7.3.4 Inject a fraction of a ul of the concentrate into the gas chromatograph to check for interferences and determine the approximate level of PCB's present. If no interferences are present dilute

7. Sample Preparation and Extraction (Cont'd)

7.3 Airborne PCB's (Cont'd)

or concentrate the sample to a known volume, as determined by the electron capture chromatogram, and proceed with the gas chromatographic analysis.

7.3.5 If interferences are present proceed with the chemical treatment and column chromatographic clean up procedures.

7.3.6 Transfer the concentrate to a 125 ml extraction flask with the aid of several small portions of solvent.

7.3.7 Evaporate the concentrate just to dryness with a gentle stream of dry filtered air and add 25 ml of 2.5% alcoholic potassium hydroxide.

7.3.8 Add a boiling chip, put a water condenser in place, and allow the solution to reflux for 45 minutes.

7.3.9 After cooling, transfer the solution to a 250 ml separation funnel with the aid of 25 ml of distilled water.

7.3.10 Rinse the extraction flask with 25 ml of hexane and add it to the separatory funnel.

7.3.11 Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

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7. Sample Preparation and Extraction (Cont'd)

7.3 Airborne PCB's (Cont'd)

7.3.12 Extract the saponification solution with a second 25 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous alcohol layer to the original separatory funnel.

7.3.13 Repeat the extraction with a third 25 ml portion of hexane. Discard the saponification solution and combine the hexane extracts.

7.3.14 Carefully add 25 ml of the sulfuric acid solution (9:1 concentrated sulfuric acid/water) to the hexane extracts.

7.3.15 Stopper the separatory funnel and shake vigorously for at least one minute. Allow the layers to separate and discard the lower aqueous acid layer. Repeat this step until the acid layer is colorless.

7.3.16 Wash the hexane with 25 ml portion of water. Discard the water wash.

7.3.17 Filter the hexane extract through a 4" funnel plugged with glass wool which is covered with a layer of sodium sulfate into a Kunderna-Danish evaporative concentrator.

7.3.18 Add a small boiling chip, put the Snyder column in place and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath.

7. Sample Preparation and Extraction (Cont'd)

7.3 Airborne PCB's (Cont'd)

7.3.19 After cooling, remove the 5 ml graduated tube and transfer the hexane extract to an alumina adsorption column washing it in with several 5 ml portions of hexane.

7.3.20 Carefully add 100 ml of hexane to the column reservoir and collect the total eluent in either a 250 ml volumetric flask or a Kunderna-Danish evaporative concentrator.

7.3.21 If the column eluent is collected in a volumetric flask, dilute to volume with hexane and proceed with the gas chromatographic analysis.

7.3.22 If the column eluent is collected in a Kunderna-Danish evaporative concentrator, reduce solvent volume, cool, dilute to volume and proceed with gas chromatographic analysis.

8. Electron Capture Gas Chromatographic Procedure

8.1 Instrument: F&M 402 Biomedial Gas Chromatograph or equivalent

8.2 Detector: High Temperature Ni^{63} Electron Capture Cell
6 mm x 6' Glass Column, 4% XE-60 on 80/100 mesh Chromosorb W, HP, AW-DMCS

8.3 Column Temperature: 160-190°C

8.4 Detector Temperature: 300°C

8.5 Injection Port Temperature: 195-215°C

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8. Electron Capture Gas Chromatographic Procedure (Cont'd)

8.6 Pulse: 150

8.7 Flow Rates: Helium Carrier 60 ml/min
Argon-Methane Purge 120 ml/min

8.8 Using EC/GC as the determinative step, inject in duplicate 1-10 ul of each solution into the chromatograph. By comparison with standard solutions injected, in duplicate, under the same operating conditions, determine the amount and type of Aroclor using the individual or total peak height and area methods.

9. Supporting Procedures

9.1 Saponification with Alcoholic Potassium Hydroxide and Extraction with Sulfuric Acid

9.1.1 Quantitatively transfer the concentrated extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

9.1.2 After cooling, remove the 5 ml graduated tube and transfer the concentrate of extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

9.1.3 Evaporate the extract just to dryness with a gentle stream of dry filtered air and add 25 ml of 2.5% alcoholic potassium hydroxide.

9.1.4 Add a boiling chip, put a water condenser in place, and allow the solution to reflux for 45 minutes.

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9. Supporting Procedures (Cont'd)

9.1 (Cont'd)

9.1.5 After cooling, transfer the solution to a 250 ml separatory funnel with the aid of 25 ml of distilled water.

9.1.6 Rinse the extraction flask with 25 ml of hexane and add it to the separatory funnel.

9.1.7 Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

9.1.8 Extract the saponification solution with a second 25 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous alcohol layer to the original separatory funnel.

9.1.9 Repeat the extraction with a third 25 ml portion of hexane. Discard the saponification solution and combine the hexane extracts.

9.1.10 CAREFULLY add 25 ml of concentrated sulfuric acid, in small portions, to the hexane extracts.

9.1.11 Stopper the separatory funnel and shake vigorously for at least one minute. Allow the layers to separate and discard the lower aqueous-acid layer. Repeat this step until the acid layer is colorless.

9.1.12 Wash the hexane with 25 ml portion of water. Discard the water wash.

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9. Supporting Procedures (Cont'd)

9.1 (Cont'd)

9.1.13 Filter the hexane extract through a 4" funnel plugged with glass wool which is covered with a layer of sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

9.2 Liquid/Solid Chromatographic Clean-Up With Alumina

9.2.1 After preparation of the alumina column, described in the "Reagents" section, quantitatively transfer the hexane extracts (5 ml or less) on to the column with the aid of several 5 ml portions of hexane (reducing the solvent level to the adsorbant level after each addition of solvent).

9.2.2 Carefully add 125 ml of hexane to the column reservoir and collect the total eluent in a Kunderna-Danish evaporative concentrator.

9.2.3 Add a small boiling chip to the Kunderna-Danish evaporative concentrator, put the Snyder column in place, and reduce the hexane volume to less 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

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10. Quantitative Determination

10.1 Quantitative determinations employing the electron capture detector are non-stoichiometric measurements made by comparing peak heights or areas for known concentrations with those for unknown compositions. Four variations of the peak height or area quantification procedures have been employed.

Case I EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with no evidence of interferences.

Case II EC gas chromatogram of PCB unknown altered with respect to standard PCB with no evidence of interference.

Case III EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with evidence of interference.

Case IV EC gas chromatogram of PCB unknown altered with respect to standard PCB with evidence of interference.

10.2 The PCB level in a Case I sample can be determined by comparison of the height of the major peak in the unknown with a calibration curve prepared by plotting the height of the major peak in the electron capture chromatogram of the corresponding standard vs the number of ng of the standard injected. With a Case II sample because of the alteration, the maximum PCB level is extimated

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10. Quantitative Determination

10.2 by comparison of the total area of the electron capture envelope of the unknown to a calibration curve prepared by plotting the total area of the electron capture envelope of the most similar standard vs the number of ng of the standard injected. Case III and IV samples are initially subjected to the chemical clean-up procedure followed by chromatography as outlined on alumina. If the interferences are removed by this treatment, the PCB levels are calculated for Case III and IV in the same manner as Case I and II, respectively. If dominant interference(s) is (are) still present, then the height of a different analytical peak, free from obvious interference, is employed to calculate the PCB level in Case III. With Case IV samples, the maximum PCB level is estimated as in Case II after correcting the total area for that of the interfering peak(s). In all cases, the response of the electron capture must be linear for quantitative analysis.

11. Calculations

11.1 Assuming that the calibration curve is prepared by plotting the electron capture response (peak height or area) vs nanograms of the PCB standard, injected, the PCB level present in the air sampled can be calculated in the following manner:

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11. Calculations (Cont'd)

11.1 (Cont'd)

$$\frac{(\text{ng PCB's from calib. curve})}{(\text{ul of sample injected})} \times (\text{ml, volume of final concentrate}) \times \left(\frac{10^3 \text{ ul}}{\text{ml}}\right) \times \left(\frac{10^{-6} \text{ mg}}{\text{ng}}\right)$$

= mg of PCB's present in volume of air sampled

$$(\text{cu ft of air sampled from meter}) \times \left(\frac{28.32 \text{ L}}{\text{cu ft}}\right) \times \left(\frac{M^3}{10^3 L}\right) \times \left(\frac{760 - \text{mm Hg}}{760}\right) \times \left(\frac{298}{273+T[^\circ C]}\right)$$

= volume of air sampled in M^3 @ 1 atm and 25°C

Results are expressed as $\frac{\text{mg}}{M^3}$ PCB's.

12. Contamination

12.1 In determining PCB's by electron capture gas chromatography, laboratory sources of contamination can be a major problem. The samples and extracts should never be allowed to come in contact with materials other than glass, Teflon or metal. Laboratory glassware should be thoroughly washed with hot, soapy water, rinsed with distilled water, acetone, and then hexane. All equipment should also be rinsed again with hexane just prior to use and blanks should be frequently carried through all steps of the procedures to insure against the possibility of contamination.

13. Discussion

13.1 Extraction

13.1.1 The extraction of PCB's from water employing hexane as the extractant was found to be quantitative and to be sufficiently simple and rapid for use as a routine procedure.

13.1.2 The evaluation of this method was based on spiking water samples with standard acetone solutions of PCB's. The spiking method consisted of adding the PCB's in acetone (25-50 ul) to 500 ml of tap water in a 32 oz. narrow neck screw cap jar. After thoroughly mixing, duplicate 225-250 ml aliquots were taken and subjected to the proposed sample preparation and work up as outlined. The results were quantified by preparing a calibration curve using standard hexane solutions of the PCB's used to spike the water samples. The total area of the electron capture chromatogram was used to construct the calibration plot.

13.1.3 The average recovery and deviation achieved substantiated the applicability of the method for the quantitative recovery and analysis of PCB's from water at the ppb-ppm level.

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13. Discussion (Cont'd)

13.2. Electron Capture Gas Chromatography

13.2.1 Columns

13.2.2 Column performance is the key to effective gas chromatographic analysis and as such the choice of column materials is particularly important. Ideally, the support employed should be inert, mechanically strong, and of high surface area. For these reasons, Chromosorb W, HP, AW-DMCS was used in all of our work.

13.2.3 A variety of polar and non-polar liquid phases were investigated. The following columns were found to provide adequate separation, etc., for use in PCB analysis by electron capture: 4% (w/w) DC-200, SF-96, OV-17, SE-30, SE-54, XE-60, Apiezon L, and 6% QF-1. DC-200 and XE-60 or QF-1 have been found to be the most suitable of these liquid phases.

13.2.4 Another important consideration when working with an extremely sensitive detector and consequently low levels of materials is column conditioning. With polar phases such as XE-60 and QF-1, we have found that operating a new column overnight at a temperature 25-50°C higher than to be used during analysis results in a more stable column. A no-flow conditioning technique is employed to

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13. Discussion (Cont'd)

13.2 Electron Capture Gas Chromatography (Cont'd)

condition non-polar columns. The column is purged with carrier gas, heated for 30 minutes at an elevated temperature without carrier flow and then cooled to room temperature. At the end of this cycle, the carrier flow is resumed and the conditioning is completed as in the case of the polar liquid phase. Two precautions: During conditioning, the column should not be connected to the detector and one should not exceed the maximum safe temperature of the liquid phase.

13.2.5 Since all liquid substrates bleed to one degree or another and columns eventually degrade, we characterize all new columns with two column performance indicators - the number of theoretical plates (N) and a tailing factor (T). p,p'-DDT is employed to check these parameters because it is known to degrade on "poor" columns. In this manner, we can determine if the performance of a new column is satisfactory and when the column performance begins to fall off. We consider a column good if the number of theoretical plates per foot is on the order of 400-500 with tailing factors of 1.0-1.3. Calculation of these parameters is

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13. Discussion (Cont'd)

13.2 Electron Capture Gas Chromatography (Cont'd)

shown in the appendix. Additionally, there should be no significant extraneous peaks upon injection of a pure p,p-DDT standard.

13.2.6 Other chromatographic conditions that can be adjusted are column temperature and flow rates. Although resolution of a mixture increases with decreasing temperature, a temperature should be chosen that allows the elution of all components within a convenient time period. The flow rates are optimum for our instrument, column and detector system and, of course, should be adjusted if better results can be achieved.

13.2.7 Two gas chromatographic systems have been used for PCB analysis - HP Model 402 and 5750. We find that any system of instrument and column suitable for chlorinated pesticides is satisfactory for PCB analysis.

13.2.8 The bulk of analyses in our laboratories was carried out using the systems outlined. The use of the high temperature Ni^{63} electron capture cell is highly recommended. The ability to operate at higher temperatures prevents maintenance problems due to contamination from high boiling components.

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