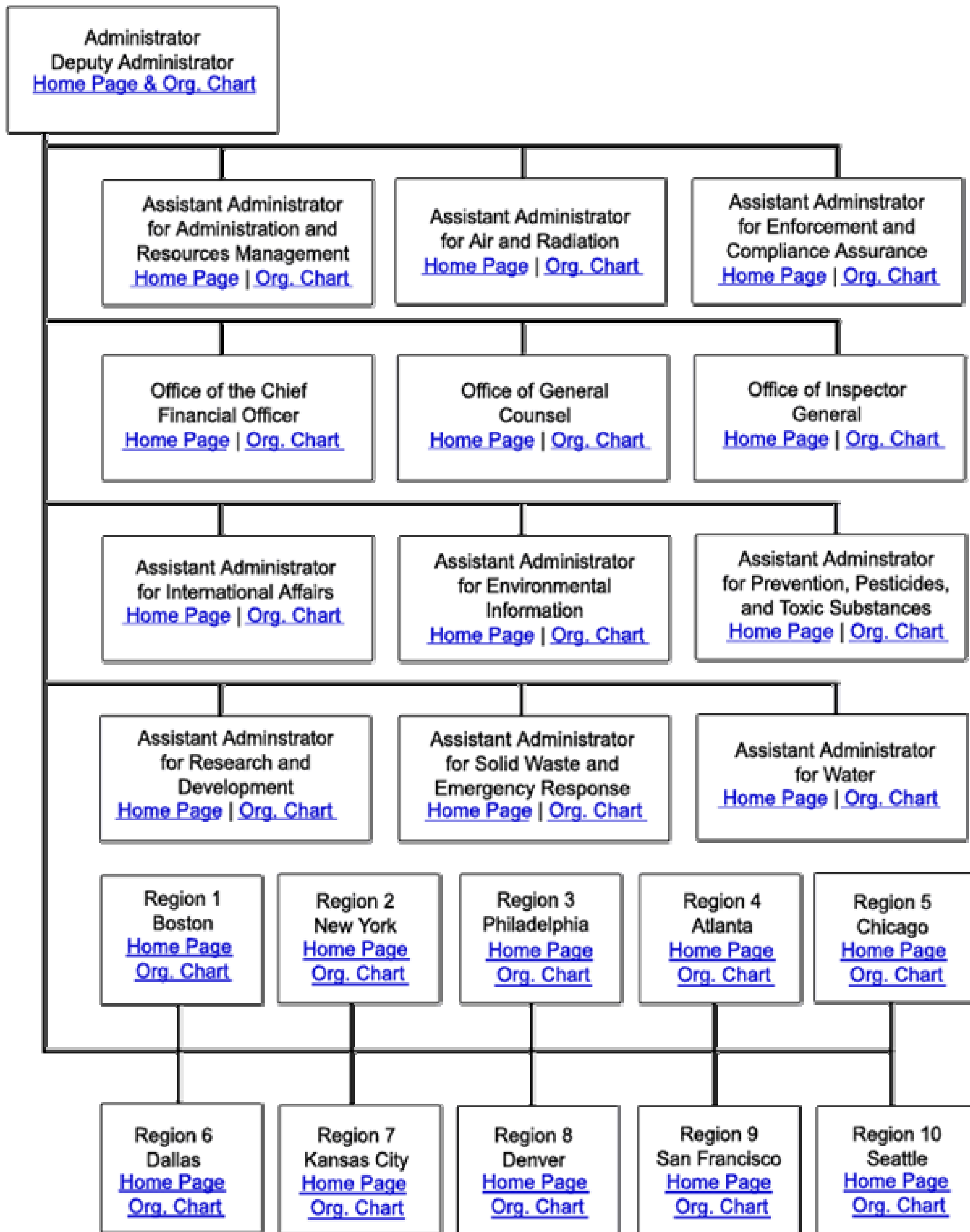
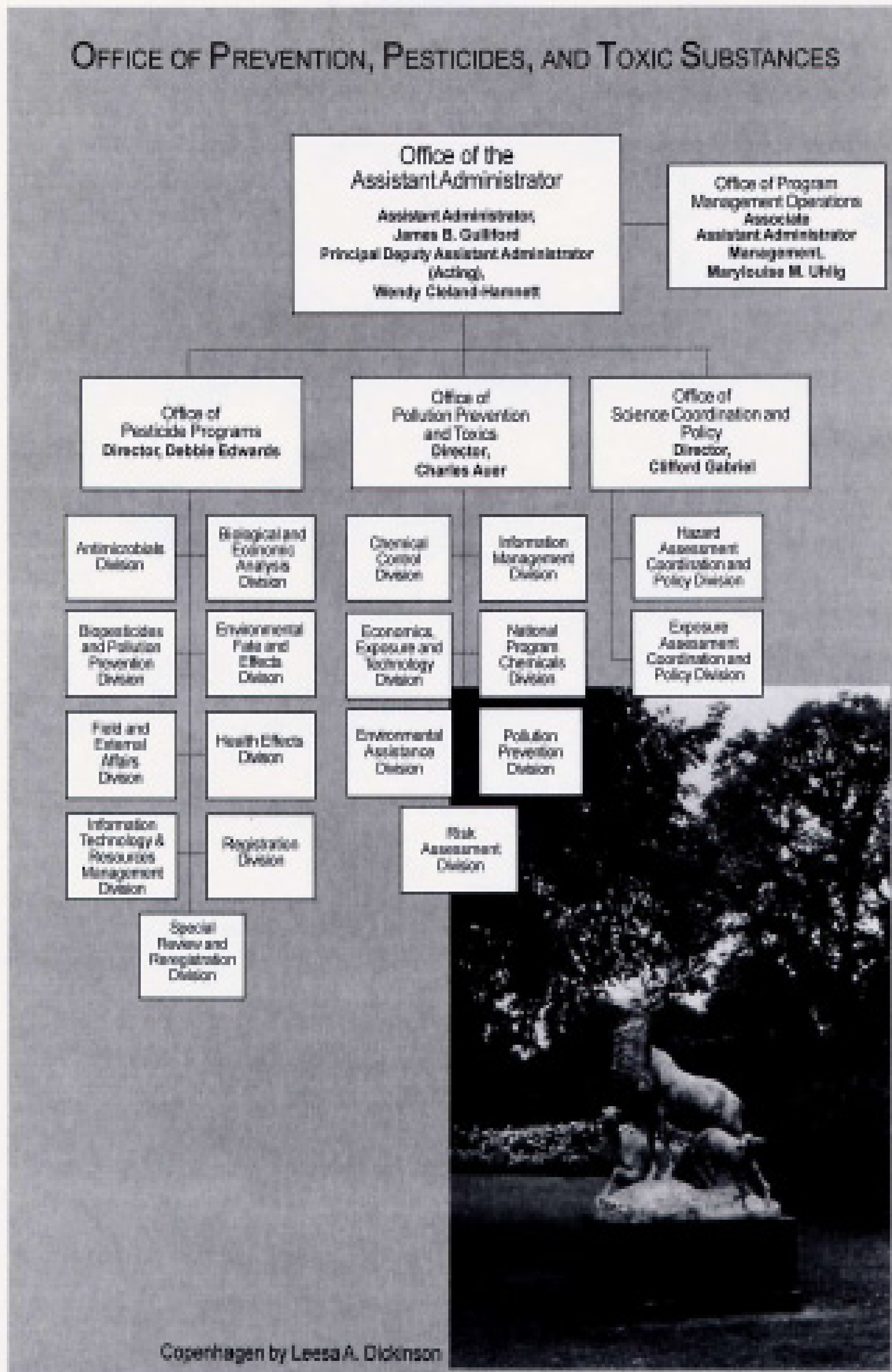
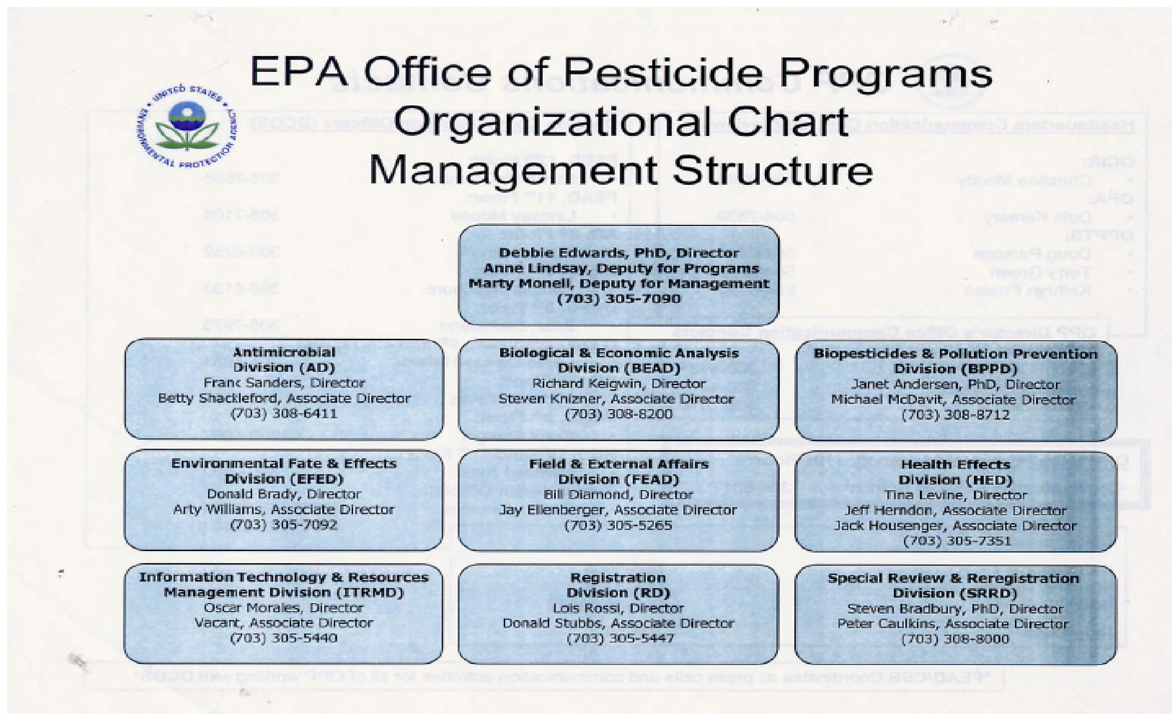
附件 1：美國環保署組織架構 **EPA Organizational Structure**



附件 1-2：美國環保署預防殺蟲劑及毒化物專案辦公室 OPPT 組織架構表



附件 **2** 殺蟲劑專案辦公室 **OPP** 組織架構表



附件 **3:**殺蟲劑註冊服務收費標準

	Action Code	Description	Fee (FY08)
Antimicrobial Pesticide	A520	Experimental Use Permit application	$5,250.00
	A380	Food use; establish tolerance exemption	$94,500.00
	A390	Food use; establish tolerance	$157,500.00
	A390	Food use; establish tolerance	$157,500.00
	A410	Non-food use; outdoor; uses other than FIFRA §2(mm)	$157,500.00
	A400	Non-food use; outdoor; FIFRA §2(mm) uses	$78,750.00
	A420	Non-food use; indoor; FIFRA §2(mm) uses	$52,500.00
	A430	Non-food use; indoor; uses other than FIFRA §2(mm)	$78,750.00
	A431	Non-food use; indoor; low-risk and low-toxicity food-grade active ingredient(s); efficacy testing for public health claims required under GLP and following DIS/TSS or AD-approved study protocol	$55,000.00
	A530	New product; identical or substantially similar in composition and use to a registered product; no data review or only product chemistry data; cite-all data citation, or selective data citation where applicant owns all required data, or applicant submits specific authorization letter from data owner. Category also includes 100% re-package of registered end-use or manufacturing-use product that requires no data submission nor data matrix.	$1,050.00
	A531	New product; identical or substantially similar in composition and use to a	$1,500.00

		registered product; registered source of active ingredient; selective data citation only for data on product chemistry and/or acute toxicity and/or public health pest efficacy, where applicant does not own all required data and does not have a specific authorization letter from data owner.	
	A532	New product; identical or substantially similar in composition and use to a registered product; registered active ingredient; unregistered source of active ingredient; cite-all data citation except for product chemistry; product chemistry data submitted	$4,200.00
	A540	New end use product; FIFRA §2(mm) uses only	$4,200.00
	A550	New end-use product; uses other than FIFRA §2(mm); non-FQPA product	$4,200.00
	A560	New manufacturing-use product; registered active ingredient; selective data citation	$15,750.00
Pay 25% of the fee and submit the application to the Agency. The Agency will determine the fee and send you an invoice or bill with any balance due.			
	A440	First food use; establish tolerance exemption	$26,250.00
	A450	First food use; establish tolerance	$78,750.00
	A490	Additional use; non-food; outdoor; uses other than FIFRA §2(mm)	$26,250.00
	A480	Additional use; non-food; outdoor; FIFRA §2(mm) uses	$15,750.00
	A510	Additional use; non-food; indoor; uses other than FIFRA §2(mm)	$10,500.00
	A500	Additional use; non-food; indoor; FIFRA §2(mm) uses	$10,500.00

	A521	Review of public health efficacy study protocol within AD; per AD Internal Guidance for the Efficacy Protocol Review Process; applicant-initiated; Tier 1	$2,000.00
	A522	Review of public health efficacy study protocol outside AD by members of AD Efficacy Protocol Review Expert Panel; applicant-initiated; Tier 2	$10,000.00
Biopesticides	B610	Food use; Experimental Use Permit application; establish temporary tolerance exemption	$10,500.00
	B620	Non-food use; Experimental Use Permit application	$5,250.00
	B621	Extend or amend Experimental Use Permit	$4,200.00
	B580	New active ingredient; food use; establish tolerance	$42,000.00
	B590	New active ingredient; food use; establish tolerance exemption	$26,250.00
	B600	New active ingredient; non-food use	$15,750.00
	B660	New product; identical or substantially similar in composition and use to a registered product; no data review or only product chemistry data; cite-all data citation, or selective data citation where applicant owns all required data, or applicant submits specific authorization letter from data owner. Category also includes 100% re-package of registered end-use or manufacturing-use product that requires no data submission nor data matrix.	$1,050.00
	B670	New product; registered source of active ingredient; all Tier I data for product chemistry, toxicology, non-target organisms, and product performance must	$4,200.00

		be addressed with product specific data or with request for data waivers supported by scientific rationales	
	B671	New product; food use; unregistered source of active ingredient; requires amendment of established tolerance or tolerance exemption; all Tier I data requirements for product chemistry, toxicology, non-target organisms, and product performance must be addressed with product-specific data or with request for data waivers supported by scientific rationales	$10,500.00
	B672	New product; non-food use or food use having established tolerance or tolerance exemption; unregistered source of active ingredient; no data compensation issues; all Tier I data requirements for product chemistry, toxicology, non-target organisms, and product performance must be addressed with product-specific data or with request for data waivers supported by scientific rationales	$7,500.00
	B630	First food use; establish tolerance exemption	$10,500.00
	B640	First food use; establish tolerance	$15,750.00
	B650	New use; non-food	$5,250.00
	B680	Label amendment requiring data submission	$4,200.00
	B681	Label amendment; unregistered source of active ingredient; supporting data require scientific review	$5,000.00
	B641	Amend established tolerance (e.g., decrease or increase)	$10,500.00
	B631	Amend established tolerance exemption	$10,500.00

	B682	Protocol review; applicant-initiated; excludes time for HSRB review (pre application)	$2,000.00

附件 **4** 各類殺蟲劑許可證申請案審查時間及費用

PRIA - Examples

Action	Decision Time (Months)					Fee
	FY04	FY05	FY06	FY07	FY08	
New ai food use	38	34	24	24	24	$516,300
New ai nonfood use	32	28	21	21	21	$358,700
Additional food use	38	30	22	15	15	$54,400
New non food use	28	24	20	15	15	$21,740
Me-too product	3	3	3	3	3	$1,300
Amendment	6	5	4	4	4	$3,280

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附件 5：美國聯邦法律電子檔入口網站 http://www.gpoaccess.gov/index.html

GPO Access Home Page - Windows Internet Explorer

http://www.gpoaccess.gov/index.html

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e-CFR Data is current as of July 10, 2008

TITLE 40--Protection of Environment

CHAPTER I--ENVIRONMENTAL PROTECTION AGENCY

SUBCHAPTER E--PESTICIDE PROGRAMS

Part	*Table of Contents*	*Headings*
150	150.17	GENERAL
151		[Reserved]
152	152.1 to 152.500	PESTICIDE REGISTRATION AND CLASSIFICATION PROCEDURES
153	153.125 to	REGISTRATION POLICIES AND

附件 6：抗菌、殺菌用途依介質不同的檢定方法

6.3.05

AOAC Official Method 966.04
Sporicidal Activity of Disinfectants

First Action 1966
Final Action 1967
Revised 2002

Method I

(Suitable for determining sporicidal activity of liquid and gaseous chemicals. Applicable to germicides for determining presence or absence of sporicidal activity against specified spore-forming bacteria in various situations and potential efficacy as sterilizing agent.)

A. Reagents

(**a**) *Culture media.*—(*1*) *Soil extract nutrient broth.*—Extract 1 lb (454 g) garden soil in 1 L H_2O, filter several times through S&S No. 588 paper, and dilute to volume (pH should be ≥5.2). Add 5 g beef extract (BD Biosciences, Codified Cat. No. 212610), 5 g NaCl, and 10 g peptone [Anatone, 955.11A(**a**) (*see* 6.1.01)]. Boil 20 min, dilute to volume, adjust with 1 M NaOH to pH 6.9, and filter through paper. Dispense in 10 mL portions into 25 × 150 mm tubes, and autoclave 20 min at 121°C. Use this broth to propagate test culture of *Bacilli.* (*2*) *Nutrient agar.*—*See* 955.11**A**(**c**) (*see* 6.1.01). Use slants of this medium to maintain stock culture of *Bacilli.* (*3*) *Modified fluid thioglycolate medium USP XX.*—Prepare as in 955.11**A**(**d**)(*2*) (*see* 6.1.01), except add 20 mL 1 M NaOH to each L before dispensing for sterilization. Use this medium to subculture spores exposed to 2.5 M HCl. For spores exposed to unknown germicides, use fluid thioglycolate medium, 955.11**A**(**d**)(*2*) (*see* 6.1.01). (*4*) *Soil extract–egg–meat medium.*—Add 1.5 g Bacto egg–meat medium dehydrated (BD Biosciences, Codified Cat. No. 242100) to 25 × 150 mm tube; then add 15 mL garden soil extract, (*1*), and sterilize 20 min at 121°C. Use this medium to propagate test cultures of *Clostridia* and maintain stock cultures of species of this genus.

(**b**) *Test organisms.*—Use *Bacillus subtilis*, ATCC No. 19659, or *Clostridium sporogenes*, ATCC No. 3584, for routine evaluation. Method is also applicable for use with other spore forming species.

(**c**) *Dilute hydrochloric acid.*—2.5 M. Use to determine resistance of dried spores. Standardize and adjust to 2.5 M as in 936.15**B** (*see* A.1.06).

B. Apparatus

(**a**) *Glassware.*—Bacteriological culture tubes, unflared, 25 × 150 mm; 100 mL glass-stoppered cylinders graduated in 1 mL divisions; 65 mm id funnels; supply of 15 × 110 mm Petri dishes matted with 2 sheets 9 cm S&S No. 597 or Whatman No. 2 filter paper. Sterilize all glassware and matted Petri dishes 2 h in air oven at 180°C.

(**b**) *Water bath.*—*See* 955.11**B**(**b**) (*see* 6.1.01).

(**c**) *Racks.*—*See* 955.11**B**(**c**) (*see* 6.1.01).

(**d**) *Transfer loop, hook.*—*See* 955.14**B**(**c**) and (**f**) (*see* 6.2.01). Forceps, *see* 961.02B(**d**) (*see* 6.3.04).

(**e**) *Tissue grinder.*—Thomas Scientific, No. 3431E20, size B, or equivalent.

(**f**) *Suture loop carrier.*—From spool of size 3 surgical silk suture (3, 6.0 metric, silk black braided SA-9G, USP, Ethicon, Inc., Rte 22, Sommerville, NJ 08876, USA), prepare standard loops by wrapping the silk around ordinary pencil 3 times, slipping coil so formed off end of pencil, and holding it firmly with thumb and index finger of left hand while passing another piece of suture through coil, knotting, and tying securely. Then shear off end of coil and knotted suture to within 2 mm. This should provide overall length of ca 65 mm of suture in 2-loop coil that can be conveniently handled in ordinary aseptic transfer procedure.

Extract loops in groups of 100–200 in Soxhlet extraction apparatus, using $CHCl_3$, for 24 h. Air-dry 12–18 h at room temperature in hood. Place 100 loops in 100 mL 0.5 M HCl for 10 min or until all loops are completely submerged in solution. Decant, and rinse repeatedly with distilled water for 15 min. Check rinse water for absence of HCl, using litmus paper. Air-dry on filter paper mats under ambient conditions or in incubator.

An inert, polyester Dacron® material (unwaxed, undyed white braid; Ashaway Line and Twine Co., Ashaway, RI 02804, USA) of the same suture size and braid as specified above may be used as an alternative to silk for evaluating the efficacy of peracetic acid-based disinfectants. These loops require no extraction prior to inoculation.

(**g**) *Cylinder carriers.*—"Penicylinders," porcelain, 8 ± 1 mm od, 6 ± 1 mm id, 10 ± 1 mm long. (Available from CeramTec Ceramic, PO Box 89, Laurens, SC 29360-0089, USA; www.ceramtec.com; Cat. No. LE15819.) Sterilize 2 h in 180°C air oven. Wash used Penicylinders with Triton X-100 and rinse with H_2O 4 times.

C. Operating Technique

Grow all *Bacilli* in soil extract nutrient broth and all *Clostridia* in soil extract–meat–egg medium. Make monthly transfer of *B. subtilis* stock culture on Nutrient Agar. *Clostridia* do not require periodic transfer. Inoculate 3 tubes, using one loop stock culture, and incubate 72 h at 37°C. Place supply of suture loops and cylinder carriers in separate Petri dishes matted with filter paper, and sterilize 20 min at 121°C. Use new loops for each test. Penicylinders must be free from chips or cracks. Filter *C. sporogenes* through funnel containing 2 × 5 × 5 cm^2 piece of moist cotton or glass wool into sterile 25 × 150 mm test tubes, using same funnel. In preparing *B. subtilis* culture, pour tube of 72 h culture into tissue grinder and macerate to break up pellicle. Filter through sterile funnel containing moist cotton or glass wool into sterile 25 × 150 mm tube, repeating operation for other 2 tubes. Place 10 sterile suture loops or Penicylinders into each of 3 tubes containing 10 mL filtrate from 72 h culture of *C. sporogenes*, agitate, and let stand 10–15 min. Using this technique, contaminate 35 loops or cylinders. Place contaminated suture loops and/or cylinders into Petri dish matted with 2 layers of filter paper. Drain. Proceed similarly for *B. subtilis*.

Place the 35 suture loops or cylinders contaminated with *C. sporogenes* or *B. subtilis* in vacuum desiccator containing $CaCl_2$ and draw vacuum of 69 cm (27 in.) Hg for 20 min. Dry 24 h under vacuum. (Spores dried and held under these conditions will retain resistance ≥7 days.)

Transfer 10 mL 2.5 M HCl, **A**(**c**), into sterile 25 × 150 mm tube. Place tube in 20°C constant temperature water bath and let come to temperature. Rapidly transfer 4 dried, contaminated loop or cylinder carriers to acid tube. Transfer remaining dried, contaminated suture loop or cylinder carriers to tube of thioglycolate subculture medium, **A**(**a**)(*3*), as viability control. After 2, 5, 10, and 20 min, withdraw individual loops or cylinders from acid and transfer to individual tubes of subculture medium. Rotate each tube vigorously 20 s and resubtransfer. Incubate 21 days at 37°C. Test spores should resist HCl ≥2 min, and many may resist HCl for full 20 min.

When testing sporicidal or sterilizing activity of gas, place carriers in polyethylene bags or in Petri dishes with lids ajar. Certain

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gases may require rehydration of spores before exposure to gas. Rehydrate spores on carriers by 1 h immersion in H_2O, using ≤20 mL H_2O/6 carriers. Drain carriers 20 min on Petri dishes matted with filter paper. After exposure to gas, remove carriers, using aseptic technique to subculture media as specified in next paragraph.

For aqueous sporicides and sterilizers, place 10 mL product at dilution recommended for use or under investigation into each of six 25 × 150 mm tubes. Place tubes in 20°C water bath and let come to temperature. Using flamed forceps, place 5 suture loops or cylinders, contaminated with *C. sporogenes* or *B. subtilis* and dried 24 h under vacuum, into each of the 6 tubes containing disinfectant, using 2-min intervals for seeding each tube. Five suture loops or cylinders can be placed into each tube within 5 s. This seeding operation will take 10 min. After contact period specified for disinfectant has been achieved, remove suture loops or cylinders, using sterilized needle hook, from each tube of disinfectant to subculture medium or other subculture medium specified in 955.11**A**(**d**) (*see* 6.1.01) (select medium containing most suitable neutralizer), placing one suture loop or cylinder per tube. Five cylinders can be removed within each 2 min interval. Flame transfer needle hook after each carrier has been transferred to subculture medium. After completing transfer, resubtransfer each suture loop or cylinder to fresh tube of thioglycolate medium and incubate 21 days at 37°C. If no growth is observed after 21 days, heat-shock tubes 20 min at 80°C and reincubate 72 h at 37°C. Report results as + (growth) or – (no growth) values.

Killing in 59 of 60 replicates on one carrier at dilution and time specified is considered evidence of sporicidal efficacy against one test spore and for confidence level of 95%. Tests with both *B. subtilis* and *C. sporogenes*, using 30 replicates with each of 2 carriers specified to provide minimum of 120 carriers, are required to presumptively support unqualified sporicidal claim or for presumptive evidence of sterilizing activity at concentration, time, and conditions specified. For sporicidal claims, no more than 2 failures can be tolerated in this 120 carrier trial. For sterilizing claims, no failures can be tolerated.

References: *JAOAC* **36,** 480(1953); **39,** 480(1956);
40, 759(1957); **49,** 721(1966); **50,** 194(1967);
61, 371(1978); **68,** 279(1985).
J. AOAC Int. 86, 407(2003).

Revised: March 1998

Method II
Revised First Action 2006

[Applicable to testing sporicidal activity of liquid disinfectants using Modified Method **966.04** against *Bacillus subtilis* on a hard surface (porcelain carrier). Performance criteria for product efficacy are not impacted. This method has been validated for products containing sodium hypochlorite, peracetic acid/hydrogen peroxide, and glutaraldehyde. *See* results of the collaborative study supporting the modifications to **966.04.**]

Caution: (*1*) All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in the institutional biosafety regulations. Use the equipment and facilities indicated for the test organism. For recommendations on safe handling of microorganisms, refer to the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* manual. (*2*) Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, and phenol. Personal protective clothing or devices are recommended during the handling of these items for purpose of activation, dilution, or efficacy testing. A chemical fume hood or other containment equipment may be employed when appropriate during performing tasks with concentrated products. The study analyst may wish to consult the *Material Safety Data Sheet* for the specific product/active ingredient to determine best course of action. (*3*) References to water mean reagent grade water, except where otherwise specified. (*4*) Commercial dehydrated media made to conform to the specified recipes may be substituted. (*5*) These microbiological methods are technique-sensitive and -oriented, thus, exact adherence to the method, good laboratory practices, and quality control (QC) are required for proficiency and validity of the results. (*6*) Detergents used in washing glassware may leave residues which are bacteriostatic. Test for inhibitory residues on glassware periodically. For procedure, refer to *Standard Methods for the Examination of Water and Wastewater*, Section 9020, Quality Assurance/Quality Control.

A. Media and Reagents

(**a**) *Culture media.*—(*1*) *Nutrient broth.*—For use in preparing nutrient agar. Add 5 g beef extract (paste or powder), 5 g NaCl, and 10 g peptone (anatone, peptic hydrolysate of pork tissues, manufactured by American Laboratories, Inc., Omaha, NE, USA) to approximately 1 L water. Boil mixture for 20 min with constant stirring. Readjust volume to 1 L with water and allow cooling to around 50°C. Adjust pH to 6.8 ± 0.2 with 1 N HCl or 1 N NaOH, if necessary. Filter through paper (e.g., Whatman No. 4). Dispense 10 mL portions into 20 × 150 mm culture tubes or 20 mL portions into 25 × 150 mm culture tubes. Dehydrated nutrient broth may be substituted; prepare according to manufacturer's instructions. (*2*) *Nutrient agar.*—For stock cultures slants. Add 1.5% (w/v) Bacto-agar to unsterilized nutrient broth. Boil mixture until agar is dissolved. Adjust pH to 7.2 ± 0.2 if necessary. Dispense 5 mL portions into 16 × 100 mm screw cap tubes. Larger tubes may be used as well. Autoclave for 20 min at 121°C. Remove from autoclave and slant tubes to form agar slopes. (*3*) *Nutrient agar with 5 μg/mL $MnSO_4$–H_2O (amended nutrient agar).*—For spore production. Suspend 11.5 g nutrient agar in 495 mL water, add 5 mL 500 ppm $MnSO_4$–H_2O. Dissolve by boiling. Adjust pH to 6.8 ± 0.2 if necessary. Autoclave for 15 min at 121°C. Pour agar into plates. (*4*) *Trypticase soy agar (TSA).*—Suspend 40 g dehydrated trypticase soy agar in 1 L water and heat gently while stirring. Boil 1 min or until completely dissolved. Adjust pH to 7.3 ± 0.2. Autoclave 15 min at 121°C. Pour agar into plates. (*5*) *Fluid thioglycolate medium (FTM).*—Suspend 29.5 g dehydrated FTM in 1 L water. Heat to boiling to dissolve completely. Adjust pH to 7.1 ± 0.2 if necessary. Dispense 10 mL portions into 20 ×150 mm culture tubes and autoclave for 15 min at 121°C. Store at room temperature. Protect from light. *Note*: If after autoclaving the aerated portion of media consumes more than one-third of tube, media must be reboiled by placing tubes in beaker of boiling water. Media can only be reboiled once. (*6*) *Fluid thioglycolate medium with 1 M NaOH (modified FTM).*—For

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subculturing spores exposed to 2.5 M HCl. Suspend 29.5 g FTM in 1 L water. Heat boiling to dissolve completely. Cool and adjust pH to 7.1 ± 0.2 if necessary. Add 20 mL 1 M NaOH and mix well. Check final pH and record (pH between 8 and 9 is typical). Dispense 10 mL into 20 × 150 mm culture tubes and autoclave for 15 min at 121°C. Store at room temperature. Protect from light. *Note*: If after autoclaving the aerated portion of media consumes more than one-third of tube, media must be reboiled by placing tubes in beaker of boiling water. Media can only be reboiled once. *Note*: Media can be stored for up to 2 months.

(**b**) *Manganese sulfate monohydrate.*—500 ppm. Add 0.25 g manganese sulfate to 500 mL water. Filter sterilize for use.

(**c**) *Dilute hydrochloric acid.*—2.5 M. Use to determine resistance of dried spores. Standardize and adjust to 2.5 M as in 936.15 (*see* A.1.06).

(**d**) *Sterile water.*—Use reagent grade water. Reagent grade water should be free of substances that interfere with analytical methods. Any method of preparation of reagent grade water is acceptable provided that the requisite quality can be met. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent grade water when used in the proper arrangement. *See Standard Methods for the Examination of Water and Wastewater* for details on reagent grade water.

(**e**) *Triton X-100.*

(**f**) *Ethanol.*—40%.

(**g**) *Test organism.—Bacillus subtilis* (ATCC No. 19659) obtained directly from a reputable supplier (e.g., ATCC).

B. Apparatus

(**a**) *Carriers.*—Penicylinders, porcelain, 8 ± 1 mm od, 6 ± 1 mm id, 10 ± 1 mm length (available from CeramTec Ceramic, Laurens, SC, USA, www.ceramtec.com; Cat. No. LE15819.)

(**b**) *Glassware.*—For disinfectant, 25 × 150 mm or 25 × 100 mm culture tubes (Bellco Glass Inc., Vineland, NJ, USA); reusable or disposable 20 × 150 mm (for cultures/subcultures); 16 × 100 mm screw cap tubes for stock cultures. Cap with closures before sterilizing. Sterilize all glassware 2 h in hot air oven at 180°C or steam sterilize for a minimum of 20 min at 121°C with drying cycle.

(**c**) *Sterile centrifuge tubes.*—Polypropylene, 15 mL conical tubes with conical bottoms (Corning), from Fisher, or equivalent.

(**d**) *Water bath/chiller unit.*—Constant temperature for test chemical, capable of maintaining 20 ± 1°C or specified temperature for conducting the test.

(**e**) *Petri dishes.*—Plastic (sterile).

(**f**) *Filter paper.*—Whatman filter paper No. 2; placed in Petri dishes for storing carriers.

(**g**) *Test tube racks.*—Any convenient style.

(**h**) *Inoculating loop.*—Any convenient inoculation/transfer loop for culture transfer.

(**i**) *Wire hook.*—For carrier transfer. Make 3 mm right angle bend at end of 50–75 mm nichrome wire No. 18 B&S gage. Have other end in suitable holder.

(**j**) *Centrifuge.*—Nonrefrigerated (e.g., Eppendorf 5804 R).

(**k**) *Sonicator.*—Ultrasonic cleaner (e.g., Branson Model 1510).

(**l**) *Orbital shaker.*—Speed range from 25 to 500 rpm (e.g., VWR DS 500).

(**m**) *Vacuum desiccator.*—For carrier storage. With adequate gauge for measuring 27 in. (69 cm) of Hg and fresh desiccant.

(**n**) *Certified BSC (Class I or II).*—Recommended for use to maintain aseptic work environment.

(**o**) *Certified timer.*—For managing timed activities; any certified timer that can display time in seconds.

C. Operating Technique

(**a**) *Culture initiation.*—Initiate *B. subtilis* culture (e.g., use nutrient broth to rehydrate a lyophilized culture, and incubate the broth culture for 24 ± 2 h at 36 ± 1°C prior to streak inoculation). Streak inoculate a set (e.g., 6) nutrient agar slopes and incubate 24 ± 2 h at 36 ± 1°C. Concurrently, perform purity and identification confirmation testing for QC (e.g., colony morphology on TSA, Gram stain, or use of other identification systems). Following incubation, store at 2–5°C. Maintain stock culture on nutrient agar slants by monthly (30 ± 2 days) transfers.

(**b**) *Production of B. subtilis spore suspension.*—Using growth from a stock culture tube, inoculate 10 mL tubes (e.g., 2 tubes, depending on the amount of spore preparation desired) of nutrient broth and incubate tubes on an orbital shaker for 24 ± 2 h at approximately 150 rpm at 36 ± 1°C. Use this culture to inoculate amended nutrient agar plates. Inoculate each plate with 500 µL broth culture and spread the inoculum with a sterile bent glass rod or suitable spreading device. Wrap each plate with parafilm or place in plastic bags. Incubate plates inverted for 12–14 days at 36 ± 1°C. Following incubation, harvest the spores by adding 10 mL cold sterile water to each plate. Using a spreader (e.g., bent glass rod), remove growth from plates and pipet suspensions into 15 mL sterile conical tubes (10 plates = 14 tubes, ~10 mL each). Centrifuge tubes at 5000 rpm for approximately 10 min at room temperature. Remove and discard supernatant. Resuspend pellet in each tube with 10 mL cold sterile water and centrifuge at 5000 rpm for approximately 10 min. Remove and discard supernatant. Repeat twice. Resuspend the pellet in each tube with 10 mL sterile water. Store the spore suspension at 2–5°C. Examine spore suspension with a phase contrast microscope or by staining to assess quality of the spores. Examine a minimum of 5 fields and determine ratio of spores to vegetative cells (or sporangia). Percentage of spores versus vegetative cells should be at least 95%. Spore suspension from multiple plates can be combined and re-aliquoted into tubes for uniformity. Prior to inoculation of carriers, determine spore titer of the concentrated spore suspension by plating serial dilutions (e.g., 1.0×10^{-6} through 1.0×10^{-8}) using pour or spread plating on TSA plates. For pour plating, add molten TSA tempered to 45–55°C to each plate, swirl, and allow agar to solidify. Incubate plates for 24 ± 2 h at 36 ± 1°C and determine titer. *Note*: When harvested and processed, 10 plates of amended nutrient agar should provide 80–100 mL concentrated spore suspension (approximately 10^9 CFU/mL). Diluting the suspension prior to carrier inoculation will be necessary; a titer of 1.0×10^8 to 5.0×10^8 CFU/mL should be adequate to achieve the target carrier count.

(**c**) *Preparation of porcelain carriers.*—Prior to use, examine porcelain carriers individually and discard those with scratches, nicks, spurs, or discolorations. Rinse unused carriers gently in water 3 times to remove loose material and drain. Place rinsed carriers into Petri dishes matted with 2 layers of filter paper in groups of 15 carriers per Petri dish. Sterilize 20 min at 121°C. Cool and store at room temperature. *Note*: Handle porcelain carriers with care when placing in Petri dishes. Minimize carrier movement and avoid excessive contact between carriers that might result in chips and cracks. Wash carriers with Triton X-100 and rinse with water 4 times for reuse.

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(d) *Inoculation of porcelain carriers*.—Dilute the concentrated spore suspension as necessary with sterile water to achieve carrier counts between 1.0×10^5 and approximately 1.0×10^6 spores/carrier. Dispense 10 mL diluted spore suspension into an appropriate number of 25 × 150 mm tubes. Add 10 sterile carriers to each tube containing 10 mL spore suspension, slightly agitate, and let stand 10–15 min. Remove each carrier with sterile hook and place upright in sterile Petri dish lined with 2 sheets of filter paper, no more than 30 carriers per Petri dish. Air dry in BSC for approximately 30 ± 2 min. Place Petri dishes containing inoculated carriers in vacuum desiccator containing $CaCl_2$ and draw vacuum of 69 cm (27 in.) Hg. Dry carriers under vacuum for 24 ± 2 h before use in HCl resistance, efficacy testing, or carrier counts. Maintain under vacuum for up to 3 months. Carriers may be used after 3 months if they meet the acceptable HCl resistance and carrier count criteria. Inoculated carriers should not be used after 1 year of storage. Sterilize and reuse if necessary [*see* **C(c)**].

(e) *Spore enumeration (carrier counts)*.—Prior to use, determine the carrier counts for each preparation of carriers. Assay 3 to 5 randomly selected carriers per preparation. Place each inoculated carrier into a 50 mL plastic, polypropylene conical centrifuge tube containing 10 mL sterile water. Sonicate carriers for 5 min ± 30 s. *Note*: For sonication, place tubes into an appropriately sized beaker with tap water to the level of sterile water in the tubes. Place beaker in sonicator so that water level in the beaker is even with water level fill line on sonicator tank. Fill tank with tap water to water level fill line. Suspend beaker in sonicator tank so it does not touch bottom of tank and so all 3 water levels (inside test tubes, inside beaker, and sonicator tank) are the same. Following sonication, mix tubes in a Vortex mixer for 2 min ± 5 s. Dilute spore suspensions by transferring 1 mL aliquots to tubes containing 9 mL sterile water. Dilute spore suspensions out to 1.0×10^{-5} and plate dilutions 1.0×10^{-2} through 1.0×10^{-5}. Plate each dilution in duplicate using pour or surface spread plating with TSA. For pour plating, add molten TSA tempered to 45–55°C to each plate. Swirl pour plates to distribute spores evenly and allow agar to solidify. Invert plates and incubate for 24–48 h at 36 ± 1°C. Count colonies (by hand or with colony counter). Use dilutions yielding between 30 and 300 CFU/plate (target counts) for enumeration; however, record all counts less than 30. Report plates with colony counts over 300 as TNTC (Too Numerous to Count). Average spore counts per carrier should be between 1.0×10^5 and approximately 1.0×10^6 spores/carrier. Do not use carriers with counts outside this range.

(f) *HCl resistance*.—Equilibrate water bath to 20 ± 1°C. Pipet 10 mL of 2.5 M HCl into two 25 × 100 mm tubes, place into water bath, and allow to equilibrate. Start timer and rapidly transfer 4 inoculated penicylinders into an acid tube (2.5 M HCl) with flamed hooks or forceps. Do not allow carriers or transfer device to contact inside of wall of acid tube. Transfer individual carriers after 2, 5, 10, and 20 min of HCl exposure to a separate tube of modified FTM. Rotate each tube vigorously by hand for approximately 20 s and then transfer carrier to a second tube of modified FTM. For viability control, place one unexposed inoculated carrier in a separate tube of modified FTM. For media sterility, use one tube of modified FTM. Incubate all test and control tubes for 21 days at 36 ± 1°C. Record results as growth (+) or no growth (–) at each time period. Spores should resist HCl for ≥2 min to be qualified as resistant test spores. Discard carriers if not resistant and repeat preparation of carriers as previously described.

(g) *Efficacy test*.—Aseptically prepare disinfectant samples as directed. Prepare all dilutions with sterile standardized volumetric glassware. For diluted products, use 1.0 mL or 1.0 g of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. For a 30 carrier test, place 10 mL product at dilution recommended for use or under investigation into each of six 25 × 150 mm or 25 × 100 mm tubes, or use appropriate number of tubes assuming 5 test carriers per tube of test chemical. Place tubes in 20 ± 1°C water bath and let equilibrate to temperature. Using a sterile hook (or forceps), transfer inoculated carriers sequentially at 2 min intervals in groups of 5 from Petri dish to test tubes containing sporicidal agent. Use a certified timer to monitor time. Flame hook and allow cooling after each transfer. When lowering carriers into test tube, neither carriers nor wire hook may touch sides of tubes. If interior sides are touched, note tube number. Do not count carrier set if any carrier from that group of 5 yields a positive result; testing another set of 5 carriers is recommended. Carriers must be deposited into test tubes within ±5 s of the prescribed drop time. Return tubes to water bath immediately after adding carriers. After contact period has been achieved, transfer carriers in same sequential timed fashion into primary subculture tubes containing appropriate neutralizer (10 mL in 20 × 150 mm test tubes). Remove the carriers one at a time from the test tube with sterile hook, tap against interior side of tube to remove excess sporicidal agent, and transfer into neutralizer tube (primary tube). All 5 carriers must be transferred during each 2 min interval. Flame hook between each carrier transfer. Move remaining carriers into their corresponding neutralizer tubes at appropriate time. Carriers may touch interior sides of neutralizer tube during transfer, but contact should be minimized. After each carrier is deposited, recap neutralizer tube and gently shake to facilitate adequate mixing and efficient neutralization. Within 1 h from when last carrier was deposited into primaries, transfer carriers using sterile wire hook to second subculture tube (secondary tube) containing 10 mL of appropriate recovery medium, one carrier per tube. Move carriers in order, but movements do not have to be timed. Gently shake entire rack of secondary tubes after all carriers have been transferred. Incubate primary (neutralizer) and secondary subculture tubes for 21 days at 36 ± 1°C. Report results as growth (+) or no growth (–). A positive result is one in which medium appears turbid. A negative result is one in which medium appears clear. Shake each tube prior to recording results to determine presence or absence of growth/turbidity. Primary and secondary subculture tubes for each carrier represent a "carrier set". A positive result in either primary or secondary subculture tube is considered a positive result for the carrier set.

Media sterility controls and system controls (check for aseptic technique during carrier transfer process) are recommended. For media controls, incubate 1–3 unopened subculture medium tubes with the test sample tubes for 21 days at 36 ± 1°C. For system controls, use sterile forceps or needle hooks to transfer 3 sterile carriers into a tube of test chemical. Transfer system control carriers to neutralizer medium as follows: At start of sample test (prior to first tube), transfer one sterile carrier to tube of neutralizer medium. After one-half of test carriers have been transferred to neutralizer tubes, transfer a second sterile carrier to tube of neutralizer medium. After all test carriers (last tube) have been transferred to neutralizer tubes, transfer third sterile carrier to tube of neutralizer medium. Transfer system control carriers to secondary subculture medium as follows: Immediately prior to initiating transfer of test carriers into

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Table 966.04. Neutralization confirmation procedure—inoculating treatment and control tubes with diluted spore suspension[a]

Neutralizer–primary subculture treatment	Secondary subculture treatment (with carrier)	Neutralizer–primary inoculated control	Secondary subculture inoculated control
1 mL of 10^{-6} → Tube 1	1 mL of 10^{-6} → Tube 1	1 mL of 10^{-6} → Tube 1	1 mL of 10^{-6} → Tube 1
1 mL of 10^{-7} → Tube 2	1 mL of 10^{-7} → Tube 2	1 mL of 10^{-7} → Tube 2	1 mL of 10^{-7} → Tube 2
1 mL of 10^{-8} → Tube 3	1 mL of 10^{-8} → Tube 3	1 mL of 10^{-8} → Tube 3	1 mL of 10^{-8} → Tube 3

[a] 1.0×10^{-6} through 1.0×10^{-8} based on an approximate starting suspension of 10^8 spores/mL.

secondary subculture medium tubes, transfer first system control sterile carrier from neutralizer medium to tube of subculture medium. After one-half of test carriers have been transferred to secondary subculture medium tubes, transfer second system control sterile carrier to tube of subculture medium. After all test carriers have been transferred to secondary subculture medium tubes, transfer third system control sterile carrier to tube of subculture medium. For each test, include a positive carrier control by placing one inoculated carrier into tube of secondary subculture medium. Incubate controls and test sample tubes together for 21 days at 36 ± 1°C.

Perform identification confirmation on a minimum of 3 positive carrier sets per test, if available, using Gram stain and/or plating on TSA. Additional confirmation may be performed using VITEK, API analysis, or comparable method. If fewer than 3 positive carrier sets, confirm growth from each positive carrier set. If both tubes are positive in carrier set, select only one tube for confirmatory testing. For test with 20 or more positive carrier sets, confirm at least 20% by Gram stain. If Gram stains are performed from growth taken directly from positive tubes, the staining should be performed within 5–7 days of conducting the efficacy test.

(h) *Neutralization confirmation procedure.*—A neutralization confirmation test must be performed in advance or in conjunction with efficacy testing. This assay is designed to simulate the conditions (i.e., neutralizer, subculture medium, contact time, diluent, concentration of test substance) of the efficacy test and to demonstrate the recovery of a low level of spores (e.g., 5–100). Diluted inoculum (e.g., spores of *B. subtilis*) is added directly to the various sets of subculture media tubes (*see* Table **966.04**). This assay provides for a quantitative approach to assessing the effectiveness of the neutralizer and any bacteriostatic action resulting from the neutralizer itself or neutralizer–disinfectant interactions.

Produce a spore preparation according to procedure for amended nutrient agar. Harvest growth from plates (e.g., 5 plates) per the method, except resuspend pellet after final centrifugation step in approximately 100 mL aqueous (40%) ethanol. Determine spore count by serial dilution and plating on TSA. Desirable target of the initial working suspension is 1.0×10^8 to 1.0×10^9 CFU/mL. The suspension may require adjustment to reach target titer. Prepare serial 10-fold dilutions of the inoculum in sterile water out to 10^{-8}. Use 10^{-6}, 10^{-7}, and 10^{-8} dilutions to inoculate the neutralizer and subculture media tubes—the target number of spores to be delivered per tube in this assay is 5–100 per tube. Determine spore titer by plating (spread plate or pour plate) each of 3 dilutions in duplicate on TSA. Incubate plates inverted for 24–48 h at 36 ± 1°C. Count colonies (by hand or with colony counter). Report plates with colony counts over 300 as TNTC. *Note*: A standardized spore preparation adjusted to deliver 5–100 spores/mL may be substituted for the 3 dilutions of spore inoculum. In addition, spores sheared from inoculated carriers may be used as a working suspension.

Use 5 sterile porcelain carriers (only 3 to be used in the assay). Within 5 s, place a set of 5 carriers into a test tube (25 × 150 mm or 25 × 100 mm) containing test chemical; transfer carriers according to (**g**). This set of tubes is the neutralizer/primary subculture treatment. Following the transfer of the last carrier into neutralizer tube, transfer each carrier, in sequence, into tube containing secondary subculture medium. This portion of assay is not timed, but should be made as soon as possible. This set is the secondary subculture treatment. Following carrier transfer, inoculate each tube (neutralizer/primary and secondary subculture treatment tubes) with 1 mL of each of 3 inoculum dilutions (10^{-6}, 10^{-7}, and 10^{-8}). For controls, use 3 fresh unexposed tubes of neutralizer and 3 tubes of the secondary subculture medium; also inoculate each control tube with 1 mL of each of 3 inoculum dilutions. Include one uninoculated tube of neutralizer and secondary subculture media to serve as sterility controls. *See* Table **966.04** for tube inoculation scheme. Incubate all tubes 5–7 days at 36 ± 1°C. Record results as growth (+) or no growth (–). *Note*: The lack of complete neutralization of the disinfectant or bacteriostatic activity of the neutralizer itself may be masked when a high level of inoculum (spores) is added to the subculture tubes.

Confirm a minimum of one positive per treatment and control (if available) using Gram staining and colony morphology on TSA. For each treatment and control group, conduct confirmation testing on growth from tube with fewest spores delivered. *B. subtilis* is a Gram positive rod and colonies on TSA are opaque, rough, dull, round, with irregular margins, and low convex. Colonial variation may be observed and is typical for this strain. Growth in the inoculated controls verifies the presence of the spores, performance of the media, and provides a basis for comparison of growth in the neutralizer and subculture treatment tubes. *Note*: There may be cases when the neutralizer is significantly different from the secondary subculture media; in these cases, growth may not be comparable. The uninoculated control tubes are used to determine sterility, and must show no growth for the test to be valid.

The occurrence of growth in the neutralizer/primary subculture and secondary subculture treatment tubes is used to assess the effectiveness of the neutralizer. No growth or growth only in tubes which received a high level of inoculum (e.g., the dilution with plate counts which are TNTC) indicates poor neutralization and/or presence of bacteriostatic properties of the neutralizer or neutralizer–disinfectant interactions. For a neutralizer to be deemed effective, growth must occur in the secondary subculture treatment tubes which received lower levels of inoculum (e.g., 5–100 CFU/tube). Growth in the secondary subculture inoculated control verifies the presence of the spores, performance of the media, and provides a basis for comparison of growth in the

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neutralizer and subculture treatment tubes. No growth or only growth in tubes which received high levels of inoculum (e.g., a dilution with plate counts which are TNTC) indicates poor media performance. Growth in the neutralizer-primary inoculated control should be comparable to the secondary subculture inoculated control if the neutralizer is the same as the secondary subculture media. There may be cases when the neutralizer is significantly different from the secondary subculture media. In these cases, growth may not be comparable to the secondary subculture inoculated control. The neutralizer-primary and secondary subculture uninoculated control tubes are used to determine sterility, and must show no growth for the test to be valid.

Note: For product registration, the EPA requires the following to demonstrate sporicidal/sterilant-level efficacy: Using **966.04**, 60 carriers representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops) must be tested separately against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 samples representing 3 different batches of product, one of which must be at least 60 days old (2 carrier types × 2 test microorganisms × 60 carriers/type = 240 carriers per batch sample; 3 product batches × 240 carriers/batch = total of 720 carriers). The product must kill all of the test spores on all of the 720 carriers without any failures.

References: *J. AOAC Int.* (future issue).

ASTM International Method E 1054–Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.

Standard Methods for the Examination of Water and Wastewater, 21st Ed., American Public Health Association, Washington, DC, USA.

Biosafety in Microbiological and Biomedical Laboratories, 4th Ed., U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health.

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6.3.06

AOAC Official Method 965.12
Tuberculocidal Activity of Disinfectants

First Action 1965
Final Action 1967
Revised First Action 1988
Revised First Action 2008

(Suitable for determining maximum tuberculocidal dilution of disinfectants used on inanimate surfaces. The microbiological method is technique-sensitive and careful adherence to the method with identified critical control points, good microbiological techniques, and quality control is required for proficiency and validity of results. The method has not been validated for glutaraldehyde-based products.)

Caution: (*1*) All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in the institutional biosafety regulations. Use the equipment and facilities indicated for the test organism. For recommendations on safe handling of microorganisms, refer to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories manual. (*2*) Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, and phenol. Personal protective clothing or devices are recommended during the handling of these items for purpose of activation, dilution, or efficacy testing. A chemical fume hood, distinct from a biological safety cabinet, or other containment equipment may be employed when appropriate during performing tasks with concentrated products. The study analyst may wish to consult the Material Safety Data Sheet for the specific product/active ingredient to determine best course of action.

Notes: (*1*) References to water (H_2O) mean reagent grade, except where otherwise specified. (*2*) Commercial dehydrated media made to conform to the specified recipes may be substituted.

I. Presumptive in vitro Screening Test Using *Mycobacterium smegmatis*

A. Reagents

(**a**) *Culture media for stock and test cultures*.—(*1*) *Modified Proskauer-Beck broth*.—Dissolve 2.5 g KH_2PO_4, 5.0 g asparagine, 0.6 g $MgSO_4 \cdot 7H_2O$, 2.5 g magnesium citrate, 20.0 mL glycerol, 0.0046 g $FeCl_3$, and 0.001 g $ZnSO_4 \cdot 7H_2O$ in 1 L H_2O. Adjust to pH 7.2–7.4 with 1 N NaOH. Filter through paper (Whatman No. 4, or equivalent), place 10 mL portions in separate 20 × 150 mm tubes, and steam sterilize 20 min at 121°C. Use this broth for propagating 48 ± 2 h initial test cultures and 6–7 day test cultures.

(*2*) *Nutrient agar*.—Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2–7.4 (blue-green with bromothymol blue), tube, and steam sterilize 20 min at 121°C and slant.

(*3*) *Nutrient broth*.—Boil 5 g beef extract (Difco; paste or powder), 5 g NaCl, and 10 g peptone (Anatone, peptic hydrolysate of pork tissues, manufactured by American Laboratories, Inc., Omaha, NE 68127, USA) in 1 L H_2O for 20 min and dilute to volume with H_2O; adjust to pH 6.8 ± 0.1. Filter through paper (Whatman No. 4, or equivalent), dispense 10 mL portions in 20 × 150 mm test tubes, and steam s 20 min at 121°C. Use to maintain stock culture.

(*4*) *Subculture media*.—Use (*1*) with addition of suitable neutralizing agents such as purified lecithin (Azolectin) or sodium thioglycolate, where necessary.

(*5*) *Sterile water*.—Prepare stock supply of H_2O in 1 L flasks with closures, steam sterilize 20 min at 121°C, and use to prepare dilutions of the test substance. Use reagent-grade water. Reagent-grade water should be free of substances that interfere with analytical methods. Any method of preparation of reagent-grade water is acceptable provided that the requisite quality can be met. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent-grade water when used in the proper arrangement. *See* Standard Methods for the Examination of Water and Wastewater for details on reagent-grade water.

(*6*) *2% Bacto-Gelatin*.—Add 2 g gelatin to 100 mL water, and steam sterilize 20 min at 121°C.

(**b**) *Test organism*.—*Mycobacterium smegmatis* (PRD No. 1; ATCC 19420[T]). Maintain on nutrient agar slants by monthly transfers. Incubate new stock transfer 48 ± 2 h at 36 ± 1°C; then store at 2–5°C. Initiate test culture by inoculating several 10 mL tubes (20 × 150 mm) of Modified Proskauer-Beck broth from a stock slant by transferring one 4 mm id loopful (or similar amount) inoculum from the stock culture onto the surface of the broth. Incubate tubes for a total of 6–7 days at 36 ± 1°C. Incubate tubes 48 ± 2 h in slanting position to provide maximum surface aeration and then in upright position 4–5 days. Using a transfer loop, transfer culture to a heat-sterilized glass tissue grinder, add 1.5 mL sterile 2.0% Bacto-Gelatin solution, and grind to break up large clumps or aggregates of bacteria. Dilute the homogenized culture with 9 mL culture medium (a)(*1*) and transfer the suspension from the tissue grinder to a sterile test tube. Allow the suspension to settle for 10–15 min. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. Dilute the pooled culture with culture medium, (**a**)(*1*), to give 20.0 ± 1% *T* at 650 nm. Use to inoculate porcelain cylinders used in test.

(**c**) *Octylphenoxypolyethoxyethanol nonionic surfactant (e.g., Triton X-100)*.

B. Apparatus

(**a**) *Pipets and glassware*.—(*1*) *Volumetric pipets and volumetric flasks*.—Various volumes for disinfectant preparation. (*2*) *Test tubes*.—For disinfectant, autoclavable 25 × 150 mm or 25 × 100 mm (Bellco Glass Inc., Vineland, NJ, USA); reusable or disposable 20 × 150 mm (for cultures/subcultures). Cap with closures before sterilizing. (*3*) *Tissue grinder*.—Thomas Scientific (Swedesboro, NJ, USA) No. 3431E20, size B, or equivalent. Sterilize all glassware 2 h in hot air oven at 180°C or steam sterilize for a minimum of 20 min at 121°C with drying cycle.

(**b**) *Water bath*.—Constant temperature, relatively deep water bath capable of maintaining 20 ± 1°C.

(**c**) *Racks or other tube holding device*.—Any convenient style.

(**d**) *Inoculating loop (transfer loop)*.—95% platinum, 3.5% rhodium alloy, 18 or 19 gauge, 4 mm loop with 75 mm shank (Baxter Scientific Products, Johnson Matthey Inc., West Chester, PA, USA; or equivalent) or 100 mm disposable loops.

(**e**) *Wire hook*.—For carrier transfer. Make 3–5 mm bend (approximately 60°) at end of suitable platinum or platinum alloy wire, No. 23 B&S gauge, in appropriate holder (Johnson Matthey Inc., or equivalent).

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(**f**) *Carriers.*—"Penicylinders," porcelain, 8 ± 1 mm od, 6 ±1 mm id, 10 ± 1 mm long (CeramTec Ceramic, Laurens, SC, USA; www. ceramtec.com; Cat. No. LE15819.) Sterilize 2 h in 180°C air oven.

(**g**) *Petri dishes.*—Matted with 2 layers of S&S No. 597 or Whatman No. 2, 9 cm sterile filter paper.

(**h**) *Timer.*—Any certified timer that can display time in seconds.

(**i**) *Spectrophotometer.*—To measure absorbance at a specified wavelength between 400 and 700 nm.

C. Operating Technique

Carrier preparation.—Prior to use, examine porcelain carriers individually and discard those with scratches, chips, or cracks. Rinse unused carriers gently in water 3 times to remove loose material and drain. Place clean carriers in multiples of 10 or 20 in capped Erlenmeyer flask or 20 × 150 mm tubes. Sterilize 20 min at 121°C. Cool and store at room temperature. *Note*: Handle porcelain carriers with care. Minimize carrier movement and avoid excessive contact between carriers that might result in damage. Wash carriers with octylphenoxypolyethoxyethanol nonionic surfactant (e.g., Triton X-100) and rinse with water 4 times for reuse.

Using a sterile hook, aseptically transfer 20 carriers prepared as above into the tube of standardized test culture (approximately 20 mL in a sterile 25 × 150 mm tube). Multiple carriers may be transferred on a single wire hook. The test culture must completely cover the carriers. If a carrier is not covered, gently shake the tube, or reposition the carrier within the tube with a sterile wire hook. Be sure to inoculate a sufficient number of carriers for the test.

After 15 min contact, remove cylinders and place on end in vertical position in sterile Petri dish matted with filter paper. Cover and place in incubator at 36 ± 1°C and let dry 20–60 min. This will provide dried test carriers in groups of 20 in individual Petri dishes. From Petri dishes, remove one dried cylinder at 30 s intervals and place into each of 20 tubes containing 10 mL dilution of germicide into a controlled temperature environment (e.g., water bath) at the appropriate temperature (20 ± 1°C or other specified temperature). Immediately after placing carrier in test tube, swirl tube 3 times before placing it back in water bath. (Thus, by time 20 carriers have been transferred, 9 min and 30 s have elapsed, leaving 30 s interval prior to subculturing series at 10 min exposure for each carrier. The 30 s interval between transfers allows adequate time for flaming and cooling transfer hook and making transfer in manner so as to drain all excess chemical from carrier.) Transfer carrier to 10 mL subculture media, **A**(**a**)(*4*). Shake all subculture tubes thoroughly and incubate 12 days at 36 ± 1°C. Report results as + (growth) or – (no growth). Where there is reason to suspect that results may be affected by bacteriostatic action of antimicrobial chemical carried over in subculture tubes, use suitable neutralizer in subculture media.

Make ≥30 carrier exposures at each of 3 relatively widely spaced dilutions of germicide under test between no response and total response dilution levels. Calculate percent of carriers on which organism is killed at each dilution. Using log percent probability paper (3 cycle logarithmic normal No. 32.376, Codex Book Co., Inc., Norwood, MA, USA), locate percent kill points on dilution lines employed (log scale). Draw best fitting straight line through these 3 points and extend to intercept 99% kill line. Read dilution line (log scale) at point of intercept. This is presumed 95% confidence end point for product. (Do not use presumptive test organism for checking validity of this presumptive end point.)

II. Confirmative in vitro Test for Determining Tuberculocidal Activity First Action 1965

D. Reagents

(**a**) *Culture media.*—(*1*) *Modified Proskauer-Beck medium.*—Dissolve 2.5 g KH_2PO_4, 5.0 g asparagine, 0.6 g $MgSO_4 \cdot 7H_2O$, 2.5 g magnesium citrate, 20.0 mL glycerol, 0.0046 g $FeCl_3$, and 0.001 g $ZnSO_4 \cdot 7H_2O$ in 1 L H_2O. Adjust to pH 7.2–7.4 with 1 N NaOH. Filter through paper, place 20 mL portions in separate 25 × 150 mm tubes, and steam sterilize 20 min at 121°C. Use this broth for propagating test cultures and for recovery of test organism from treated carriers.

(*2*) *Middlebrook 7H9 Agar (dehydrated M7H9 medium + agar).*—Dissolve 4.7 g in 900 mL H_2O containing 2 mL glycerol and 15.0 g agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Middlebrook ADC Enrichment (Difco) under aseptic conditions, and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm screw-capped tubes and slant or dispense into sterile Petri plates. Use slants to maintain stock culture and plates for inoculum enumeration.

(*3*) *Middlebrook 7H9 Broth (Difco dehydrated M7H9 medium).*—Dissolve 4.7 g in 900 mL H_2O containing 2 mL glycerol and 1.0 g Bacto agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Middlebrook ADC Enrichment (Difco) under aseptic conditions and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm tubes. Use for recovery of test organism from treated carriers.

(*4*) *Kirchners medium.*—Dissolve 5 g asparagine (Difco), 2.5 g sodium citrate, 0.6 g magnesium sulfate, 2.5 g monopotassium phosphate, and 1.5 g dipotassium phosphate, in 900 mL H_2O containing 20 mL glycerol and 1.0 g Bacto agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Middlebrook ADC Enrichment under aseptic conditions, and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm tubes. Use for recovery of test organism from treated carriers.

(*5*) *TB broth base.*—Dissolve 2.0 g Yeast Extract, 2.0 g Proteose peptone No. 3, 2.0 g Casitone, 1.0 g potassium phosphate monobasic, 2.5 g sodium phosphate dibasic, 1.5 g sodium citrate, and 0.6 g magnesium sulfate (heptahydrate) in 900 mL H_2O containing 50 mL glycerol and 1.0 g Bacto-agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Dubos Medium Serum (Difco) under aseptic conditions, and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm tubes. Use for recovery of test organism from treated carriers.

(**b**) *Test organism.*—*Mycobacterium bovis* (BCG) (Organon Teknika Corp., Durham, NC, USA, or equivalent). For stock culture, streak inoculate M7H9 slants. Incubate 15–20 days at 36 ± 1°C. Following incubation, maintain at 2–5°C for 4–6 weeks.

(**c**) *Sterile water.*—*See* **A**(**a**)(*5*).

(**d**) *Neutralizer.*—Normal horse serum or other chemical to inactivate the germicide.

(**e**) *0.1% Polysorbate 80 in saline.*—Add 0.1 mL polysorbate 80 to 100 mL sterile 0.85% aqueous saline (sodium chloride) solution, filter sterilize. Used in test culture preparation.

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(f) *Octylphenoxypolyethoxyethanol nonionic surfactant (e.g.Triton X-100).*

E. Apparatus

(a) *Pipets and glassware.*—(*1*) *Volumetric pipets and volumetric flasks.*—Various volumes for disinfectant preparation. (*2*) *Test tubes.*—For disinfectant, autoclavable 25 × 150 mm or 25 × 100 mm (Bellco Glass Inc., or equivalent); reusable or disposable 20 × 150 mm (for cultures/subcultures). Cap with closures before sterilizing. (*3*) *Tissue grinder.*—Thomas Scientific No. 3431E20, size B, or equivalent. Sterilize all glassware 2 h in hot air oven at 180°C or steam sterilize for a minimum of 20 min at 121°C with drying cycle.

(b) *Water bath.*—Constant temperature, relatively deep water bath capable of maintaining 20 ± 1°C.

(c) *Racks or other tube holding device.*—Any convenient style.

(d) *Inoculating loop (transfer loop).*—95% platinum, 3.5% rhodium alloy, 18 or 19 gauge, 4 mm loop with 75 mm shank (Baxter Scientific Products, or equivalent) or 100 mm disposable loops.

(e) *Wire hook.*—For carrier transfer. Make 3–5 mm bend (approximately 60°) at end of suitable platinum or platinum alloy wire, No. 23 B&S gauge, in appropriate holder (Johnson Matthey Inc., or equivalent).

(f) *Carriers.*—"Penicylinders," porcelain, 8 ± 1 mm od, 6 ±1 mm id, 10 ± 1 mm long (CeramTec Ceramic; Cat. No. LE15819.) Sterilize 2 h in 180°C air oven. Wash used Penicylinders with Triton X-100 and rinse with H_2O 4 times.

(g) *Petri dishes.*—Matted with 2 layers of S&S No. 597 or Whatman No. 2, 9 cm filter paper.

(h) *Timer.*—Any certified timer that can display time in seconds.

F. Operating Technique

(a) *Carrier preparation.*—Prior to use, examine porcelain carriers individually and discard those with scratches, chips, or cracks. Rinse unused carriers gently in water 3 times to remove loose material and drain. Place clean carriers in multiples of 10 or 20 in capped Erlenmeyer flask or 20 × 150 mm tubes. Sterilize 20 min at 121°C. Cool and store at room temperature. *Note*: Handle porcelain carriers with care. Minimize carrier movement and avoid excessive contact between carriers that might result in damage. Wash carriers with octylphenoxypolyethoxyethanol nonionic surfactant (e.g., Triton X-100) and rinse with water 4 times for reuse.

(b) *Test culture preparation.*—Initiate test culture by inoculating several 20 mL tubes (25 × 150 mm) of Modified Proskauer-Beck broth from a M7H9 stock agar slant by transferring one 4 mm id loopful or equivalent inoculum from the stock culture onto the surface of the broth. Incubate the tubes 21–25 days undisturbed at 36 ± 1°C preferably in a slanted position to increase surface area. (*Note*: The test cultures must be carefully managed. Over-inoculation of Modified Proskauer-Beck broth may lead to reduced viability due to excessive growth after 21–25 days, and the resulting carrier counts may be negatively impacted. Inoculation of Modified Proskauer-Beck broth with a smaller amount of inoculum (i.e., a partial loopful) may lead to higher quality cultures.) Using a transfer loop, transfer culture to a heat-sterilized glass tissue grinder, add 1.0 mL 0.1% polysorbate 80 in saline solution, grind to break up large clumps or aggregates of the test organism. Dilute the homogenized culture with 9 mL Modified Proskauer-Beck broth and transfer the suspension from the tissue grinder to a sterile test tube. Allow the suspension to settle for 10–15 min. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. Dilute the pooled culture with Modified Proskauer-Beck broth to achieve 20.0 ± 1% *T* at 650 nm. Use standardized culture to inoculate porcelain cylinders.

Transfer 10 sterile carriers, using flamed wire hook, into enough (ca 15–20 mL) standardized test culture in 25 × 150 mm test tube. The test culture must completely cover the carriers. If a carrier is not covered, gently shake the tube, or reposition the carrier within the tube with a sterile wire hook. Be sure to inoculate a sufficient number of carriers for the test.

After 15 ± 1 min contact period, remove cylinders, using flamed wire hook; shake carrier vigorously against side of the tube to remove excess culture, and place on end in vertical position in sterile Petri dish matted with 2 layers of Whatman No. 2 (or equivalent) filter paper, making sure that carriers do not touch to prevent improper drying. Place no more than 12 carriers in a Petri dish. Carriers that touch or fall over cannot be used for testing and must be removed and recleaned. Once all of the carriers have been transferred, cover and place in incubator at 36 ± 1°C, and let dry 30 ± 2 min. Inoculated carriers should be used for testing as soon as possible on the day of preparation.

(c) *Disinfectant sample preparation.*—Equilibrate water bath and allow it to come to 20 ± 1°C or the temperature specified (±1°C) by the manufacturer. Prepare the disinfectant dilutions within 3 h of performing the assay unless test parameters specify otherwise. Ready-to-use products are tested as received; no dilution is required.

Aseptically prepare disinfectant samples as directed. Prepare all dilutions with sterile standardized volumetric glassware. For diluted products, use ≥1.0 mL or 1.0 g of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Dispense 10 mL aliquots of the diluted disinfectant or ready-to-use product into 25 × 100 mm (or 25 × 150 mm) test tubes, one tube per carrier. Place tubes in the equilibrated water bath for approximately 10 min to allow test solution to come to specified temperature.

(d) *Test procedure.*—After the required drying time, carriers are sequentially transferred from Petri dish to test tubes containing disinfectant at appropriate intervals. Use a timer to monitor the transfers. Modify intervals to accommodate exposure times other than 10 min.

One carrier is added per tube. The carrier must be deposited in the tube within ±5 s of the prescribed drop time. Using alternating hooks, sterilize the hook and allow it to cool after each carrier transfer. When lowering the carrier into the disinfectant tubes, neither the carrier nor the wire hook should touch the interior sides of the tube. (*Note:* Proper execution of transfer step is one of the most critical technique-sensitive areas of the method. False positives may result from the inadvertent transfer of live organism to sides of the tube followed by contact with the treated carrier during removal.)

After the carriers have been deposited into the disinfectant, and the exposure time is complete, the carriers are then transferred in a sequentially timed fashion into the 10 mL neutralizer (e.g., horse serum) in 20 × 150 mm tubes with a sterile hook. Drain excess disinfectant from the carrier prior to transfer. Shake tube containing carrier in neutralizer thoroughly and immediately transfer the carrier to the tube containing 20 mL Modified Proskauer-Beck broth. Sterilize hook after each carrier transfer. Contact of the carrier

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to the interior of the tube during transfer should be avoided as much as possible.

Once all carriers have been transferred, sequentially transfer 2 mL aliquots from each neutralizer tube into 2 additional subculture media, Middlebrook 7H9 Broth, Kirchners medium, or TB broth, as specified. This portion of the assay is not timed, but the aliquots should be sequentially transferred to the subculture media within 30 ± 5 min. Repeat this with each of the 10 carriers. Incubate 1 tube of each subculture medium with 2 mL sterile neutralizer for quality control purposes. Shake each subculture tube thoroughly; incubate 60 days at 36 ± 1°C, and report results as + (growth) or – (no growth). If no growth or occasional (insufficient for confirmation) growth occurs within a tube, incubate an additional 30 days and record the results. Growth should be checked by using standard confirmation procedures (e.g., acid fast staining and growth on selective media) to ensure that no contamination is present. Maximum dilution of germicide which kills test organism on the 10 carriers, and no growth in each of the 2 mL aliquots for 2 extra media, represents maximum safe use-dilution for practical tuberculocidal disinfection.

(**e**) *Viability controls*.—On the day of testing, place a dried inoculated carrier into a tube of Modified Proskauer-Beck and a tube of each subculture media. Incubate tubes as in the test. Growth in tubes validates test system viability.

References: *JAOAC* **48**, 635(1965); **50**, 767(1967); **53**, 860(1970); **70**, 318(1987).
ASTM International Method E 1054—Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.
Standard Methods for the Examination of Water and Wastewater (2005) 21st Ed., American Public Health Association, Washington, DC, USA.
Biosafety in Microbiological and Biomedical Laboratories (BMBL) (2007) 5th Ed., U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm

Additional Guidance

(The information provided in this section is not considered a component of the official test; rather it serves as procedural guidance to augment the confirmative test.)

A. Neutralization Confirmation

A neutralization confirmation test must be performed in advance or in conjunction with the confirmatory test. Historical use of neutralizer media for specific active ingredients may be taken into consideration when a neutralizer is selected. A neutralization confirmation procedure must demonstrate the recovery of a low level (e.g., ≤100 CFU/tube) of the test organism in the subculture media. For example, in a separate assay to simulate actual test conditions, sterile carriers are exposed to the disinfectant as in the efficacy test. Carriers are transferred in a timed fashion from the disinfectant into the neutralizer and finally into the subculture medium. From the neutralizer tube, 2 mL portions are placed in the 2 additional subculture media. In addition, sterile carriers are exposed to the neutralizer only and transferred aseptically to the subculture medium and 2 mL portions of the neutralizer are again transferred to the additional subculture media. Untreated subculture media are inoculated as controls for comparison. Diluted, standardized *M. bovis* (BCG) inoculum is added directly to the various sets of subculture media tubes. The standardized inoculum is quantified by plating on Middlebrook 7H9 agar. Shake each subculture tube thoroughly, incubate at 36 ± 1°C. Incubate tubes showing no growth up to 60 days at and report results as + (growth) or – (no growth). Incubate plates at 36 ± 1°C for 21–25 days and record the number of colonies. Growth in treatment tubes (disinfectant and neutralizer only) indicates effective product neutralization and lack of bacteriostatic effects of the neutralizer. Test organism may not grow in all specified media. Control tubes are used for baseline media performance. Also, *see* Method ASTM E 1054.

B. Quantitation of Test Organism on Carriers

Perform count of at least 3 carriers for each set of carriers used in testing. Place each dried inoculated carrier into 10 mL of subculture medium (e.g., Modified Proskauer-Beck medium). Recover bacteria from the carrier by sonication (ultrasonic cleaner) for 10 min. Alternatively, carriers may be pooled in subculture medium at the same 1 carrier per 10 mL ratio prior to sonication. Prepare serial dilutions in phosphate buffer dilution water and plate in duplicate on M7H9 agar using standard plating procedures. Incubate at 36 ± °C for 21–25 days and record the number of colonies.

C. Hard Water

For products requiring hard water, *see* 960.09E (*see* 6.3.03).

D. Organic Burden

For one-step cleaner disinfectants, measure standardized culture and add appropriate amount of organic burden. Swirl to mix and proceed with carrier inoculation. For a 5% preparation, pipet 19 mL of culture and 1 mL of organic soil/serum into a 25 × 150 mm test tube (this will allow testing of 20 carriers).

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(**f**) *Octylphenoxypolyethoxyethanol nonionic surfactant (e.g.Triton X-100).*

E. Apparatus

(**a**) *Pipets and glassware*.—(*1*) *Volumetric pipets and volumetric flasks*.—Various volumes for disinfectant preparation. (*2*) *Test tubes*.—For disinfectant, autoclavable 25 × 150 mm or 25 × 100 mm (Bellco Glass Inc., or equivalent); reusable or disposable 20 × 150 mm (for cultures/subcultures). Cap with closures before sterilizing. (*3*) *Tissue grinder*.—Thomas Scientific No. 3431E20, size B, or equivalent. Sterilize all glassware 2 h in hot air oven at 180°C or steam sterilize for a minimum of 20 min at 121°C with drying cycle.

(**b**) *Water bath*.—Constant temperature, relatively deep water bath capable of maintaining 20 ± 1°C.

(**c**) *Racks or other tube holding device*.—Any convenient style.

(**d**) *Inoculating loop (transfer loop)*.—95% platinum, 3.5% rhodium alloy, 18 or 19 gauge. 4 mm loop with 75 mm shank (Baxter Scientific Products, or equivalent) or 100 mm disposable loops.

(**e**) *Wire hook*.—For carrier transfer. Make 3–5 mm bend (approximately 60°) at end of suitable platinum or platinum alloy wire, No. 23 B&S gauge, in appropriate holder (Johnson Matthey Inc., or equivalent).

(**f**) *Carriers*.—"Penicylinders," porcelain, 8 ± 1 mm od, 6 ±1 mm id, 10 ± 1 mm long (CeramTec Ceramic; Cat. No. LE15819.) Sterilize 2 h in 180°C air oven. Wash used Penicylinders with Triton X-100 and rinse with H_2O 4 times.

(**g**) *Petri dishes*.—Matted with 2 layers of S&S No. 597 or Whatman No. 2, 9 cm filter paper.

(**h**) *Timer*.—Any certified timer that can display time in seconds.

F. Operating Technique

(**a**) *Carrier preparation*.—Prior to use, examine porcelain carriers individually and discard those with scratches, chips, or cracks. Rinse unused carriers gently in water 3 times to remove loose material and drain. Place clean carriers in multiples of 10 or 20 in capped Erlenmeyer flask or 20 × 150 mm tubes. Sterilize 20 min at 121°C. Cool and store at room temperature. *Note*: Handle porcelain carriers with care. Minimize carrier movement and avoid excessive contact between carriers that might result in damage. Wash carriers with octylphenoxypolyethoxyethanol nonionic surfactant (e.g., Triton X-100) and rinse with water 4 times for reuse.

(**b**) *Test culture preparation*.—Initiate test culture by inoculating several 20 mL tubes (25 × 150 mm) of Modified Proskauer-Beck broth from a M7H9 stock agar slant by transferring one 4 mm id loopful or equivalent inoculum from the stock culture onto the surface of the broth. Incubate the tubes 21–25 days undisturbed at 36 ± 1°C preferably in a slanted position to increase surface area. (*Note*: The test cultures must be carefully managed. Over-inoculation of Modified Proskauer-Beck broth may lead to reduced viability due to excessive growth after 21–25 days, and the resulting carrier counts may be negatively impacted. Inoculation of Modified Proskauer-Beck broth with a smaller amount of inoculum (i.e., a partial loopful) may lead to higher quality cultures.) Using a transfer loop, transfer culture to a heat-sterilized glass tissue grinder, add 1.0 mL 0.1% polysorbate 80 in saline solution, grind to break up large clumps or aggregates of the test organism. Dilute the homogenized culture with 9 mL Modified Proskauer-Beck broth and transfer the suspension from the tissue grinder to a sterile test tube. Allow the suspension to settle for 10–15 min. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. Dilute the pooled culture with Modified Proskauer-Beck broth to achieve 20.0 ± 1% *T* at 650 nm. Use standardized culture to inoculate porcelain cylinders.

Transfer 10 sterile carriers, using flamed wire hook, into enough (ca 15–20 mL) standardized test culture in 25 × 150 mm test tube. The test culture must completely cover the carriers. If a carrier is not covered, gently shake the tube, or reposition the carrier within the tube with a sterile wire hook. Be sure to inoculate a sufficient number of carriers for the test.

After 15 ± 1 min contact period, remove cylinders, using flamed wire hook; shake carrier vigorously against side of the tube to remove excess culture, and place on end in vertical position in sterile Petri dish matted with 2 layers of Whatman No. 2 (or equivalent) filter paper, making sure that carriers do not touch to prevent improper drying. Place no more than 12 carriers in a Petri dish. Carriers that touch or fall over cannot be used for testing and must be removed and recleaned. Once all of the carriers have been transferred, cover and place in incubator at 36 ± 1°C, and let dry 30 ± 2 min. Inoculated carriers should be used for testing as soon as possible on the day of preparation.

(**c**) *Disinfectant sample preparation*.—Equilibrate water bath and allow it to come to 20 ± 1°C or the temperature specified (±1°C) by the manufacturer. Prepare the disinfectant dilutions within 3 h of performing the assay unless test parameters specify otherwise. Ready-to-use products are tested as received; no dilution is required.

Aseptically prepare disinfectant samples as directed. Prepare all dilutions with sterile standardized volumetric glassware. For diluted products, use ≥1.0 mL or 1.0 g of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Dispense 10 mL aliquots of the diluted disinfectant or ready-to-use product into 25 × 100 mm (or 25 × 150 mm) test tubes, one tube per carrier. Place tubes in the equilibrated water bath for approximately 10 min to allow test solution to come to specified temperature.

(**d**) *Test procedure*.—After the required drying time, carriers are sequentially transferred from Petri dish to test tubes containing disinfectant at appropriate intervals. Use a timer to monitor the transfers. Modify intervals to accommodate exposure times other than 10 min.

One carrier is added per tube. The carrier must be deposited in the tube within ±5 s of the prescribed drop time. Using alternating hooks, sterilize the hook and allow it to cool after each carrier transfer. When lowering the carrier into the disinfectant tubes, neither the carrier nor the wire hook should touch the interior sides of the tube. (*Note:* Proper execution of transfer step is one of the most critical technique-sensitive areas of the method. False positives may result from the inadvertent transfer of live organism to sides of the tube followed by contact with the treated carrier during removal.)

After the carriers have been deposited into the disinfectant, and the exposure time is complete, the carriers are then transferred in a sequentially timed fashion into the 10 mL neutralizer (e.g., horse serum) in 20 × 150 mm tubes with a sterile hook. Drain excess disinfectant from the carrier prior to transfer. Shake tube containing carrier in neutralizer thoroughly and immediately transfer the carrier to the tube containing 20 mL Modified Proskauer-Beck broth. Sterilize hook after each carrier transfer. Contact of the carrier

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6.2.04

AOAC Official Method 955.15
Testing Disinfectants against *Staphylococcus aureus*
Use-Dilution Method
First Action 1955
Final Action 1959
Revised 2006

(Applicable to testing disinfectants with H_2O to determine maximum dilutions effective for practical disinfection. These microbiological methods are technique-sensitive methods in which careful adherence to the method with identified critical control points, good microbiological techniques, and quality controls is required for proficiency and validity of results. These methods have been validated using distilled water only without soil challenge; *see* **A**(**c**) for detailed information on H_2O.)

Notes: (*1*) All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in the institutional biosafety regulations. Use the equipment and facilities indicated for the test organism. For recommendations on safe handling of microorganisms refer to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories manual. (*2*) Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, and phenol. Personal protective clothing or devices are recommended during the handling of these items for purpose of activation, dilution, or efficacy testing. A chemical fume hood or other containment equipment may be employed when appropriate during performing tasks with concentrated products. The study analyst may wish to consult the Material Safety Data Sheet for the specific product/active ingredient to determine best course of action. (*3*) References to water (H_2O) mean reagent grade, except where otherwise specified. (*4*) Commercial dehydrated media made to conform to the specified recipes may be substituted.

A. Reagents

(**a**) *Culture media for stock and test cultures.*—(*1*) *Nutrient broth.*—Boil 5 g beef extract (Difco; paste or powder), 5 g NaCl, and 10 g peptone (Anatone, peptic hydrolysate of pork tissues, manufactured by American Laboratories, Inc., Omaha, NE 68127, USA) in 1 L H_2O 20 min and dilute to volume with H_2O; adjust to pH 6.8 ± 0.1. (If colorimetric method is used, adjust broth to give dark green with bromothymol blue.) Filter through paper (Whatman No. 4, or equivalent), place 10 mL portions in 20 × 150 mm test tubes, and steam sterilize 20 min at 121°C. Use this broth for daily transfers of test cultures.

(*2*) *Synthetic broth.*—*Solution A.*—Dissolve 0.05 g L-cystine, 0.37 g DL-methionine, 0.4 g L-arginine·HCl, 0.3 g DL-histidine, 0.85 g L-lysine·HCl, 0.21 g L-tyrosine, 0.5 g DL-threonine, 1.0 g DL-valine, 0.8 g L-leucine, 0.44 g DL-isoleucine, 0.06 g glycine, 0.61 g DL-serine, 0.43 g DL-alanine, 1.3 g L-glutamic acid·HCl, 0.45 g L-aspartic acid, 0.26 g DL-phenylalanine, 0.05 g DL-tryptophan, and 0.05 g L-proline in 500 mL H_2O containing 18 mL 1 N NaOH.

Solution B.—Dissolve 3.0 g NaCl, 0.2 g KCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 1.5 g KH_2PO_4, 4.0 g Na_2HPO_4, 0.01 g thiamine·HCl, and 0.01 niacinamide in 500 mL H_2O.

Mix Solutions A and B, final pH should be 7.1 ± 0.1, dispense in 10 mL portions in 20 × 150 mm tubes, and steam sterilize 20 min at 121°C. Before using for daily transfers of test cultures, aseptically add 0.1 mL sterile 10% glucose solution per tube. Grow cultures with tube slanted.

(*3*) *Nutrient agar.*—Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2–7.4 (blue-green with bromothymol blue) or in synthetic broth, tube, steam sterilize, and slant.

(*4*) *Subculture media.*—Use (i), (ii), or (iii).

(i) *Nutrient broth.*—Described in (**a**)(*1*).

(ii) *Fluid thioglycolate medium USP.*—Mix 0.5 g L-cystine, 0.75 g agar, 2.5 g NaCl, 5.5 g glucose·H_2O, 5.0 g H_2O soluble yeast extract, and 15.0 g pancreatic digest of casein with 1 L H_2O. Heat on water bath to dissolve, add 0.5 g Na thioglycolate or 0.3 g thioglycolic acid, and adjust with 1 N NaOH to pH 7.1 ± 0.2. If filtration is necessary, reheat without boiling and filter hot through moistened filter paper. Add 1.0 mL freshly prepared 0.1% Na resazurin solution, transfer 10 mL portions to 20 × 150 mm tubes, and steam sterilize 20 min at 121°C. Cool at once to 25°C and store at 20–30°C, protected from light.

(iii) *Letheen broth.*—Dissolve 0.7 g lecithin (American Lecithin Co., Oxford, CT 06478, USA) and 5.0 g polysorbate 80 (Tween 80, or equivalent) in 400 mL hot water, and boil until clear. Add 600 mL solution of 5.0 g beef extract (Difco; paste or powder), 10 g peptone (Anatone), **a**(*1*), and 5 g NaCl in H_2O and boil 10 min. Adjust with 1 N NaOH and/or 1 N HCl to pH 7.0 ± 0.2 and filter through coarse paper; transfer 10 mL portions to 20 × 150 mm tubes, and steam sterilize 20 min at 121°C.

(iv) *Other subculture media.*—Use (*4*)(ii) with 0.7 g lecithin (Alcolec Granules, American Lecithin Co.) and 5.0 g polysorbate 80 (Tween 80, or equivalent) added; or suspend 29.8 g dehydrated fluid thioglycolate medium (Difco), 0.7 g lecithin and 5.0 g polysorbate 80 in 1 L H_2O, and boil until solution is clear. Cool, dispense in 10 mL portions in 20 × 150 mm tubes, and steam sterilize 20 min at 121°C. Store at 20–30°C. Protect from light.

(**b**) *Test organism, Staphylococcus aureus.*—ATCC 6538. Maintain stock culture on nutrient agar slants by monthly (30 ± 2 days) transfers. Every 10 to 12 months, initiate new stock cultures from lyophilized culture obtained directly from a reputable supplier (ATCC or equivalent). Initiate cultures according to supplier recommendations or equivalent. For stock cultures, streak inoculate nutrient agar slants (inoculum taken from the initial reconstituted culture) and incubate at 36 ± 1°C for 48 ± 2 h. Every 30 ± 2 days inoculate a new set of stock culture tubes from a current stock culture tube. Incubate the new stock cultures 36 ± 1°C for 48 ± 2 h. Following incubation, store the cultures at 2–5°C for 30 ± 2 days. Repeat cycle for 1 year.

(**c**) *Sterile water.*—Prepare stock supply of H_2O in 1 L flasks with closures, sterilize 20 min at 121°C, and use to prepare dilutions of the test substance. Use reagent grade water, which should be free of substances that interfere with analytical methods. Any method of preparation of reagent grade water is acceptable provided that the requisite quality can be met. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent grade water when used in the proper arrangement. *See* Standard Methods for the Examination of Water and Wastewater for details on reagent grade water.

(**d**) *Sodium hydroxide solution.*—Approximately 1 M (4%). (For cleaning metal carriers before use.)

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B. Apparatus

(**a**) *Pipets and glassware*.—(*1*) *Volumetric pipets and volumetric flasks*.—Various volumes for disinfectant preparation.

(*2*) *Test tubes*.—For disinfectant, autoclavable 25 × 150 mm or 25 × 100 mm (Bellco Glass Inc., Vineland, NJ 08360-3493, USA); reusable or disposable 20 × 150 mm (for cultures/subcultures). Cap with closures before sterilizing.

Sterilize all glassware 2 h in hot air oven at 180°C or steam sterilize for a minimum of 20 min at 121°C with drying cycle.

(**b**) *Water bath*.—Constant temperature, relatively deep water bath capable of maintaining 20 ± 1°C.

(**c**) *Racks or other tube holding device*.—Any convenient style.

(**d**) *Transfer loops and hooks or equivalent*.—(*1*) *Transfer loop*.—Make 4 mm id single loop at end of 50–75 mm (2–3 in.) Pt or Pt alloy wire No. 23 B&S gage or 4 mm loop fused on 75 mm (3 in.) shaft (available from Johnson Matthey, West Chester, PA 19380, USA). Fit other end in suitable holder. Bend loop at 30° angle with stem. Volumetric transfer devices may be used instead of transfer loops. (*2*) *Hooks*.—Make 3 mm right angle bend at end of 50–75 mm nichrome wire No. 18 B&S gage. Have other end in suitable holder.

(**e**) *Carriers*.—Polished stainless steel cylinders (penicillin cups), 8 ± 1 mm od, 6 ± 1 mm id, length 10 ± 1 mm, of type 304 stainless steel, SS 18-8 (S&L Aerospace Metals, Maspeth, NY 11378, USA, or Fisher Scientific, e.g., Cat. No. 7-907-5 as of January 2006). Discard carriers that are visibly damaged (dull, chipped, dented, or gouged). Before the stainless steel carriers can be used for use-dilution testing, each individual carrier must be screened biologically. This is accomplished by performing an AOAC use-dilution test on each carrier using a 48–54 h old culture of *Staphylococcus aureus* (ATCC 6538) and 500 ppm alkyl dimethyl benzyl ammonium chloride with alkyl chain distribution C14, 50%, C12, 40%, C16, 10% (e.g., BTC-835 Stepan Co., Northfield, IL 60093, USA). Dilute chemical in water to 500 ppm and use as the test disinfectant. Test at 20 ± 1°C with a 10 min exposure period. Discard those carriers giving positive results. In subsequent testing of samples, carriers in tubes showing growth must be rescreened and may not be reused unless screen tests result in no growth.

(**f**) *Positive displacement pipet*.—With corresponding sterile tips able to deliver 10 µL.

(**g**) *Timer*.—Any certified timer that can display time in seconds.

(**h**) *Petri dishes*.—Matted with 2 layers of S&S No. 597 or Whatman No. 2, 9 cm filter paper.

C. Operating Technique

(**a**) *Carrier preparation*.—Visually screen carriers. Discard cylinders that are visibly damaged (dull, chipped, dented, or gouged). Soak carriers overnight (approximately 12 h) in 1 N NaOH, rinse several times with tap water. Collect a portion of the last rinse water and add 2–3 drops of 1% phenolphthalein; if any NaOH remains, the phenolpthalein turns pink, indicating the need for additional rinsing. Continue to rinse the carriers until the addition of phenolpthalein does not produce a color change, then rinse twice more with H_2O. Place cleaned carriers in 25 × 150 mm test tubes with closures, cover with water, sterilize 20 min at 121°C, cool, and hold at room temperature up to 3 months; then reclean and sterilize prior to use.

(**b**) *Test culture preparation*.—Initiate test culture by inoculating a 10 mL tube (20 × 150 mm) of nutrient broth or synthetic broth from a stock slant. Transfer one 4 mm id loopful (or use a 10 µL certified transfer loop or a calibrated positive displacement pipet to deliver 10 µL) of inoculum from the stock culture into the broth. Make at least 3 consecutive 24 ± 2 h transfers (use one 4 mm id loopful, or a 10 µL certified transfer loop, or a calibrated positive displacement pipet to deliver 10 µL) in 10 mL nutrient broth or synthetic broth incubated at 36 ± 1°C. Up to 30 ± 2 total transfers are allowed. If only one of the consecutive 24 h transfers has been missed, it is not necessary to repeat the previous 3-day sequence prior to the inoculation of the 48–54 h test culture. For this final subculture step, inoculate for the test procedure, a sufficient number of 25 × 150 mm tubes containing 20 mL nutrient or synthetic broth; incubate 48–54 h at 36 ± 1°C. Using a Vortex-style mixer, mix nutrient broth test cultures 3–4 s and let stand 10 min at room temperature before continuing. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. Aliquot 20 mL portions into sterile 25 × 150 mm test tubes.

Using a sterile hook, aseptically transfer 20 carriers prepared as above into each of the tubes containing the test culture. Drain the water from the carriers by tapping them against the side of the tube before transferring. Multiple carriers may be transferred on a single wire hook. The test culture must completely cover the carriers. If a carrier is not covered, gently shake the tube, or reposition the carrier within the tube with a sterile wire hook. Be sure to inoculate a sufficient number of carriers for the test. (Alternately, the water may be siphoned off the carriers and the 20 mL test culture added directly to the carriers without transferring.)

After 15 ± 2 min contact period, remove carriers using flamed nichrome wire hook; shake carrier vigorously against side of the tube to remove excess culture, and place on end in vertical position in sterile Petri dish matted with 2 layers of Whatman No. 2 (or equivalent) filter paper, making sure that carriers do not touch to prevent improper drying. Place no more than 12 carriers in a Petri dish. Carriers that touch or fall over cannot be used for testing and must be removed and recleaned. Once all of the carriers have been transferred, cover and place in incubator at 36 ± 1°C and let dry 40 ± 2 min. Inoculated carriers must be used on day of preparation.

(**c**) *Disinfectant sample preparation*.—Equilibrate the water bath and allow it to come to 20 ± 1°C or the temperature specified (±1°C). Prepare the disinfectant dilutions within 3 h of performing the assay unless test parameters specify otherwise. Ready-to-use products are tested as received; no dilution is required.

Aseptically prepare disinfectant samples as directed. Prepare all dilutions with sterile standardized volumetric glassware. For diluted products, use ≥1.0 mL or 1.0 g of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Round to 2 decimal places toward a stronger product. Dispense 10 mL aliquots of the diluted disinfectant or ready-to-use product into 25 × 100 mm (or 25 × 150 mm) test tubes, one tube per carrier. Place tubes in the equilibrated water bath for approximately 10 min to allow test solution to come to specified temperature.

(**d**) *Test procedure*.—After the required drying time, the carriers are sequentially transferred from the Petri dish to the test tubes containing the disinfectant at appropriate intervals. Use a certified timer to time the transfers. Modify intervals to accommodate exposure times other than 10 min. (*Note*: Proper execution of

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transfer step is one of the most critical, technique-sensitive areas of method. False positives will result if sides of tube are touched.)

One carrier is added per tube. Immediately after placing carrier in the test tube, briefly swirl tube before placing it back in the bath. The carrier must be deposited in the tube within ±5 s of the prescribed drop time. Using alternating hooks, flame the hook and allow it to cool after each carrier transfer. When lowering the carriers into the disinfectant tubes, neither the carrier itself nor the tip of the wire hook can touch the interior sides of the tube. Individual manipulation of carriers is required; the use of semi-automated ring carrier is prohibited. (*Note*: Above step is one of the most critical, technique-sensitive areas of method. False positives can result from transfer of live organisms to sides of tubes due to contact or aerosol formation.) If the side is touched, mark or note the tube; the tube is not counted if it yields a positive result.

After the carriers have been deposited into the disinfectant, and the exposure time is complete, the carriers are then transferred in a sequentially timed fashion into the subculture tubes containing the appropriate neutralizer (10 mL in 20 × 150 mm tubes). The carrier is removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant and transferred into the subculture tube. Flame hook after each carrier transfer. The remaining carriers are moved into their corresponding subculture tubes at the appropriate time. As with the transfers to the disinfectant tubes, transfers into subculture tubes should be within ±5 s of the actual transfer. Contact of the carrier to the interior sides of the subculture tube during transfer should be avoided as much as possible.

After the carrier is deposited in the subculture tube, recap the subculture tube and shake thoroughly. Place subculture tubes into 36 ± 1°C incubator and incubate for 48 ± 2 h.

The subculture medium (primary subculture tube) must serve as a suitable neutralizer for the test substance as well as an adequate growth medium which must be confirmed in advance or concurrently with the use-dilution test. Report results as + (growth), or – (no growth) as determined by presence or absence of turbidity. Growth in tubes should be checked by Gram stain to ensure that no contamination is present. Check ≥20% of positive tubes. In the event that there are positive carriers present in the test, the test may be repeated in order to confirm the outcome. Once the results are recorded, it is important that the carriers be reprocessed before use in another study.

Note: If a secondary subculture tube is deemed necessary to achieve complete neutralization, then transfer carrier from the primary tube to a secondary tube of sterile medium after a minimum of 30 ± 5 min from the end of the initial transfer. Transfer the carriers using a sterile wire hook to a second subculture tube containing 10 mL of the appropriate subculture medium which may contain a suitable neutralizer. Move the carriers in order but the movements do not have to be timed. Thoroughly shake the subculture tubes after all of the carriers have been transferred. Incubate both the primary and secondary subculture tubes 48 ± 2 h at 36 ± 1°C. Record the results from both tubes (a carrier set) after this time.

(**e**) *Viability controls*.—On the day of testing, place 2 dried inoculated carriers into separate tubes containing 10 mL neutralizing subculture broth. Incubate tubes for 48 ± 2 h at 36 ± 1°C. Positive growth in each tube validates test system viability.

(**f**) *Verification of positive carriers*.—Positive carriers are examined for test organism by inoculating onto TSA and selective media. Incubate TSA and selective media plates 18–24 h at 36 ± 1°C. Examine plates for colonial morphology characteristic to the test organism (conforming to the morphology in Bergeys manual). Growth from subculture media should be checked by Gram stain. Any suitable identification can also be done.

Maximum dilution of germicide which kills test organism on 10 carriers in 10 min interval represents presumed maximum safe use-dilution for practical disinfection.

Note: While killing in 10 of 10 replicates specified provides reasonably reliable index in most cases, killing in 59 of 60 replicates is necessary for confidence level of 95%.

References: *J. Bacteriol.* **49**, 526(1945).
Am. J. Vet. Res. **9**, 104(1948).
JAOAC **36**, 466(1953); **70**, 318(1987);
71, 117(1988); **72**, 116(1989).
ASTM International Method E 1054–Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.
Standard Methods for the Examination of Water and Wastewater (2005) 21st Ed., American Public Health Association, Washington, DC, USA.
Biosafety in Microbiological and Biomedical Laboratories (1999) 4th Ed., U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health.

Additional Guidance

The information provided in this section is not considered a component of the official test; rather it serves as procedural guidance to augment use-dilution testing of specific antimicrobial products and specific test conditions as the need arises.

A. Neutralization Confirmation

A neutralization confirmation test must be performed in advance or in conjunction with the use-dilution test. Historical use of neutralizer media for specific active ingredients may also be taken in consideration. A neutralization confirmation procedure must demonstrate the recovery of a low level (e.g., 10–100 CFU) of the test organism in the subculture media. For example:

(**a**) At the conclusion of the incubation period, randomly select at least one negative tube for each 10 tubes tested to be used in neutralization confirmation. Dilute a 24–48 h culture of the test organism using phosphate buffer dilution water to achieve 100–1000 CFU/mL. Add 0.1 mL diluted suspension to each tube. Confirm number of cells in the suspension in duplicate by pour plate or spread plates. Incubate tubes and plates for 48 ± 2 h at 36 ± 1°C. Count colonies on plates to determine inoculum level. Examine tubes for growth. Growth in tubes indicates effective neutralization.

(**b**) In a separate assay to simulate actual test conditions, expose a sterile carrier to the test material and transfer to subculture medium (or both primary and secondary tubes if used in the efficacy test) as in the test procedure. Immediately following the transfer, inoculate the tube(s) with 10–100 CFU/tube of the specified culture and incubate 48 ± 2 h at 36 ± 1°C. Confirm number of cells in the suspension in duplicate by pour plate or spread plates. Count colonies on plates to determine inoculum level. Examine tubes for growth. Growth in tubes indicates effective neutralization.

(**c**) *See also* Method ASTM E 1054.

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B. Quantitation of Test Organism on Carriers

Perform count on at least 3 carriers for each set of carriers used in testing. Place each dried inoculated carrier into 10 mL neutralizing broth (e.g., letheen broth). Recover bacteria from the carrier by sonication for 30 s to 5 min or mixing on a Vortex mixer for 30 s. Alternatively, carriers may be pooled in neutralizing broth at the same 1 carrier per 10 mL ratio prior to sonication or mixing on a Vortex mixer. Prepare serial dilutions in phosphate buffer dilution water and plate in duplicate using standard plating procedures. Incubate at 36 ± 1°C for 24–48 h and record the number of colonies.

C. Hard Water

For products requiring hard water, *see* 960.09E (*see* 6.3.03).

D. Organic Burden

For one-step cleaner disinfectants, mix on a Vortex mixer the 48–54 h test culture. Allow culture to stand for ≥10 min before using. For a 5% preparation, pipet 19 mL culture and 1 mL organic soil/serum into a 25 × 150 mm test tube (this volume will allow testing of 20 carriers), mix, or use 9.5 mL culture and 0.5 mL organic load/serum (this volume will allow testing of 10 carriers).

Posted: April 3, 2006

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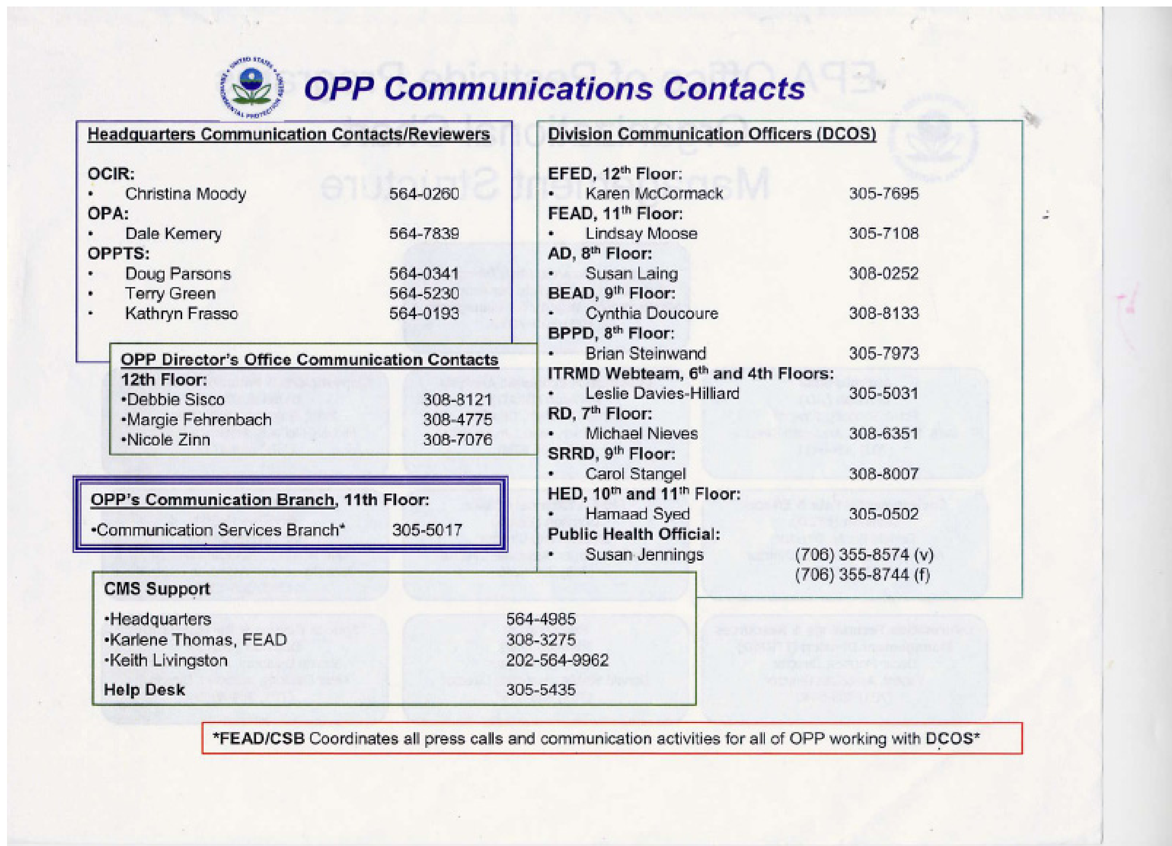
附件 **7**：殺蟲劑專案辦公室 **OPP** 各部門負責溝通的聯絡窗口。



附件 8：殺蟲劑專案辦公室 OPP 業務類別架構表

Office of Pesticide program

Immediate Office
Director：Debbie Edwards
Deputy Director：Marty Monell；Deputy Director：Anne Lindsay

Information & Support	Risk Assessment	Risk Management	Policy & Field Implementation
Information Technology & Resources man Division (ITRD)	Environmental Fate& Effects Division (EFED)	Registration Division (RD)	Field&External Affair (FEAD)
staff：70	staff：90	staff：105	staff：70
Biological & Economic Analysis Division (BEAD)	Health Effects Divisionr (HED)	Special Review & Reregistration Division (SRRD)	
staff：80	staff：175	staff：75	
	Antimicrobial Division (AD)		
	staff：60		
	Biopesticide & Pollution Prevention Division(BPPD)		
	staff：50		

附件 **9**：高溫噴藥注意事項

United States
Environmental Protection Agency
Prevention, Pesticides and
Toxic Substances (7506C)

EPA 750-F-95 001
September 1995

Occupational Safety and Health Administration

Controlling Heat Stress In Agriculture

KEY ELEMENTS

1. Drinking enough water to replace body fluid lost through sweating.
2. Gradually adjusting to working in the heat.
3. Taking periodic rest breaks in a shaded or air conditioned area whenever possible.
4. Monitoring by supervisors of environmental conditions and workers.

BASIC STEPS

- Training in how to control heat stress and to recognize, prevent, and treat heat illnesses.
- Accounting for the weather, workload, protective gear to be worn, and condition of the workers.
- Determining minimum amounts of water workers should drink.
- Adjusting work practices for the conditions of each day.
- Giving first aid when workers become ill.

INDIVIDUAL RESPONSIBILITIES

- Carrying out instructions and training for controlling heat stress, including being alert to signs of heat illness in yourself and others.
- Drinking enough water before, during, and after work.
- Reporting and responding to heat stress problems.
- Personal health, not using drugs, getting adequate rest and sleep.

Taken from EPA/OSHA's "A Guide to Heat Stress in Agriculture." This card is also available in Spanish. The full Heat Stress Guide is available from the U.S. Government Printing Office (document number 055- 000-00474-9). An English/Spanish summary chart is also available (document number 055-000-00544-3). For additional copies of this card in English, refer to document number 055-000-00557-5; in Spanish, document number 055-000-00558-3. To order, write GPO, Superintendent of Documents, Washington, DC 20402, or call (202) 512-1800.

附件10：噴藥訓練紀錄單

WORKER or HANDLER PESTICIDCE SAFETY TRAINING

Verification for the Worker Protection Standard

Establishment Name: ______________________________________
Mailing Address of Establ.: ________________________________
City: ________________________ State: ____________ Zip Code: ___________
Employer Name: _________________________
Date of Training: ______________ (mm/day/yr) [training expires in 5 yrs]
Trainer Name: _________________________
TYPE OF TRAINING
[ ] WORKER [ ] HANDLER
Trainer Employer (if different than above): __________________________
Signature of Trainer: ________________________________
Trainer Qualifications:

[ ] Cert. as Applicator of RUPs (State Cert. Card #: ___________________)
[ ] Trained as WPS Handler (may train Workers only)
(WPS Handler Card #: ______________; State: ____________; Exp. Date: ______________)
[ ] Designated as Trainer of Cert. Appls or WPS Handlers by State/Tribe/EPA
[ ] Completed a WPS Train-the-Trainer Program approved by State/Tribe/EPA
(TtT date:_____________; Trainer: _______________________)

Training Methods (check all that apply):

[ ] audiovisual [ ] oral from written material [ ] language of training: [ ] English [ ] Spanish [ ] Other: __________________

{this training record may be retained for 5 yrs, after which all employees must be retrained}

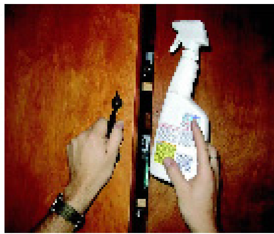
List of Training Materials Used [attach outline of training program]	**EPA Approved YES**	**NO**
1.		
2.		
3.		
4.		
5.		

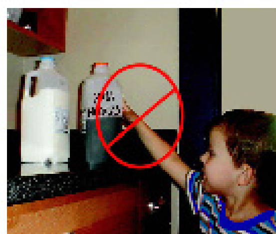
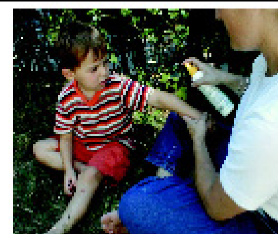
Employee Name (Nombre del Empleado en Letra de Molde)	**Employee Signature (Firma del Empleado)**	**WPS Card Number (if applicable)**
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		

Duplicate as Needed

附件11：10個秘訣保護兒童免於殺蟲劑和鉛暴危害

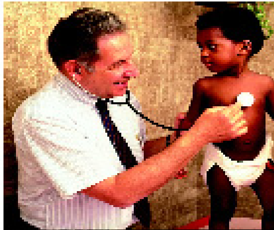
Ten Tips to Protect Children from Pesticide and Lead Poisonings

<table>
<tr>
<td>
1. Always store pesticides and other household chemicals, including chlorine bleach, out of children's reach -- preferably in a locked cabinet.</td>
<td>
2. Read the Label FIRST! Pesticide products, household cleaning products, and pet products can be dangerous or ineffective if too much or too little is used.</td>
</tr>
<tr>
<td>

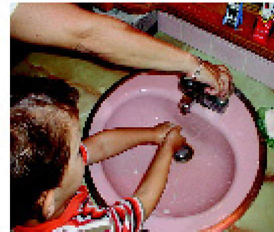
3. Before applying pesticides or other household chemicals, remove children and their toys, as well as pets, from the area. Keep children and pets away until the pesticide has dried or as long as is recommended on the label.</td>
<td>
4. If your use of a pesticide or other household chemical is interrupted (perhaps by a phone call), properly reclose the container and remove it from children's reach. Always use household products in child-resistant packaging.</td>
</tr>
<tr>
<td>
5. Never transfer pesticides to other containers that children may associate with food or drink (like soda bottles), and never place rodent or insect baits where small children can get to them.</td>
<td>
6. When applying insect repellents to children, read all directions first; do not apply over cuts, wounds, or irritated skin; do not apply to eyes, mouth, hands, or directly on the face; and use just enough to cover exposed skin or clothing, but do not use under clothing.</td>
</tr>
</table>

7. Many homes built before 1978 have lead-based paint. If you plan to remodel or renovate, **get your home tested** . Don't try to remove lead paint yourself.

8. Ask about lead when buying or renting a home. Sellers and landlords must disclose known lead hazards in houses or apartments built before 1978.



9. Get your child tested for lead. There are no visible symptoms of lead poisoning, and children may suffer behavior or learning problems as a result of exposure to lead hazards.



10. Wash children's hands, toys, and bottles often. Regularly clean floors, window sills, and other surfaces to reduce possible exposure to lead and pesticide residues.

附件12：優良實驗室查核結果查詢網頁

GLPS Inspection Reports | Compliance Monitoring | US EPA - Windows Internet Explorer

http://www.epa.gov/compliance/monitoring/programs/fifra/glpinspections.html

Compliance Monitoring

Recent Additions | Contact Us **Search:** All EPA Compliance and Enforcement Go

You are here: EPA Home » Compliance and Enforcement » Compliance » Compliance Monitoring » Statutory Programs » FIFRA » Good Laboratory Practice Standards » GLPS Inspection Reports

- Compliance and Enforcement Home
- Compliance Home
- Compliance Monitoring Home
- Basic Information
- Newsroom
- Statutory Programs
- Inspections & Evaluations
- Civil Investigations
- Records Review
- Information Requests

GLPS Inspection Reports

EPA conducts inspections as part of its GLP program:

- to monitor compliance with the regulations
- to assure that studies submitted to the Agency in support of a pesticide registration or under a testing consent agreement for an industrial chemical were done with integrity, are of good quality and valid

GLP inspectors:

- conduct inspections and investigations to detect violations and collect evidence necessary to successfully prosecute FIFRA and TSCA violators
- collect physical samples and documentary evidence

EPA provides a list of the testing facilities (PDF) (5 pp, 310K, About PDF) that have been inspected since October 1, 2005. The list provides the name and address of the testing facility, the date of inspection and the status of the inspection. It is updated biannually.

For more information contact:

Richard Cooney
Laboratory Data Integrity Branch (2225A)
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460
Telephone 202-564-4202
Fax 202-564-0029
cooney.richard@epa.gov

FIFRA Topics

- Good Laboratory Practices
- FIFRA Grants
- Inspections
- Worker Protection Safety
- Pesticide Producing Establishments
- Pesticide Imports and Exports
- Information Resources

附件13：美國環保署風險評估指引

EPA/630/P-03/001FMarch 2005

Guidelines for Carcinogen Risk Assessment

Risk Assessment Forum U.S. Environmental Protection Agency Washington, DC

2.5. WEIGHT OF EVIDENCE NARRATIVE

The *weight of evidence narrative* is a short summary (one to two pages) that explains an agent's human carcinogenic potential and the conditions that characterize its expression. It should be sufficiently complete to be able to stand alone, highlighting the key issues and decisions that were the basis for the evaluation of the agent’s potential hazard. It should be sufficiently clear and transparent to be useful to risk managers and non-expert readers. It may be useful to summarize all of the significant components and conclusions in the first paragraph of the narrative and to explain complex issues in more depth in the rest of the narrative.

2-49

The weight of the evidence should be presented as a narrative laying out the complexity of information that is essential to understanding the hazard and its dependence on the quality, quantity, and type(s) of data available, as well as the circumstances of exposure or the traits of an exposed population that may be required for expression of cancer. For example, the narrative can clearly state to what extent the determination was based on data from human exposure, from animal experiments, from some combination of the two, or from other data. Similarly, information on mode of action can specify to what extent the data are from *in vivo* or *in vitro* exposures or based on similarities to other chemicals. The extent to which an agent's mode of action occurs only on reaching a minimum dose or a minimum duration should also be presented. A hazard might also be expressed disproportionately in individuals possessing a specific gene; such characterizations may follow from a better understanding of the human genome. Furthermore, route of exposure should be used to qualify a hazard if, for example, an agent is not absorbed by some routes. Similarly, a hazard can be attributable to exposures during a susceptible lifestage on the basis of our understanding of human development.

The weight of evidence-of-evidence narrative should highlight:

- the quality and quantity of the data;
- all key decisions and the basis for these major decisions; and
- any data, analyses, or assumptions that are unusual for or new to EPA.

To capture this complexity, a weight of evidence narrative generally includes

C conclusions about human carcinogenic potential (choice of descriptor(s), described below),
2-50

C

a summary of the key evidence supporting these conclusions (for each descriptor used), including information on the type(s) of data (human and/or animal, *in vivo* and/or *in vitro*) used to support the conclusion(s),

C

available information on the epidemiologic or experimental conditions that characterize expression of carcinogenicity (e.g., if carcinogenicity is possible only by one exposure route or only above a certain human exposure level),

C a summary of potential modes of action and how they reinforce the conclusions,

C indications of any susceptible populations or lifestages, when available, and

C a summary of the key default options invoked when the available information is inconclusive.

To provide some measure of clarity and consistency in an otherwise free-form narrative, the weight of evidence descriptors are included in the first sentence of the narrative. Choosing a descriptor is a matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence. These descriptors and narratives are intended to permit sufficient flexibility to accommodate new scientific understanding and new testing methods as they are developed and accepted by the scientific community and the public. Descriptors represent points along a continuum of evidence; consequently, there are gradations and borderline cases that are clarified by the full narrative. Descriptors, as well as an introductory paragraph, are a short summary of the complete narrative that preserves the complexity that is an essential part of the hazard characterization. **Users of these cancer guidelines and of the risk assessments that result from the use of these cancer guidelines should consider the entire range of information included in the narrative rather than focusing simply on the descriptor.**

2-51

In borderline cases, the narrative explains the case for choosing one descriptor and discusses the arguments for considering but not choosing another. For example, between “suggestive” and “likely” or between “suggestive” and “inadequate,” the explanation clearly communicates the information needed to consider appropriately the agent's carcinogenic potential in subsequent decisions.

Multiple descriptors can be used for a single agent, for example, when carcinogenesis is dose- or route-dependent. For example, if an agent causes point-of-contact tumors by one exposure route but adequate testing is negative by another route, then the agent could be described as likely to be carcinogenic by the first route but not likely to be carcinogenic by the second. Another example is when the mode of action is sufficiently understood to conclude that a key event in tumor development would not occur below a certain dose range. In this case, the agent could be described as likely to be carcinogenic above a certain dose range but not likely to be carcinogenic below that range.

Descriptors can be selected for an agent that has not been tested in a cancer bioassay if sufficient other information, e.g., toxicokinetic and mode of action information, is available to make a strong, convincing, and logical case through scientific inference. For example, if an agent is one of a well-defined class of agents that are understood to operate through a common mode of action and if that agent has the same mode of action, then in the narrative the untested agent would have the same descriptor as the class. Another example is when an untested agent's effects are understood to be caused by a human metabolite, in which case in the narrative the untested agent could have the same descriptor as the metabolite. As new testing methods are developed and used, assessments may increasingly be based on inferences from toxicokinetic and mode of action information in the absence of tumor studies in animals or humans.

When a well-studied agent produces tumors only at a point of initial contact, the descriptor generally applies only to the exposure route producing tumors unless the mode of action is relevant to other routes. The rationale for this conclusion would be explained in the narrative.

When tumors occur at a site other than the point of initial contact, the descriptor generally applies to all exposure routes that have not been adequately tested at sufficient doses. An

exception occurs when there is convincing information, e.g., toxicokinetic data that absorption does not occur by another route.

When the response differs qualitatively as well as quantitatively with dose, this information should be part of the characterization of the hazard. In some cases reaching a certain dose range can be a precondition for effects to occur, as when cancer is secondary to another toxic effect that appears only above a certain dose. In other cases exposure duration can be a precondition for hazard if effects occur only after exposure is sustained for a certain duration. These considerations differ from the issues of relative absorption or potency at different dose levels because they may represent a discontinuity in a dose-response function.

When multiple bioassays are inconclusive, mode of action data are likely to hold the key to resolution of the more appropriate descriptor. When bioassays are few, further bioassays to replicate a study's results or to investigate the potential for effects in another sex, strain, or species may be useful.

When there are few pertinent data, the descriptor makes a statement about the database, for example, "Inadequate Information to Assess Carcinogenic Potential," or a database that provides "Suggestive Evidence of Carcinogenic Potential." With more information, the descriptor expresses a conclusion about the agent's carcinogenic potential to humans. If the conclusion is positive, the agent could be described as "Likely to Be Carcinogenic to Humans" or, with strong evidence, "Carcinogenic to Humans." If the conclusion is negative, the agent could be described as "Not Likely to Be Carcinogenic to Humans."

Although the term "likely" can have a probabilistic connotation in other contexts, its use as a weight of evidence descriptor does not correspond to a quantifiable probability of whether the chemical is carcinogenic. This is because the data that support cancer assessments generally are not suitable for numerical calculations of the probability that an agent is a carcinogen. Other health agencies have expressed a comparable weight of evidence using terms such as "Reasonably Anticipated to Be a Human Carcinogen" (NTP) or "Probably Carcinogenic to Humans" (International Agency for Research on Cancer).

The following descriptors can be used as an introduction to the weight of evidence narrative. The examples presented in the discussion of the descriptors are illustrative. The

2-53

examples are neither a checklist nor a limitation for the descriptor. The complete weight of evidence narrative, rather than the descriptor alone, provides the conclusions and the basis for them.

"Carcinogenic to Humans"

This descriptor indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

C This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

C Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when all of the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, and (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on mode of action and also an indication of the relative weight that each source of information carries, e.g., based on human information, based on limited human and extensive animal experiments.

"Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor

"Carcinogenic to Humans." Adequate evidence consistent with this descriptor covers a broad spectrum. As stated previously, the use of the term "likely" as a weight of evidence descriptor does not correspond to a quantifiable probability. The examples below are meant to represent the broad range of data combinations that are covered by this descriptor; they are illustrative and provide neither a checklist nor a limitation for the data that might support use of this descriptor. Moreover, additional information, e.g., on mode of action, might change the choice of descriptor for the illustrated examples. Supporting data for this descriptor may include:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments;
- an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case.

2-55

"Suggestive Evidence of Carcinogenic Potential"

This descriptor of the database is appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples include:

- a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system (see discussions of *conflicting evidence* and *differing results*, below);
- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed. (When there is a high background rate of a specific tumor in animals of a particular sex and strain, then there may be biological factors operating independently of the agent being assessed that could be responsible for the development of the observed tumors.) In this case, the reasons for determining that the tumors are not due to the agent are explained;

evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally

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flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships); or

- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend.

"Inadequate Information to Assess Carcinogenic Potential"

This descriptor of the database is appropriate when available data are judged inadequate for applying one of the other descriptors. Additional studies generally would be expected to provide further insights. Some examples include:

- little or no pertinent information;
- conflicting evidence, that is, some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative. *Differing results*, that is, positive results in some studies and negative results in one or more different experimental systems, do not constitute *conflicting evidence,* as the term is used here. Depending on the overall weight of evidence, differing results can be considered either suggestive evidence or likely evidence; or
- negative results that are not sufficiently robust for the descriptor, "Not Likely to Be Carcinogenic to Humans."

"Not Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the available data are considered robust for deciding that there is no basis for human hazard concern. In some instances, there can be positive results in experimental animals when there is strong, consistent evidence that each mode of action in experimental animals does not operate in humans. In other cases, there can be convincing

evidence in both humans and animals that the agent is not carcinogenic. The judgment may be based on data such as:

- animal evidence that demonstrates lack of carcinogenic effect in both sexes in well-designed and well-conducted studies in at least two appropriate animal species (in the absence of other animal or human data suggesting a potential for cancer effects),
- convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans,
- convincing evidence that carcinogenic effects are not likely by a particular exposure route (see Section 2.3), or
- convincing evidence that carcinogenic effects are not likely below a defined dose range.

A descriptor of "not likely" applies only to the circumstances supported by the data. For example, an agent may be "Not Likely to Be Carcinogenic" by one route but not necessarily by another. In those cases that have positive animal experiment(s) but the results are judged to be not relevant to humans, the narrative discusses why the results are not relevant.

Multiple Descriptors

More than one descriptor can be used when an agent's effects differ by dose or exposure route. For example, an agent may be "Carcinogenic to Humans" by one exposure route but "Not Likely to Be Carcinogenic" by a route by which it is not absorbed. Also, an agent could be "Likely to Be Carcinogenic" above a specified dose but "Not Likely to Be Carcinogenic" below that dose because a key event in tumor formation does not occur below that dose.

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2.3.2. Structure-Activity Relationships (SARs)

SAR analyses and models can be used to predict molecular properties, surrogate biological endpoints, and carcinogenicity (see, e.g., Richard, 1998a, b; Richard and Williams, 2002; Contrera et al., 2003). Overall, these analyses provide valuable initial information on agents, they may strengthen or weaken concern, and they are part of the weight of evidence.

Currently, SAR analysis is most useful for chemicals and metabolites that are believed to initiate carcinogenesis through covalent interaction with DNA (i.e., DNA-reactive, mutagenic, electrophilic, or proelectrophilic chemicals) (Ashby and Tennant, 1991). For organic chemicals, the predictive capability of SAR analysis combined with other toxicity information has been

demonstrated (Ashby and Tennant, 1994). The following parameters are useful in comparing an agent to its structural analogues and congeners that produce tumors and affect related biological processes such as receptor binding and activation, mutagenicity, and general toxicity (Woo and Arcos, 1989):

C nature and reactivity of the electrophilic moiety or moieties present;

C potential to form electrophilic reactive intermediate(s) through chemical, photochemical, or metabolic activation;

C contribution of the carrier molecule to which the electrophilic moiety(ies) is attached;

C physicochemical properties (e.g., physical state, solubility, octanol/water partition coefficient, half-life in aqueous solution);

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structural and substructural features (e.g., electronic, stearic, molecular geometric);

C

metabolic pattern (e.g., metabolic pathways and activation and detoxification ratio); and

C

possible exposure route(s) of the agent.

Suitable SAR analysis of non-DNA-reactive chemicals and of DNA-reactive chemicals that do not appear to bind covalently to DNA should be based on knowledge or postulation of the probable mode(s) of action of closely related carcinogenic structural analogues (e.g., receptor mediated, cytotoxicity related). Examination of the physicochemical and biochemical properties of the agent may then provide the rest of the information needed in order to make an assessment of the likelihood of the agent's activity by that mode of action.

Assessment of Residential Exposure to Pesticides

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
September 21, 1999

SESSION I - Issues Pertaining to the Assessment of Residential Exposure to Pesticides

PARTICIPANTS

Chair

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Designated Federal Official

Mr. Larry Dorsey, FIFRA Scientific Advisory Panel, Office of Prevention, Pesticides and Toxic

Substances, Environmental Protection Agency, Washington, DC

PUBLIC COMMENTERS

Oral statements were made by:

Dr. Muhilan Pandian, American Crop Protection Association

Ms. Leah Rosenheck, Outdoor Residential Exposure Task Force

Dr. John Deprosto, Outdoor Residential Exposure Task Force

Dr. Susan Youngren, Responsible Industry for a Sound Environment

Dr. Dave Esterly, Spray Drift Task Force
Ms. Usha Vedula, Indoor Residential Exposure Joint Venture
Mr. William Perlberg, Hartz Mountain Corporation
Dr. Jim Clark, BASF Corporation
Dr. David Wallinga, Natural Resources Defense Council
Dr. Larry Smith

Written statements were received from:

Dr. Muhilan Pandian, American Crop Protection Association
Ms. Leah Rosenheck, Outdoor Residential Exposure Task Force
Dr. John Deprosto, Outdoor Residential Exposure Task Force
Dr. Susan Youngren, Responsible Industry for a Sound Environment
Dr. Dave Esterly, Spray Drift Task Force
Ms. Usha Vedula, Indoor Residential Exposure Joint Venture
Mr. William Perlberg, Hartz Mountain Corporation

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency regarding issues pertaining to the assessment of residential exposure to pesticides. Advance notice of the meeting was published in the Federal Register on September 3, 1999. The review was conducted in an open Panel meeting held in Arlington, Virginia, on September 21, 1999. The meeting was chaired by Ronald J. Kendall, Ph.D, Professor and Director, The Institute of Environmental and Human Health, Texas Tech University/Texas Tech University Health Sciences Center, Lubbock, TX. Mr. Larry Dorsey served as the Designated Federal Official.

The 1996 Food Quality Protection Act (FQPA) requires the Agency to routinely address nondietary and non-occupational pesticide exposure for the general population. These are exposures that can occur in a residential setting (or other areas frequented by the general population) and that do not occur as part the diet or as a result of participation in occupational practices. In response to FQPA, the Agency developed Standard Operating Procedures (SOPs) for residential exposure assessment, which it brought before the SAP on September 9, 1997. Today's meeting does not present a revised version of the 1997 SOPs. Instead, the Office of Pesticide Programs (OPP) is presenting the most critical issues for discussion prior to developing a revised SOP document. These critical issues are: calculating percent dislodgeability of available pesticide residues from lawns, indoor surfaces, and pets; use of choreographed activities as surrogates for estimating children's dermal exposure; characterizing hand (or object)-to mouth activities; calculating exposure to pesticides that may result from track-in, spray drift, bathing or showering; estimating exposure of children of farmers or farm workers to pesticides; exposure to drift; and calculating exposure from use of pesticides in schools, day-care center, and other public

places. OPP requests the Panel's input on these issues and responses to specific questions concerning these exposure issues.

CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document, Overview of Issues Related to the Standard Operating Procedures for Residential Exposure Assessment, dated August 5, 1999 and are presented as follows:

Issue #1 - Percent Transferable Residues

1. OPP is proposing to change the default assumptions in its SOPs for "percent transferable residues" of pesticides on lawns, indoor surfaces and pets. Does the Panel find these changes reasonable and scientifically defensible, based upon the available data? In particular, does the Panel agree with OPP's proposed assumption of 5% transferability for indoor surfaces, recognizing that data for carpet and desktops support this level, but data for vinyl surfaces show 10% to 20% transferability? Similarly, should OPP consider using a higher "percent transferable residue" factor for wet surfaces and/or sticky hands or not?

Issue #2 - Surrogates for Estimating Dermal Exposure to Children

2. OPP has indicated the intention to continue to use choreographed activities by adults as surrogates for estimating dermal exposure to children. Specifically, OPP has proposed the use of 20 minutes of Jazzercise as a surrogate for up to 4 hours of mixed activities. This position is based on comparisons to biological monitoring studies with adults performing choreographed activities. The Panel is asked to comment upon this approach and its utility when addressing short-term exposures (1 - 7 days) or exposures of longer durations. In addition, the SOPs currently do not account for potential differences in permeability of children's skin compared to adult skin and the Agency has found no scientific data to document such differences. How does the Panel think that the SOPs should address the concern that infants' and children's skin may absorb pesticides at a greater rate than adult skin?

Issue #3 - Frequency of Events

3. OPP has adopted the SAP's previous recommendations concerning the frequency of hand-to-mouth events (20/hr) and available hand surface area (20 cm2). Are these assumptions protective of teething toddlers (8-18 months old), particularly concerning the amount of the hand placed in the mouth (two to three fingers; 20 cm2)? The frequency of 20 events per hour is the 90th percentile from a study involving observations of children at home and in day care centers. The mean in that study is ~10 events per hour. Panel is also asked to comment on the use of these values when addressing short-term exposures (1 - 7 days) or exposures of longer durations.

Issue #4 -Estimating Exposures from Secondary Sources

4. Given the relatively low magnitude of exposures from track-in, bathing or showering relative to other scenarios, should OPP estimate exposure to pesticides that may result from

these sources? If so, have we identified the most critical scenarios and approaches to be used to do the estimation?

Issue #5 - Estimating Exposures from Non-residential Pathways

5. OPP proposes to address exposure of children living on or near farms where pesticides are used by estimating deposition on lawns resulting from pesticide drift; OPP is developing a drift model for this purpose. Does the Panel consider this approach reasonable and are there other important non-residential pathways of potential pesticide exposure that should be evaluated for farm children?

Issue #6 - Addressing Exposure from Spray Drift

6. OPP is proposing to initiate the use of a spray drift model to estimate the likely magnitude of unintentional exposure to pesticide residues as a result of direct exposure to sprays. What is the Panel's opinion concerning the introduction of this new source of exposure into the risk assessment process?

Issue #7 - Twenty- four Hour Assumption Used in Estimating Risk in Schools, Day Care Centers, and Other Public Places

7. OPP currently assumes 24 hour residential exposure as a basis for its exposure assessments. OPP believes that this assumption is sufficiently conservative to protect from exposures that are likely to be encountered in other non-residential settings such as schools, day care centers, or other public places where the use patterns are comparable. Does the Panel agree or disagree and why?

PANEL RECOMMENDATION

Medically, a screening tool is designed to be highly sensitive (e.g., few false negatives) often requiring a trade-off in being less specific (e.g., allowing more false positives). If the Standard Operating Procedures (SOPs) are to be used as a screening tool, they should reflect this orientation and choices should err on the side of overestimating exposures. Thus, using means and other measures of central tendency would not be appropriate. Rather choosing “numbers” that reflect the right side of all distributions, be it the upper limits of the range of measurements when few data are available or the upper bound of a 95th or 99th percentile, is much more conservative and protective.

DETAILED RESPONSE TO THE CHARGE

Issue #1 - Percent Transferable Residues

1. OPP is proposing to change the default assumptions in its SOPs for "percent transferable residues" of pesticides on lawns, indoor surfaces and pets. Does the Panel find these changes reasonable and scientifically defensible, based upon the available data? In particular, does the Panel agree with OPP's proposed assumption of 5% transferability for indoor surfaces, recognizing that data for carpet and desktops support this level, but data for vinyl surfaces show 10% to 20% transferability? Similarly, should OPP consider using a higher "percent transferable residue" factor for wet surfaces and/or sticky hands or not? In general, additional research is required to develop a realistic percentage of

pesticide transfer from different surface types. A survey of peer-reviewed articles should be conducted to determine a realistic range of transfer rates. In addition, formulation types (e.g., microencapsulated) should be evaluated and compared (e.g., microencapsulated versus emulsifiable concentrate). Until such data have been evaluated or studies conducted to determine transfer rates from specific surfaces, the Agency should consider using a more conservative approach, (i.e., 20% from surfaces that have no supporting data).

The need exists for more discussion about studies validating methods used to estimate transferable residues from hard surfaces, such as floors, counters, decks, etc. Of fundamental concern is whether methods show a linear relationship between pesticide removal and the area wiped. If the relationship is not linear, comparison of wipe data across different studies employing different sampling designs becomes problematic.

(1) Lawns: Although based on most of the current literature cited in the report, the 5% transferable residue is most likely an overestimation. Furthermore, studies by Cowell et al. (1993) and Hurto and Prinster, (1993) for two separate pesticides suggest that 5% may not provide a suitable margin of error for all pesticides potentially applied outdoors. In these two cases, the percent transferable residue was 4% for isofenfos, and for Dithiopyr-Microencapsulated the dislodgeable residues were 3.19%. The Agency should determine what would be a suitable margin of error in this case. The microencapsulated pesticide raises another interesting issue that the Agency needs to address, because the formulation of the pesticide may play an important role in its transferability. A microencapsulated for333mulation could have a greater attachment potential, because it "sticks" to an insect's body and is ingested by grooming.

However, one Panel member expressed that the 5% transfer rate was too low, as more material could be transferred from hard surfaces and toys. There was concern that variation between a hard surface (e.g., floor) and clothing may not be equal. A member of the public commented that the use of a simulated dermal press removed all pesticide residues (compound not named) from plastic but only 4% from carpet. A Panel member asked if different carpet types had been tested to determine removal efficiency. It was stated that a few types had been examined, but the primary material was nylon.

A Panel member questioned if there were data on validating the removal of pesticides from surfaces by the use of repetitive motions for X number of times on the same area. The Agency stated that there were limited data available on validations. It was pointed out that wetness of a surface, human or turf, could have an effect on transferability of residues.

One Panel member questioned the rationale for choosing the PUF roller as the standard for surface transfer residuals since the method seems to affect the values greatly. For example, the shoe method may be more realistic than the roller for activities like playing soccer or football on a recently treated field. Having the rationale fully

explained along with biomonitoring data showing the correlations with equivalent surfaces tested with the various methods coupled with urinary metabolite data would be very useful. Even if the PUF roller method is more routinely used and easier to standardize, if it does not accurately reflect the transfer residuals when compared to biomonitoring data, then it is not the best method to choose.

(2) Indoor Surfaces: Why does the Agency assume uniformity in "percent transferable residues" for all indoor surfaces? The validity of the proposed change depends upon the relative frequency of touches involving hard and soft surfaces. What defaults are built into Stochastic Human Exposure and Dose Simulation (SHEDS)? Shouldn't the SOPs and SHEDS reflect similar (if not exactly equivalent) assumptions? The 5% transfer rate for indoor surfaces would be inappropriate based on current literature. Both Camann (1995) and Fenske (1990) found substantially higher transferable residues (23.5% and 11.4%, respectively). Homes contain both smooth and textured surfaces. Since the range of surfaces available in homes are variable, the more conservative approach would be to accept the higher residue transfer values. A question was asked if different types of carpet had an effect on surface residue levels, and it was stated that no data were available to address this concern. More data are needed from surfaces other than carpet.

(a) In support of a more conservative value, it should be noted that the median contact rates by 30 preschool children with smooth surfaces was 80 times per hour; while for textured surfaces such as carpet, the median contact rate was only 16 times per hour (Reed et al. 1999). In addition, the Minnesota Children's Pesticide Exposure Study observed 19 older children (ages 3-12 years) and showed a similar directional difference (Freeman et al. 1999). The greater contact with surfaces with higher transferability should mean that a more conservative figure (i.e., a higher transfer rate) be used rather than a less conservative figure.

(b) Children often have wet and sticky hands, feet, faces, abdomens and chests from saliva. Moist and sticky hands of children seem to be an important concern. Saliva extracts residue with higher efficiency in some studies for some chemicals. They can also be wet in the groin/buttocks area due to urine, or all over the body due to activities or other bodily secretions. This potentially affects non-dietary ingestion as well as dermal absorption. With respect to moist or sticky hands, there are not enough available data to make a determination whether using a higher "percent transferable residue" factor is justifiable. Therefore, it would be better for the Agency to err on the side of the higher transfer rate until further data are available.

(3) Pet applications: We agree that more research is needed with respect to pet applications. Thus far, the transfer models are based on the assumption that the major route of exposure is from petting the animal followed by licking or mouthing the hand. The Agency should also consider that children kiss, mouth, lick, cuddle and sleep with their pets, as well as handle and eat food with hands that have just contacted the pet. The

comment was made that it may be safe to assume that children would not play with animals the first 24 hours after they are treated. This is counter-intuitive, because within hours after treatment would be the most pleasant time to play with a clean, fluffy, nice smelling, flea free dog. Therefore, this is not a justifiable assumption.

One Panel member indicated that choosing the mean of 20% for the pet transfer (as stated on page 39 of the Agency's background document) is not consistent with the concept of screening, where central tendencies should be avoided and cut offs at the upper bounds should be chosen.

Issue #2 - Surrogates for Estimating Dermal Exposure to Children

2. OPP has indicated the intention to continue to use choreographed activities by adults as surrogates for estimating dermal exposure to children. Specifically, OPP has proposed the use of 20 minutes of Jazzercise as a surrogate for up to 4 hours of mixed activities. This position is based on comparisons to biological monitoring studies with adults performing choreographed activities. The Panel is asked to comment upon this approach and its utility when addressing short-term exposures (1 - 7 days) or exposures of longer duration.

In addition, the SOP's currently do not account for potential differences in permeability of children's skin compared to adult skin and the Agency has found no scientific data to document such differences. How does the Panel think that the SOP's should address the concern that infants' and children's skin may absorb pesticides at a greater rate than adult skin?

A comparative study, using children and adults in similar activities, is required to assess dermal exposure in children using the adults as surrogates. Children's behavior patterns (i.e., aggressive vs. passive behavior) must be taken into account when determining potential exposures. The Agency must consider children's ages when assessing dermal exposure, because the literature describes variability when age is used as a criterion in the evaluation process. Future studies involving school children must include the collection of like samples (e.g., urine) from their parents in order to determine if the amount of exposure is greater than other age groups in the study.

It is illogical for the Agency to assume that short, vigorous activity is equivalent in exposure potential to longer periods of more passive behavior. Without adequate evaluation of the adult's Jazzercize behavior relative to activities actually conducted by children (i.e., the types, frequency and duration of contacts in a twenty minute period, and concurrent inhalation and cardiovascular rates), it is unclear that adult Jazzercise tells much, other than what an adult is exposed to during a 20 minute routine. Some sort of comparative study is needed to assure that the extrapolations proposed by the Agency are reasonable. The proposed conversions are based on adult-adult not adult-child comparisons. Some of the adult surrogate activities compared to Jazzercise (e.g., picnicking on a blanket) appear to mimic very passive behavior. An approach that took into account the relative

frequency (among children) of aggressive and passive behavior would be more convincing than the flat assumption of equivalence without examination of behavioral patterns.

Comments were made suggesting the use of a probabilistic approach to determine dermal exposures by looking at both aggressive and passive activities. It was pointed out that industry has dermal exposure studies which it is sharing with the Agency. A question was raised on both bioavailability and persistence of a pesticide over time and how this would affect transfer to a body surface. No specific answer was given.

Some of the logic presented by the Agency concerning lawn contact is flawed. There is very little contact with grass as it is being mowed. In contrast, sitting or lying on grass provides a longer period of contact. Perspiration on the limbs in contact with the grass could facilitate transfer.

It should be noted that studies can be conducted with children in “real” environments where parents routinely use pesticides without raising the ethical issues that concern the Agency. It should also be noted that there are always logistical problems when doing studies with people in homes (regardless of age) in comparison to using employees in a laboratory. The laboratory studies, such as Jazzercise provide only an initial approximation. Without real world validation, it is difficult to interpret the Jazzercise results. There are post-application studies being conducted now with children in real world situations that might illuminate these issues, but the results will not be available for some time.

One Panel member raised the question, "are weight and surface area the appropriate scaling factors for back extrapolating Jazzercise results to children"?

Of particular interest in the reported Jazzercise data, the participants' exposures are as great or greater at 9 hours after application as they were 3 hours after application. Since the approved re-entry time post-application is typically 1-2 hours, what does this say about reasonableness of current re-entry standards? The study by Shah (1987) which the Agency uses to argue that there is no “major” difference in permeability between children’s skin compared to adult skin does not support the Agency's argument of no "major" difference in skin permeability. Some of the pesticides showed higher uptake in younger animals, and others did not, while still others showed higher uptake in the older animals. It is believed that this shows that we need to learn more about how the various pesticides act and that it may not be appropriate to try to make a “one size fits all” dermal exposure model for pesticides.

It should be noted that the Shah study used 33 day-old rats for its young group. This is akin to using a pre-pubertal adolescent as a surrogate for an infant or toddler. One would not expect many differences between a nearly mature rat of 33 days and an adult rat of 84 days; thus, the fact that differences were found is very interesting. In addition, pediatricians are taught that children absorb more through the skin than adults because of the increased surface area/volume ratio as well as differences in skin permeability. The Agency appears to

be satisfied that these differences are related to sampling technique, but the primary data were not supplied. Thus, this judgement was made after a conversation with the authors. Explicitly, further discussion of this point is warranted, because it goes against "established wisdom." One also wonders if a rat is an appropriate dermal model for a human.

The Minnesota Children's Pesticide Exposure Study conducted in collaboration between the University of Minnesota and the RTI/EOHSI/NHEXAS Consortia has produced some very interesting pesticide exposure information. When compared to the NHANES chlorpyrifos metabolite data, the children in the NHEXAS study had higher metabolite levels. Unfortunately, urine samples were not collected from adults in the same NHEXAS families for comparison with their children. The chlorpyrifos loading on the hands of the NHEXAS children did not differ across ages; however, there was a slight but significant negative correlation between the age of the child and metabolite levels suggesting that the dermal absorption was greater in the younger children or that the younger children's activity patterns increased their exposure relative to older children.

It was stated that it would be useful to have access to the studies related to children's exposure on the Internet. This would allow the Panel members the opportunity to review the primary data and foster judgements about the choices entertained by the Agency, rather than requiring the Panel members to rely either on summary data, or search for articles independently.

Issue #3 - Frequency of Events

3. OPP has adopted the SAP's previous recommendations concerning the frequency of hand-to-mouth events (20/hr) and available hand surface area (20 cm2). Are these assumptions protective of teething toddlers (8-18 months old), particularly concerning the amount of the hand placed in the mouth (two to three fingers; 20 cm2)? The frequency of 20 events per hour is the 90th percentile from a study involving bservations of children at home and in day care centers. The mean in that study is ~10 events per hour. The Panel is also asked to comment on the use of these values when addressing short-term exposures (1 - 7 days) or exposures of longer duration.

Additional data are required in order to determine the frequency of hand-to-mouth (also hand-to-feet in infants) contact among age groups. Since the sample size is so small from the few studies published, the upper range of the distribution from these studies should be used when determining absorption from hand-to-mouth activities. Although 20 hand-to-mouth events appear to be an appropriate measure over a one- to six-day period, it is not known what the effects of developmental changes are having on this value. Also, the dorsal surface of babies' hands must be taken into account, because this portion of the hand is used when on flat surfaces. Additional studies are underway n Arizona and Texas, which might provide some insight into this important aspect of research. To the extent current data on hand-to-mouth activity reveals the number of events where hands (or objects) come into contact with the lips, but not necessarily resulting in entry into the

mouth, then the Agency should consider separating their estimate of frequency of events into two terms. One term would represent the number of events where body parts and objects contact the lips and another term would represent the fraction of these events that result from contact with the interior of the mouth. The former is directly available from empirical data, whereas the latter may or may not be and consequently, one must rely on assumptions. Separation into two distinct terms may allow a less ambiguous use of empirical data and provide more transparency of that we know well and that we may not know well.

Unfortunately, there are few data on older infants and young toddlers. The Dutch study cited by the Agency has the only relevant data on these very young children (5 children, 3 to 6 months; 14 children, 6 to 12 months). It only reports duration of mouthing activities with no means of determining frequency of mouthing activities. No data are presented on activities that might contaminate the child's hand or what types of objects are put into the mouth. In addition, no data are presented on ethnicity, gender, region, season of year or other conditions indicating that these few children are representative of a whole population.

The use of 20 events per hour for hand to mouth events is a reasonable 90th percentile for older toddlers and preschool children based on the work of Zartarian and Reed. However, it is not protective since many of the children sampled had much higher rates (up to 62 hand-to-mouth and 39 objects-to-mouth in Reed). Furthermore, object-to- mouth events are not represented in this number, and with data from Gurunatnan (1998), it is clear that this can be a very important route of exposure. At present, there are few data available on 8 to 18 monthold children to determine if this is a meaningful frequency for the younger age group. Thus, a more realistic and protective cutoff of 95% should be used.

Also, one Panel member did not view the 20 events per hour as a reasonable 90th percentile for older toddlers and preschool children stating that sample size in all ages in extremely small, variance is extremely large, the samples are too small to reflect differences (i.e., seasonal or regional behavior) and do not look at intra-individual variation. The absence of sufficient data, specifically for children 8 to 18 months was noted. One Panel member stated that since the sample size of the hand-to-mouth activity was small, the Standard Deviation associated with absorption would be large and that the upper range of the distribution should be used to add an additional precaution. Further, a 90th percentile cut off is not considered sufficiently conservative and protective for a "screening" function, because by definition it leaves 10% of the population unprotected.

The surface area noted for fingers would be appropriate for toddlers. The surface area of three fingers for a 15-kg child is not appropriate for all children. Additional information is needed about the portion and surface area of hand that the younger children put in the mouth. Very young infants can put a larger portion of the hand in the mouth than toddlers, but of course the hand is smaller. Whether 20 cm2 is appropriate for the younger children needs to

be determined. One can not do a linear back calculation from the toddler data. Infants also suck toes and arms. To some extent it appears that children can be classified as "mouthers" or "nonmouthers." This is not to say that there are oung children who don't put fingers in the mouth, only that there are differences in the frequency and duration of these activities across children. Over the short term (1-6 days), it is believed that the 20 events per hour is an appropriate measure; however, for longer term exposures of young children, we are concerned about the issue of developmental changes that may influence the appropriateness of that value. Longitudinal studies are beginning in Texas and Arizona this fall that will eventually provide some answers. There may be other studies on-going that also are directed towards resolving this issue.

On page 68 of the overview document, a discussion of Wester (not Webster) et al. misstates the percentage of chlorine in Aroclor mixtures as the percent PCB, and then misinterprets that quantity as indicative of mass rather than type of chemical on skin.

Two Panel members commented on the importance of the dorsal surface of the hand to babies as they are somewhat immobile, and they use this portion of the hand while on a flat surface like a floor. Page 64 of the Agency's background document suggests that infants are at less risk when then are not yet mobile, but the opposite is also true. Pediatricians teach parents to place their children on the floor as a safe place when playpens and cribs are not available. An infant placed on a contaminated surface or near a crack or crevice treated area might end up with a higher exposure because of his/her inability to move. Babies often have both dorsal and ventral contact with surfaces including hands, arms, trunk, abdomen etc., depending upon the temperature and their clothing. This assumption is not safe and data are definitely required for the younger children for the micro-analysis. In addition, it was pointed out that a baby's feet are accessible for mouth contact. There are peak periods of teething, and finger-to-mouth contact will vary by tooth type.

Issue #4 -Estimating Exposures from Secondary Sources

4. Given the relatively low magnitude of exposures from track-in, bathing or showering relative to other scenarios, should OPP estimate exposure to pesticides that may result from these sources? If so, have we identified the most critical scenarios and approaches to be used to do the estimation?

Data are insufficient to state that exposures from bathing, showering or "track in" are of "relatively low magnitude" and should be minimized. Applications to homes, schools and yards do not result in a uniform distribution of residue levels, and the Agency should focus on postapplication exposures from these types of scenarios. Thus, a "total daily exposure estimate" would be provided for assessing these types of exposures.

One Panel member thought that the primary focus of estimating exposures should focus on post application aspects of lawn or broadcast applications, providing the Agency with a "total daily exposure estimate".

Generally, the contact frequency for small children will be considerably greater in home than outdoors; it is not immediately obvious that the tracking-in exposure route will indeed be that inconsequential. There is insufficient information in the documents provided to determine whether track-in, bathing or showering pathways can be dropped a priori. One Panel member commented that residue levels resulting from "track in," showers or dust might become so minor that these potential routes of exposure would "drop out" (i.e., diminish to insignificant contributions to exposure). Pending the availability of better information that would justify ignoring these additional pathways, the Agency should continue working on developing models for estimating exposures by these routes.

Issue #5 - Estimating Exposures from Non-residential Pathways

5. OPP proposes to address exposure of children living on or near farms where pesticides are used by estimating deposition on lawns resulting from pesticide drift; OPP is developing a drift model for this purpose. Does the Panel consider this approach reasonable and are there other important non-residential pathways of potential pesticide exposure that should be evaluated for farm children?

Too few data are available to rely solely on pesticide residues on lawns in farming or adjacent residences to use in a drift model. A child's movement outdoors through pesticidetreated areas and deposition of residues indoors, from clothing worn by parents working in treated areas have to be considered. In addition, pesticide use patterns, housecleaning children's behavior in these environments must be addressed.

Concerns were raised that there are a variety of exposure pathways that represent potential exposures in addition to deposition on lawn. It was suggested that examples of children's exposures may include field exposures from walking to school through a field, from playing in fields along with working parents or on their own, from swimming in drainage ditches containing runoff, from residues entering a home, from residues on an applicator's clothing, and from residue and spills along paths and roadsides near houses or paths. While a previous presentation did not emphasize important exposures via drinking water, some areas are known to be contaminated in farming communities and this should be considered.

It was pointed out that while the distribution of household dust and mass loading samples shown in Tables 31 and 32 of the Agency's background document are higher in the farmer and farm worker houses than in reference houses, the high range of samples were often more than a magnitude higher in these families. This speaks to the significant degree that some children might be at increased exposure. Also stressed was the importance of individual behaviors, including inappropriate use patterns, pica in children, and house cleaning. A study of lead-poisoned children was cited that found that none of the families with elevated blood lead levels had a vacuum cleaner in the house.

University of Washington researchers are currently investigating a population of agricultural community children. Residential proximity to active orchards is associated with higher body burdens (urinary organophosphate metabolites). Urinary metabolite levels in the

children cannot be explained by contamination in diet, drinking water, house dust or indoor air. Exposure to spray drift (direct inhalation or dermal contact with deposited residues) is one possible explanation. Other hypotheses are also plausible.

(1) The child may be mobile and have access to treated areas, not just drift-impacted areas.

(2) Parental occupation in agriculture may result in "take home" of pesticides. (The actual mechanism involved is not well defined. Contaminated clothing is a plausible source of exposure to co-habitants of occupationally exposed persons.) Since conventional pathways do not predict observed biomonitoring results, additional pathways must be considered (and inclusion of spray drift in the SOP appears prudent). However, the research that should help clarify these exposure issues is ongoing. The missing pathway(s) has not been identified.

A number of general comments were made by the Panel members regarding estimating exposures from non-residential pathways. For example, one Panel member stated that there could be regional differences in exposures based upon commodities, application types, …. It was interjected that illegal pesticide applications could result in exposure. Mention was made of pesticide drift along the edge of fields and roadways that could result in exposures to children playing in these areas. One Panel member stated that pesticides had been found stored in well houses in North Carolina. As a result, North Carolina has data that demonstrates wells have sometimes become contaminated with pesticides. Another Panel member stated that children's exposures in "hot spots" resulting from application drift might be more important than exposures from diet and dust. The Agency responded affirmatively to the question whether aerial, air blast, and boom sprayer applications were used in the exposure models.

Issue #6 - Addressing Exposure from Spray Drift

6. OPP is proposing to initiate the use of a spray drift model to estimate the likely magnitude of unintentional exposure to pesticide residues as a result of direct exposure to sprays. What is the Panel's opinion concerning the introduction of this new source of exposure into the risk assessment process?

Generally, the Panel concluded that using occupational exposures to determine exposure to non-occupational individuals was unrealistic. A question was raised as to how the model would deal with post-application exposure. Presently, the model does not have a means to include post-application exposures. In light of this, the use of dislodgeable residues might be used. The model assumes legal applications following label directions, but legal applications do not always happen and allowances should be made for this possibility in the model, (e.g., allow lawn residues to become dry before allowing children access).

Issue #7 - Twenty- four Hour Assumption Used in Estimating Risk in Schools, Day Care Centers, and Other Public Places

7. OPP currently assumes 24 hour residential exposure as a basis for its exposure assessments. OPP believes that this assumption is sufficiently conservative to protect from exposures that are likely to be encountered in other non-residential settings such as schools, day care centers, or other public places where the use patterns are comparable. Does the Panel agree or disagree and why?

Overall, since children's activities vary greatly between day care, home, school and other public places, the Agency should not assume a 24-hour residential exposure to be the sole basis for risk assessment. Micro-activity data, collected during school-time activities should be used as exposures might be greater in this environment compared to those in the home.

It is not a legitimate assumption that school, daycare and other public place exposures would be equal to or less than residential exposures. Activities of children vary greatly by location. School and daycare activities are substantially different from home activities. It seems that, for example, re-entry into a school after a three day weekend during which an insecticide was sprayed on Friday afternoon, might result in even higher exposures than re-entry into a home after 4-8 hours (Gurunathan, 1998). Furthermore, activities might well bring children into contact with residues at either higher or lower rates. Micro-activity data, if the model of micromacro activity is found to be useful, would be necessary to decide on this issue. Since the number of items manipulated by children in school (e.g., computer keys, formica surfaces, vinyl tile floors) is greater than those in a house, it was felt that "micro" studies should be performed in schools.

One Panel member stated that, from an exposure standpoint, schools and homes were not equal. It was pointed out that individual variations existed regarding cleanliness in both schools and homes. It was mentioned that children at school could be playing on soils with exposures resulting from both dust and soil while they could be playing on grassy areas at home.

While use patterns might be comparable both in the physical environment and home, the behaviors and activities of the children are likely to be significantly different. It was noted that no supporting evidence was presented to back up the assumptions that activity patterns would be the same in both environments.

The use of videography in schools shows promise to obtain behavior patterns of interest that currently elude researchers. Videography in schools might actually be easier than in homes, because many schools are equipped with video equipment. A cross section of many children over several hours might enrich the database in terms of capturing intra- and inter-individual variation on hand-to-mouth, object-to-mouth and other behaviors of interest. Interesting things like the differences in hygiene, use of baby wipes, child- to-child contacts could be studied.

http://www.epa.gov/OSA/spc/pdfs/rchandbk.pdf

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RISK CHARACTERIZATION HANDBOOK

Prepared for the U.S. Environmental Protection Agency by members of the Risk Characterization Implementation Core Team, a group of EPA's Science Policy Council

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1.4 Overview Presentation of TCCR Principles

The following table presents an encapsulated overarching presentation of the TCCR principles and their criteria for a good risk characterization. It is meant to serve as a stand alone summary one-page guide for the use of TCCR throughout the risk assessment process.

Principle	Definition	Criteria for a Good Risk Characterization
Transparency	Explicitness in the risk assessment process.	✓ Describe assessment approach, assumptions, extrapolations and use of models ✓ Describe plausible alternative assumptions ✓ Identify data gaps ✓ Distinguish science from policy ✓ Describe uncertainty ✓ Describe relative strength of assessment
Clarity	The assessment itself is free from obscure language and is easy to understand.	✓ Employ brevity ✓ Use plain English ✓ Avoid technical terms ✓ Use simple tables, graphics, and equations
Consistency	The conclusions of the risk assessment are characterized in harmony with other EPA actions.	✓ Follow statutes ✓ Follow Agency guidance ✓ Use Agency information systems ✓ Place assessment in context with similar risks ✓ Define level of effort ✓ Use review by peers
Reasonableness	The risk assessment is based on sound judgment.	✓ Use review by peers ✓ Use best available scientific information ✓ Use good judgment ✓ Use plausible alternatives

b) Risk assessors that generate site- or medium-specific risk assessments – these assessors usually rely on existing databases and site- or media-specific exposure information (e.g., IRIS, HEAST, OPP database, Exposure Factors Handbook)

Regardless of which group you are in, your major responsibility as a risk assessor is to communicate your key risk findings and conclusions and your confidence in them in the risk characterization section of your assessment. Your basic job is to write the risk assessment with the technical risk characterization (see section 4.2.1).

Your specific responsibilities are to:

a) Explain what is the risk, what individuals, populations or systems are affected and by what route of exposure

b) Describe your level of comfort with the conclusions and what degree of certainty you place in them

   1) Summarize and identify the key pieces of information critical to your evaluation

   2) Let your manager know whether the key data used for the assessment are considered experimental, state-of-the art or generally accepted scientific knowledge

c) Describe quantitative risk estimates in plain English; the use of tables and graphics may be helpful as a supplement

d) Describe the uncertainties inherent in the risk assessment and the default positions used to address these uncertainties or gaps in the assessment

e) Refer the reader to an Agency risk assessment guideline or other easily obtainable reference that explains terminology (e.g., how a RfC was developed)

f) Put this risk assessment into a context with other similar risks that are available to you and describe how the risk estimated for this stressor, agent or site compares to others regulated by EPA

g) Describe how the strengths and weaknesses of EPA's assessment compare with other assessments prepared by EPA in the past

b) Risk assessors that generate site- or medium-specific risk assessments – these assessors usually rely on existing databases and site- or media-specific exposure information (e.g., IRIS, HEAST, OPP database, Exposure Factors Handbook)

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  1) Summarize and identify the key pieces of information critical to your evaluation

  2) Let your manager know whether the key data used for the assessment are considered experimental, state-of-the art or generally accepted scientific knowledge

c) Describe quantitative risk estimates in plain English; the use of tables and graphics may be helpful as a supplement

d) Describe the uncertainties inherent in the risk assessment and the default positions used to address these uncertainties or gaps in the assessment

e) Refer the reader to an Agency risk assessment guideline or other easily obtainable reference that explains terminology (e.g., how a RfC was developed)

f) Put this risk assessment into a context with other similar risks that are available to you and describe how the risk estimated for this stressor, agent or site compares to others regulated by EPA

g) Describe how the strengths and weaknesses of EPA's assessment compare with other assessments prepared by EPA in the past

h) Describe the rationale and bases for the conclusions drawn by those outside EPA about this agent, stressor or site

   1) If their conclusions differ from yours, let the manager know whether theirs is a reasonable alternative

   2) Can their conclusions reasonably be derived from the data set

   3) Inform the manager of the strengths and weaknesses of their evaluations compared to yours

i) If you have developed specific assessments for one or more risk management alternatives, let the risk manager know what changes in risk would occur under these various candidate risk management alternatives

j) Highlight areas in the assessment which might be overlooked or misinterpreted by the risk manager

k) Keep the decision maker informed of the status of your risk assessment and risk characterization

l) Organize, conduct, and complete the risk characterization following Agency procedures

m) Archive the risk characterization record in a manner consistent with your organization's archiving procedures

1.5.4 What Are My Responsibilities as a Risk Manager?

Risk managers are generally the decision makers in their organization. The AA/RA is the ultimate decision maker for his/her organization and is accountable for both the risk characterization process and products in his/her office. The AA/RA may designate Office Directors, Division Directors, and/or Branch Chiefs (or other appropriate level line-managers) as the front-line decision makers. Generally, the decision makers commit the resources needed to ensure a proper risk assessment which includes a complete risk characterization.

As a risk manager, you are responsible for ensuring that risk assessments, containing risk characterizations, are properly performed and documented. You are also responsible for ensuring

that the key information from each risk characterization is honestly and clearly elevated up the management chain and communicated to senior management. As a decision maker, you integrate the risk characterization with other considerations specified in applicable statutes, Agency and office policies, executive orders, and other factors (e.g., see Chapter 5) to make and justify regulatory decisions.

Your specific responsibilities are:

a) Promote a culture supportive of preparing risk characterizations and ensure that all risk assessment work products produced by or submitted to your organization are well characterized

b) Provide advice, guidance, and support for the preparation, conduct, and completion of a full risk characterization for each assessment

c) Play a major role in determining the scope of the risk assessment

d) Ensure that sufficient funds are designated in the office's budget request to conduct a risk characterization for each risk assessment

e) Establish a realistic risk assessment schedule that includes risk characterization

f) Designate the stage(s) of product development where risk characterization is appropriate

g) Ensure that the characterizations prepared by individual risk assessors for their portion of each risk assessment document are integrated into a complete risk characterization for each risk assessment

h) Provide proper risk assessment training for your staff including how to write risk assessments and their characterizations

i) Establish systems to maintain records of the risk assessments, including risk characterizations, prepared by risk assessors under your supervision

j) Ensure that the key points from the risk characterization are carried forward in all deliberations or considerations for decision making

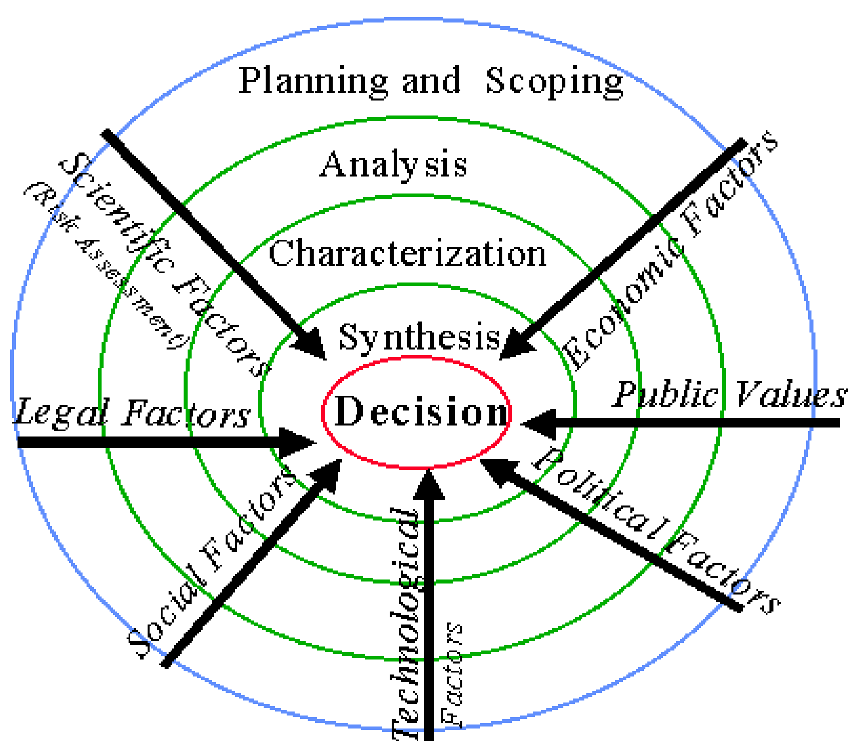

Figure 5.1 Risk Management Decision Framework. At least seven factors (represented by the arrows) affect and inform risk management decisions. Each factor passes through four analytical steps to integrate the information for a risk management decision.

http://www.epa.gov/OSA/pdfs/ratf-final.pdf

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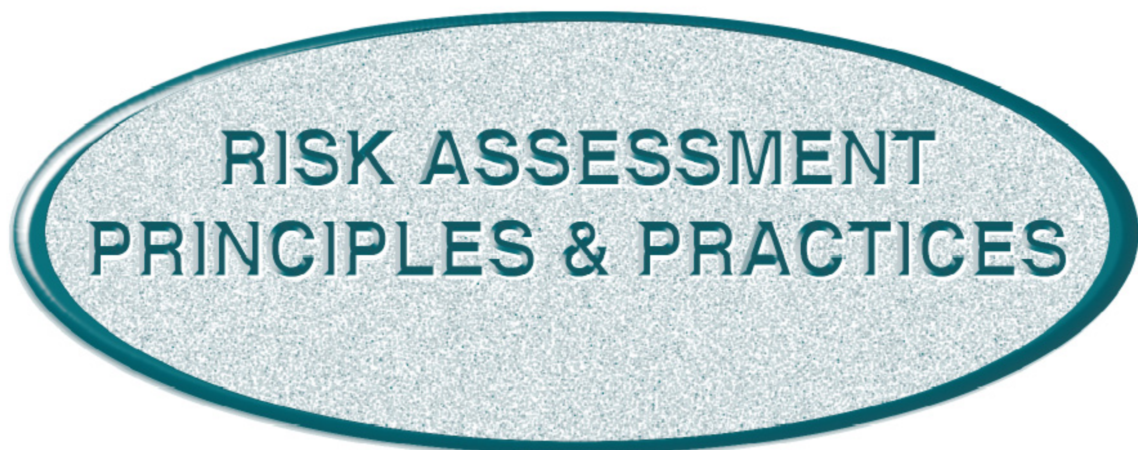

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U.S. Environmental Protection Agency

AN EXAMINATION OF EPA RISK ASSESSMENT PRINCIPLES AND PRACTICES

Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force

Office of the Science Advisor
U.S. Environmental Protection Agency
Washington, DC 20460

DISCLAIMERS

This document has been reviewed in accordance with United States Environmental Protection Agency policy and approved for publication and distribution. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This document presents an analysis of EPA's general risk assessment practices, based on typical historic and current practice. The document does not establish new Agency policy or guidance or amend any existing Agency policy or guidance. Nor does the document attempt to present binding prospective requirements, necessarily applicable to future agency actions. The use of the words "should," "can," "would," and "may" in this document means that something is suggested or recommended, but not required.

A particular risk assessment practice described in this document may not apply to an individual situation based upon the circumstances. Interested parties are free to raise questions and objections about the substance of the practices discussed in this document and the propriety of the application of those practices to a particular situation. Any individual or site-specific risk management decision will be based on the applicable statute and regulations, and on facts specific to the circumstances at issue. Variance from the approaches outlined in this document does not necessarily have any significance. EPA and other decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those described in this document where appropriate.

Risk assessments discussed in this staff paper reflect a "snapshot" in time and may not be reflective of any further assessment activity past the time of a particular description. For example, the Integrated Risk Information System (IRIS) descriptions, particularly of past assessments, may not be reflective of the current IRIS data base, as assessments are continuously updated. Further, it is important to note that current IRIS health assessments are conducted using the 1999 draft cancer guidelines (as of this examination), and not the 2003 draft final cancer guidelines.

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附件14：有機磷劑陶斯松再註冊結果

Chlorpyrifos Facts

EPA 738-F-01-006

February 2002

EPA has assessed the risks of chlorpyrifos and reached an Interim Reregistration Eligibility Decision (IRED) for this organophosphate (OP) pesticide. Provided that risk mitigation measures are adopted, chlorpyrifos fits into its own "risk cup"-- its individual, aggregate risks are within acceptable levels. Chlorpyrifos also is eligible for reregistration, pending a full reassessment of the cumulative risk from all OPs.

Used on a variety of food and feed crops, golf courses, as a non-structural wood treatment, and as an adult mosquitocide, chlorpyrifos residues in food and drinking water do not pose risk concerns. With mitigation eliminating virtually all homeowner uses, chlorpyrifos fits into its own "risk cup." With other mitigation measures, chlorpyrifos worker and ecological risks also will be below levels of concern for reregistration.

EPA's next step under the Food Quality Protection Act (FQPA) is to complete a cumulative risk assessment and risk management decision encompassing all the OP pesticides, which share a common mechanism of toxicity. The interim decision on chlorpyrifos cannot be considered final until this cumulative assessment is complete. Further risk mitigation may be warranted at that time.

EPA is reviewing the OP pesticides to determine whether they meet current health and safety standards. Older OPs need decisions about their eligibility for reregistration under FIFRA. OPs with residues in food, drinking water, and other non-occupational exposures also must be reassessed to make sure they meet the new FQPA safety standard.

The chlorpyrifos interim decision was made through the OP pilot public participation process, which increases transparency and maximizes stakeholder involvement in EPA's development of risk assessments and risk management decisions. EPA worked extensively with affected parties to reach the decisions presented in this interim decision document, which concludes the OP pilot process for chlorpyrifos.

Uses

- Chlorpyrifos is an organophosphate insecticide, acaricide and miticide used to control foliage and soil-borne insect pests on a variety of food and feed crops.
- Approximately 10 million pounds are applied annually in agricultural settings. The largest agricultural market for chlorpyrifos in terms of total pounds ai is corn (~5.5 million).

Health Effects

- Chlorpyrifos can cause cholinesterase inhibition in humans; that is, it can overstimulate the nervous system causing nausea, dizziness, confusion, and at very high exposures (e.g., accidents or major spills), respiratory paralysis and death.

Risks

- Dietary exposures from eating food crops treated with chlorpyrifos are below the level of concern for the entire U.S. population, including infants and children. Drinking water risk estimates based on screening models and monitoring data from both ground and surface water for acute and chronic exposures are generally not of concern.
- **In June, 2000, the Agency entered into an agreement with the technical registrants to eliminate virtually all homeowner uses, except ant and roach baits in child resistent packaging.**
- **Residential postapplication exposures may occur after termiticide use in residential structures. To mitigate risks from this use, the technical registrants agreed in June 2000 to limit termiticide treatments to 0.5% solution, and cancel all postconstruction uses. Pre-construction use will remain until 2005, unless acceptable exposure data are submitted that show that residential postapplication risks from this use are not a concern.**
- Occupational exposure to chlorpyrifos is of concern to the Agency. Exposures of concern include mixing/loading liquids for aerial/chemigation and groundboom application, mixing wettable powder for groundboom application, aerial application, and application by backpack sprayer, high-pressure handwand, and hand-held sprayer or duster. Generally, these risks can be mitigated by a combination of additional personal protective equipment and engineering controls, and by reductions in application rates. Additionally, the Agricultural Handler Task Force will be developing exposure data to better characterize the risk from certain uses (e.g., applying granulars by air).
- Risk quotients indicate that a single application of chlorpyrifos poses risks to small mammals, birds, fish and aquatic invertebrate species for nearly all registered outdoor uses. Multiple applications increase the risks to wildlife and prolong exposures to toxic concentrations. To address these risks, a number of measures including reduced application rates, increased retreatment intervals, reduced seasonal maximum amounts applied per acre, and no-spray setback zones around water bodies will be needed.

Risk Mitigation

In order to support a reregistration eligibility decision for chlorpyrifos, the following risk mitigation measures are necessary:

- To mitigate risks to agricultural workers PPE consisting of double layers, chemical resistant gloves, chemical resistant shoes plus socks, chemical resistant headgear for overhead exposure, chemical resistant apron when cleaning and mixing or loading and a dust/mist respirator are required for the following scenarios: mixing/loading liquids for groundboom and airblast application, loading granulars for ground application, tractor drawn granular spreader, and low pressure handwand.
- engineering controls are required for the following scenarions: mixing wettable powder for groundboom application (water soluble packaging), mixing wettable powder for airblast application (water soluble packaging), and aerial application of sprays (enclosed cockpit).
- There are still some occupational risk scenarios that are still below the target MOE of 100, even with all feasible PPE or engineering controls. The risk assessments for these uses will be refined with additional data.
- To mitigate ecological risks the technical registrants have agreed to label amendments which include the use of buffer zones to protect water quality, fish and wildlife, reductions in application rates, number of applications per season, seasonal maximum amounts applied, and increases in the minimum intervals for retreatment.
- The mitigation measures prescribed in the IRED along with mitigation that is already being implemented as a result of the June, 2000, Memorandum of Agreement, will reduce risk to both terrestrial and aquatic species. For example, many of the reported incidents of wildlife mortality associated with chlorpyrifos use were related to residential lawn and termite uses and use on golf courses. The residential uses have been eliminated, the termiticide use is being phased out, and the application rate on golf courses has been reduced from 4 to 1 lb/ai/A. Additionally, no-spray buffers around surface water bodies, as well as rate reductions for agricultural uses will be implemented as a result of this IRED and will further reduce the environmental burden of chlorpyrifos.

The OP Pilot Public Participation Process

The organophosphates are a group of related pesticides that affect the functioning of the nervous system. They are among EPA's highest priority for review under the Food Quality Protection Act.
EPA is encouraging the public to participate in the review of the OP pesticides. Through a six-phased pilot public participation process, the Agency is releasing for review and comment

its preliminary and revised scientific risk assessments for individual OPs. (Please contact the OP Docket, telephone 703-305-5805, or see EPA's web site, Pesticide Reregistration Status.) EPA is exchanging information with stakeholders and the public about the OPs, their uses, and risks through Technical Briefings, stakeholder meetings, and other fora. USDA is coordinating input from growers and other OP pesticide users.

Based on current information from interested stakeholders and the public, EPA is making interim risk management decisions for individual OP pesticides, and will make final decisions through a cumulative OP assessment.

Next Steps

- Numerous opportunities for public comment were offered as this decision was being developed. In addition, the chlorpyrifos IRED has been issued with a public comment period (see Pesticide Reregistration Status).
- When the cumulative risk assessment for all organophosphate pesticides is completed, EPA will issue its final tolerance reassessment decision for chlorpyrifos and may request further risk mitigation measures. The Agency will revoke the tomato tolerance and amend the grape and apple tolerances for chlorpyrifos. For all OPs, raising and/or establishing tolerances will be considered once a cumulative assessment is completed.

附件15：美國環保署禁用殺蟲劑清單

UN PIC & U.S. PIC-Nominated Pesticides List

Following is a list of 22 UN PIC pesticides, 4 UN Severely Hazardous Pesticide Formulations (SHPF), 6 UN PIC pesticides added during the interim period, and 36 additional U.S. actions reported, originally nominated for inclusion on the PIC list, and based on PIC definitions of the voluntary program. (Two of the six interim pesticides were included in the original U.S. list, bringing the total to 64.)

#	Pesticide	UN PICList	Banned	Severely Restricted	SHPF
1	*aldrin*	x	x		
2	arsenic trioxide			x	
3	***asbestos all forms (Interim)***	x	x		
4	***benzene hexachloride[BHC]***	x	x		
5	*binapacryl (Interim)*		x		
6	2,3,4,5-Bis(2-butylene)tetrahydro-2-furaldehyde [Repellent-11]		x		
7	bromoxynil butyrate		x		
8	cadmium compounds		x		
9	calcium arsenate		x		
10	*captafol*	x	x		
11	carbofuran (granular only)			x	
12	carbon tetrachloride		x		
13	chloranil		x		
14	*chlordane*	x	x		
15	chlordecone (kepone)		x		
16	*chlordimeform*	x	x		
17	*chlorobenzilate*	x	x		
18	chloromethoxypropylmercuric acetate [CPMA]		x		
19	copper arsenate		x		
20	daminozide/alar			x	
21	DBCP		x		
22	*DDT*	x	x		
23	*dieldrin*	x	x		
24	*dinoseb*and salts	x	x		
25	Di(phenylmercury)dodecenylsuccinate [PMDS]		x		

26	*DNOC (Interim)*	x	x		
27	*1,2-dibromoethane ethylene dibromide - EDB)*	x	x		
28	*ethylene dichloride (EDC) (Interim)*		x		
29	*ethylene oxide (ETO) (Interim) agricultural uses only*			x	
30	endrin		x		
31	EPN		x		
32	ethyl hexyleneglycol [6-12]		x		
33	*fluoroacetamide*	x	x		
34	*heptachlor*	x		x	
35	*hexachlorobenzene [HCB]*	x	x		
36	lead arsenate		x		
37	leptophos		x		
38	*lindane*	x		x	
39	*mercury compounds* (mercurous chloride and mercuric chloride)	x	x		
40	*methamidophos*	x			x
41	*methyl parathion*	x			x
42	mevinphos		x		
43	mirex		x		
44	*monocrotophos*	x	x		
45	nitrofen (TOK)		x		
46	OMPA (octamethylpyrophosphoramide)		x		
47	*parathion*(ethyl)	x			x
48	*pentachlorophenol*	x		x	
49	phenylmercury acetate [PMA]		x		
50	phenylmercuric oleate [PMO]		x		x
51	*phosphamidon*	x			
52	potassium 2,4,5-trichlorophenate [2,4,5-TCP]		x		
53	pyriminil [Vacor]		x		
54	safrole		x		
55	silvex		x		
56	sodium arsenate			x	
57	sodium arsenite		x		
58	TDE		x		
59	Terpene polychlorinates [Strobane]		x		
60	thallium sulfate		x		

61	*toxaphene (chlorinated camphene) (Interim)*	x	x		
62	tributyltin compounds			x	
63	*2,4,5-Trichlorophenoxyacetic acid [2,4,5-T]*	x	x		
64	vinyl chloride		x		

* Pentachlorophenol is still registered for use in the U.S. as a wood preservative.

附件16：美國環保署使用的結構活性相關系統市售軟體網站資料

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Data is available for 382,048 records.
Toxicity
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(mg/kg or ppm)
Species: (any)
Route: (any)
Effect: (any)
Toxicity data is available for 139,354 records.
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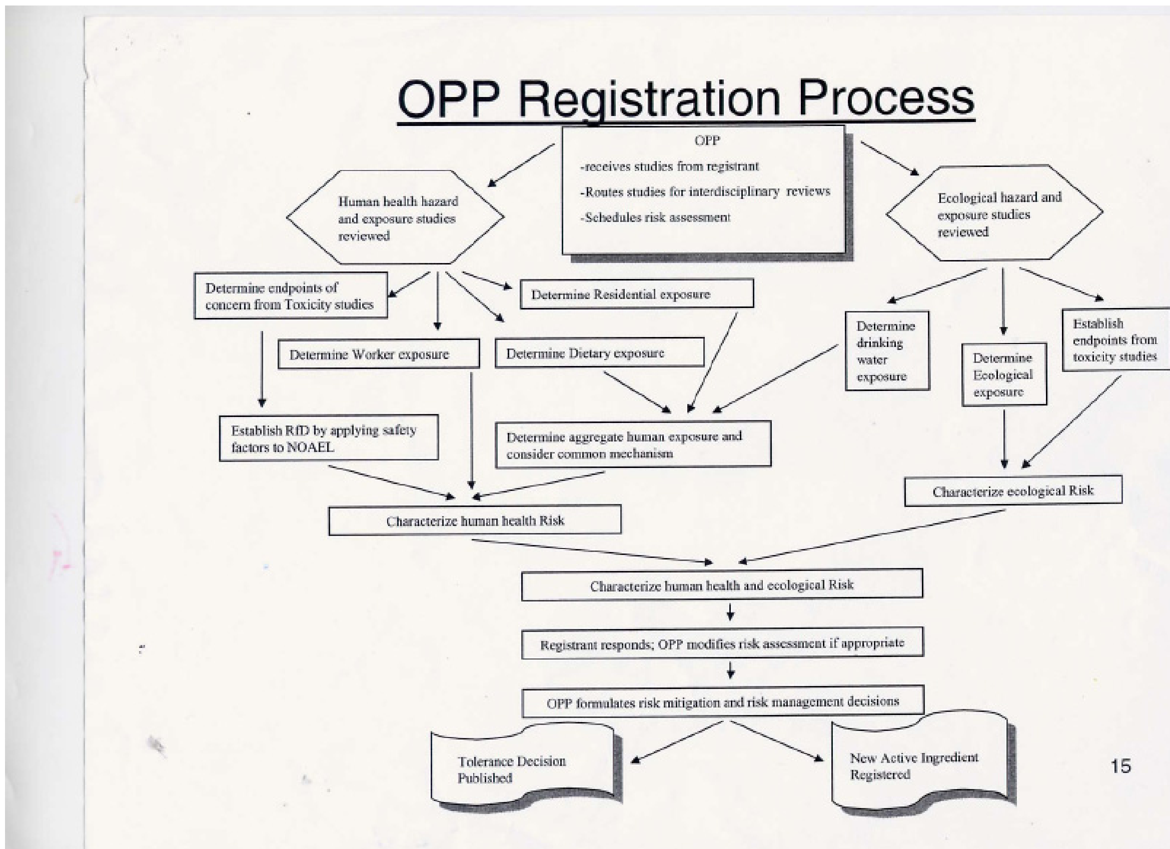
附件17：殺蟲劑註冊申請登記流程



附件18:美國環保署殺鼠劑風險減輕措施

Final Risk Mitigation Decision for Ten Rodenticides

Resources

- Rodenticides Reregistration Web page
- Rodenticides Background
- Controlling Rodents

Current as of May 28, 2008

After fully assessing human health and ecological effects, as well as benefits, EPA is announcing measures to reduce risks associated with ten rodenticides:

Brodifacoum
Bromadiolone

Bromethalin

Chlorophacinone

Cholecalciferol

Difenacoum

Difethialone

Diphacinone

Warfarin

Zinc phosphide

New safety measures announced by the U.S. Environmental Protection Agency will protect children from accidental exposure to rodent-control products. These measures will also reduce the risk of accidental poisonings of pets and wildlife. With the Agency's risk mitigation measures in place, rodenticide products will be safe, effective, and affordable for all consumers.

On this page:

Rodenticide Safety Concerns

Final Risk Mitigation Measures

Summary of New Restrictions

Proposed Mitigation Measures are Protective and Flexible

Integrated Pest Management Will Improve Effectiveness

More Information

Rodenticide Safety Concerns

Rodenticides are important products for controlling mice, rats and other rodents that pose threats to public health, critical habitats, native plants and animals, crops, and food supplies. However, these products also present human and environmental safety concerns.

Exposures to Children - Rodenticides are an important tool for public health pest control, including controlling mice and rats around the home; however, the use of these products has been associated with accidental exposures to thousands of children each year. Fortunately, only a small number of exposed children experience medical symptoms or suffer adverse health effects as a result of their exposure.

The Agency believes, however, that the number of exposure incidents is unacceptably high. Further, data indicate that children in low income families are disproportionately exposed. EPA's risk mitigation

measures address this situation by significantly reducing the likelihood of rodenticide exposure to children, including those children who may be disproportionately at risk for exposure.

Risks to Wildlife - Rodenticides pose significant risks to non-target wildlife including birds, such as hawks and owls, and mammals, including raccoons, squirrels, skunks, deer, coyotes, foxes, mountain lions, and bobcats. Rodenticides applied as bait products pose risks to wildlife from primary exposure (direct consumption of rodenticide bait) and secondary exposure (predators or scavengers consuming prey with rodenticides present in body tissues). Several reported incidents have involved Federally listed threatened and endangered species, for example the San Joaquin kit fox and Northern spotted owl, in addition to the Bald eagle, which is protected under the Bald and Golden Eagle Act.

Differences Among the Rodenticides - The ten rodenticide active ingredients covered by this action can be divided into three categories:

first-generation anticoagulants: warfarin, chlorophacinone, and diphacinone;
second-generation anticoagulants: brodifacoum, bromadiolone, difenacoum, and difethialone; and
non-anticoagulants: bromethalin, cholecalciferol and zinc phosphide.

The anticoagulants interfere with blood clotting, and death can result from excessive bleeding. Bromethalin is a nerve toxicant that causes respiratory distress. Cholecalciferol is vitamin D3, which in small dosages is needed for good health in most mammals, but in massive doses is toxic, especially to rodents. Zinc phosphide causes liberation of toxic phosphine gas in the stomach.

The second-generation anticoagulants are especially hazardous for several reasons. They are highly toxic, and they persist a long time in body tissues. The second-generation anticoagulants are designed to be toxic in a single feeding, but since time-to-death is several days, rodents can feed multiple times before death, leading to carcasses containing residues that may be many times the lethal dose. Predators or scavengers that feed on those poisoned rodents may consume enough to suffer harm.

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Final Risk Mitigation Measures

EPA's decision reduces rodenticide exposures to children and wildlife, while still allowing residential users, livestock producers, and professional applicators access to a variety of effective and affordable rodent control products.

Childrens' Risk Mitigation - To minimize children's exposure to rodenticide products used in homes, EPA is requiring that all rodenticide bait products available for sale to consumers be sold only in bait stations. Loose bait such as pellets will be prohibited as a bait form. A range of different types of bait stations will meet the new requirements, providing flexibility in cost.

Tiered Bait Station Requirements for Consumer-Use Products

Tier 1 – Tamper-resistant for children and dogs; weather resistant; tested according to EPA protocols; indoor and outdoor use;
Tier 2 – Tamper-resistant for children and dogs; tested according to EPA protocols; indoor use only;
Tier 3 – Tamper-resistant for children; tested according to EPA protocols; indoor use only; and,
Tier 4 – Self-certification; packaging not reasonably anticipated to release other than small quantities of bait; resistant to opening by a child less than six years old; indoor use only; non-refillable (one-time-use only).

Ecological Risk Mitigation - To reduce wildlife exposures and ecological risks, EPA will require sales and distribution and packaging restrictions for products containing four of the ten rodenticides that pose the greatest risk to wildlife (the second-generation anticoagulants – brodifacoum, bromadiolone, difenacoum, and difethialone) to prevent purchase on the consumer market.

Sale and Distribution Restrictions

The terms and conditions of registration for products containing brodifacoum, bromadiolone, difenacoum, and difethialone must be amended to specify that the registrants will control distribution of the products so that they shall only be distributed to or sold in agricultural, farm and tractor stores or directly to PCOs and other professional applicators, and that registrants will not sell or distribute products containing brodifacoum, bromadiolone, difenacoum, and difethialone in channels of trade likely to result in retail sale in hardware and home improvement stores, grocery stores, convenience stores, drug stores, club stores, big box stores, and other general retailers.

Minimum Package Size Requirements

The Agency is requiring second-generation anticoagulant bait products to be sold in packages that contain ≥ 8 pounds of bait for

products that are labeled for use only inside of and around agricultural buildings, and not for use in and around homes.
For second-generation anticoagulant bait products intended for use by professional applicators, the minimum permissible amount of bait per package is 16 pounds.

Use Site Restriction

For second-generation anticoagulant bait products in packages with at least 8 but not more than 16 pounds of bait, labels must state that products may only be used in and around agricultural buildings (e.g., barns, hen houses), and bear the statement "Do not use this product in homes or other human residences."

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Summary of New Restrictions

"Consumer Size" Products (Products containing ≤ 1 pound of bait)

May not contain brodifacoum, difethialone, bromadiolone, or difenacoum (the second-generation anticoagulants)
Loose bait forms such as pellets are prohibited
Each retail unit must include a pre-loaded bait station
Bait refills may be sold with pre-loaded bait stations in a single retail unit

Second-Generation Anticoagulant Products for Use Around Agricultural Buildings

Products must contain at least eight pounds of bait.
Bait stations are required for all outdoor, above-ground placements of second-generation anticoagulant products.
Bait stations are required indoors if exposure to children, pets, or non-target animals is possible.
Product labels must indicate that the product is for use only in and around agricultural buildings and that use in residential use sites is prohibited.
Distribution to and sales in "consumer" stores including grocery stores, drug stores, hardware stores, club stores will be prohibited.

Second-Generation Anticoagulant Products for Professional Applicators

Products must contain at least 16 pounds of bait.
Bait stations are required for all outdoor, above-ground placements of second-generation anticoagulants.
Bait stations are required indoors if exposure to children, pets, or non-target animals is possible.
Distribution to and sales in "consumer" stores including grocery stores, drug stores, hardware stores, club stores will be prohibited.

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Proposed Mitigation Measures are Protective and Flexible

In January 2007, to decrease the incidence of children's accidental exposures to rodenticides, EPA proposed a requirement that all rodenticides sold "over the counter" for residential use be available only in tamper-resistant bait stations. The proposal also included a requirement that the second-generation anticoagulants be classified for restricted use, to minimize impacts on non-target wildlife.

EPA's final rodenticide decision achieves the same goal of protection of children and wildlife. In response to comments concerning the costs of tamper-resistant bait stations to protect children and pets, the Agency adopted a tiered bait station system that allows for a variety of effective bait stations at a range of prices. Provisions are also being put into place to prevent the sale and distribution of the more highly toxic products on the consumer market, while maintaining their availability for agricultural production and pest control operators. EPA believes that these steps will significantly reduce the amount of product in the environment, providing additional protection for wildlife from poisonings by these more toxic and persistent products.

The Agency also evaluated and incorporated comments in its final decision from a wide range of stakeholders, and continues its discussions with several federal agencies, including the Centers for Disease Control, the the Department of Housing and Urban Development, the U.S. Department of Agriculture, and the U.S. Fish and Wildlife Service.

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Integrated Pest Management Will Improve Effectiveness

Integrated pest management (IPM), a multi-faceted approach to pest control, is essential for effective management of rodents in and around households. In most situations, mice and rats cannot be controlled using rodenticides alone. Effective rodent control also requires sanitation, rodent-proofing, and removal of rodent harborage. Without habitat modification to make an area less attractive to rodents, even eradication will not prevent new populations from recolonizing the area. Non-chemical devices such as snap traps are also affordable and effective methods for rodent control.

EPA is working in partnerships with the Centers for Disease Control and Prevention (CDC) and the Department of Housing and Urban Development (HUD) to promote IPM in low-income housing and other settings where pest pressures are significant.

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For More Information

EPA's Final Risk Mitigation Decision for Ten Rodenticides (May 28, 2008) and supporting documents are available in docket EPA-HQ-OPP-2006-0955 at Regulations.gov.

The Final Risk Mitigation Decision for Ten Rodenticides (May 28, 2008) (PDF) (60 pp, 2.8 MB, about PDF) is available from the docket in Regulations.gov.

The Controlling Rodents Web page provides information about preventing, identifying, and treating rodent infestations. It also addresses regulation of rodent-control products and safe pesticide use.

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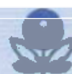

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