§ 4130.7-1 Payment of fees.

* * * * *

(e) Failure to pay the grazing bill on or before the date specified in the bill will result in a late fee assessment of $25.00 or 10 percent of the grazing bill, whichever is greater. Thirty days from the grazing bill due date will be allowed to make payment of the grazing bill and late fee. Failure to make payment within this 30 days may result in action by the authorized officer under § 4160.1–2.

22. Section 4140.1 is amended by reviewing paragraph (a)(3) to read:

§ 4140.1 Acts prohibited on public lands.

(a) * * *

(3) Placing supplemental feed on these lands without authorization.

* * * * *

23. Section 4150.3 is amended by revising the introductory paragraph and paragraphs (a), (b), and (c) to read as follows:

§ 4150.3 Settlement.

The authorized officer shall determine whether the violation is nonwillful, willful, or repeated willful. Where violations are repeated willful, the authorized officer may take action under § 4170.1–1 (a) or (b) of this title. The amount due for all settlements shall include the value of forage consumed as determined by paragraphs (a), (b), or (c) of this section. Settlement for willful and repeated willful violations shall also include the full value for all damages to the public lands and other property of the United States; and all reasonable expenses incurred by the United States in detecting, investigating, and resolving violations.

(a) For nonwillful violation: 5 times the public land grazing fee per AUM established annually by the Secretary of the Interior for each AUM consumed.

(b) For willful violations: 2 times the penalty per AUM established annually for a nonwillful violation for each AUM consumed.

(c) For repeated willful violations: 3 times the penalty per AUM established annually for a nonwillful violation for each AUM consumed.

* * * * *

24. A new § 41709.1–3 is revised to read as follows:

§ 4170.1-3 Bald Eagle Protection Act and Endangered Species Act.

Violation of the Bald Eagle Protection Act or the Endangered Species Act may result in penalty under § 4170.1–1 where:

(a) Public land administered by the Bureau of Land Management is involved or affected;

(b) Such violation is related to grazing use authorized by permit or lease;

(c) The permittee or lessee has been convicted or otherwise found to be in violation of the law or regulations by a court or by final determination of any agency charged with the administration of the law where no further appeals are outstanding.

J. Steven Griles,

Assistant Secretary of the Interior.

April 23, 1987.

[FR Doc. 87–11493 Filed 5–19–87; 8:45 am]

BILLING CODE 4310-84-M

federal register

Wednesday
May 20, 1987

Part IV

Office of Management and Budget

Cumulative Report on Rescissions and Deferrals; Notice

OFFICE OF MANAGEMENT AND BUDGET

Cumulative Report on Rescissions and Deferrals

May 1, 1987.

This report is submitted in fulfillment of the requirements of section 1014(e) of the Impoundment Control Act of 1974 (Pub. L. 93–344). Section 1014(e) provides for a monthly report listing all budget authority for this fiscal year for which, as of the first day of the month, a special message has been transmitted to the Congress.

This report gives the status as of May 1, 1987, of 57 deferrals contained in the five special messages of FY 1987. These messages were transmitted to the Congress on September 26, and December 15, 1986, and January 5 and 28, and March 4, 1987.

Rescissions (Table A and Attachment A)

As of May 1, 1987, there were no rescission proposals pending before the Congress.

Deferrals (Table B and Attachment B)

As of May 1, 1987, $6,660.8 million in 1987 budget authority was being deferred from obligation and $3.0 million in 1987 outlays was being deferred from expenditure. Attachment B shows the history and status of each deferral reported during FY 1987.

Information From Special Messages

The special message containing information on the deferrals covered by this cumulative report is printed in the **Federal Register** listed below:

Vol. 51, FR p. 35976, Tuesday, October 7, 1986
Vol. 51, FR p. 47356, Wednesday, December 31, 1986
Vol. 52, FR p. 964, Friday, January 9, 1987
Vol. 52, FR p. 3552, Wednesday, February 4, 1987
Vol. 52, FR p. 8046, Friday, March 13, 1987.

James C. Miller III,

Director.

BILLING CODE 3110-01-M

TABLE A

STATUS OF 1987 RESCISSIONS

	Amount (In millions of dollars)
Rescissions proposed by the President	$5,835.8
Accepted by the Congress	0
Rejected by the Congress	5,835.8
Pending before the Congress	0

****

TABLE B

STATUS OF 1987 DEFERRALS

	Amount (In millions of dollars)
Deferrals proposed by the President	11,457.6
Routine Executive releases through May 1, 1987 (OMB/Agency releases of $4,765.9 million and cumulative adjustments of $0.7 million)	-4,765.2
Overturned by the Congress	-28.6
Currently before the Congress	6,663.8 a/

a/ This amount includes $3.0 million in outlays for a Department of the Treasury deferral (D87-21).

Attachments

Attachment A - Status of Rescissions - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Rescission Number	Amount Previously Considered by Congress	Amount Currently before Congress	Date of Message	Amount Rescinded	Amount Made Available	Date Made Available	Congressional Action
DEPARTMENT OF AGRICULTURE								
Agricultural Research Service								
Buildings and facilities	R87-1 R87-1A	28,000		1-5-87 1-28-87		28,000	3-16-87	
Agricultural Stabilization and Conservation Service								
Rural clean water program	R87-2	6,000		1-5-87		6,000	3-16-87	
Agricultural conservation program	R87-3	164,356		1-5-87		164,356	3-16-87	
Water bank program	R87-4	8,166		1-5-87		8,166	3-16-87	
Emergency conservation program	R87-5	10,000		1-5-87		10,000	3-16-87	
Farmers Home Administration								
Rural water and waste disposal grants	R87-6	79,500		1-5-87		79,500	3-16-87	
Rural community fire protection grants	R87-7	2,300		1-5-87		2,300	3-16-87	
Rural housing for domestic farm labor	R87-8	7,400		1-5-87		7,400	3-16-87	
Mutual and self-help housing	R87-9	8,000		1-5-87		8,000	3-16-87	
Very low income housing repair grants	R87-10	9,400		1-5-87		9,400	3-16-87	
Compensation for construction defects	R87-11	500		1-5-87		500	3-16-87	
Rural housing preservation grants	R87-12	14,400		1-5-87		14,400	3-16-87	
Soil Conservation Service								
Watershed and flood prevention operations	R87-13	96,000		1-5-87		96,000	3-16-87	
Great Plains conservation program	R87-14	8,000		1-5-87		8,000	3-16-87	
Resource conservation and development	R87-15	5,000		1-5-87		5,000	3-16-87	
Forest Service								
Land acquisition	R87-16	49,030		1-5-87		49,030	3-16-87	
DEPARTMENT OF COMMERCE								
Economic Development Administration								
Economic development assistance programs	R87-17 R87-17A	169,718 -50		1-5-87 1-28-87		169,668	3-16-87	
International Trade Administration								
Operations and administration	R87-18	11,400		1-5-87		11,400	3-16-87	
National Oceanic and Atmospheric Administration								
Operations, research, and facilities	R87-19	58,857		1-5-87		58,857	3-16-87	
National Telecommunications and Information Administration								
Public telecommunications facilities, planning and construction	R87-20	19,300		1-5-87		19,300	3-16-87	

Attachment A - Status of Rescissions - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Rescission Number	Amount Previously Considered by Congress	Amount Currently before Congress	Date of Message	Amount Rescinded	Amount Made Available	Date Made Available	Congressional Action
DEPARTMENT OF DEFENSE - MILITARY								
Procurement								
Procurement of weapons and tracked combat vehicles, Army	R87-21	15,000		1-5-87		15,000	3-16-87	
Other procurement, Navy	R87-22	116,000		1-5-87		116,000	3-16-87	
Military Construction								
Military construction, Air Force	R87-23	2,750		1-5-87		2,750	3-16-87	
DEPARTMENT OF DEFENSE - CIVIL								
Corps of Engineers - Civil								
Construction, general	R87-24	7,715		1-5-87		7,715	3-16-87	
DEPARTMENT OF EDUCATION								
Office of Elementary and Secondary Education								
Compensatory education for the disadvantaged	R87-25	7,500		1-5-87		7,500	3-16-87	
Impact aid	R87-26	17,500		1-5-87		17,500	3-16-87	
Special programs	R87-27	54,980		1-5-87		54,980	3-16-87	
Office of Bilingual Education and Minority Languages Affairs								
Bilingual education	R87-28	45,886		1-5-87		45,886	3-16-87	
Office of Special Education and Rehabilitative Services								
Education for the handicapped	R87-29	288,659		1-5-87		288,659	3-16-87	
Rehabilitation services and handicapped research	R87-30	127,455		1-5-87		127,455	3-16-87	
Office of Vocational and Adult Education								
Vocational and adult education	R87-31	432,319		1-5-87		432,319	3-16-87	
Office of Postsecondary Education								
Student financial assistance	R87-32	1,269,000		1-5-87		1,269,000	3-16-87	
Higher education	R87-33	203,050		1-5-87		203,050	3-16-87	
	R87-33A			1-28-87				
Office of Educational Research and Improvement								
Libraries	R87-34	34,500		1-5-87		34,500	3-16-87	

Attachment A - Status of Rescissions - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Rescission Number	Amount Previously Considered by Congress	Amount Currently before Congress	Date of Message	Amount Rescinded	Amount Made Available	Date Made Available	Congressional Action
DEPARTMENT OF ENERGY								
Energy Programs								
Energy supply, research and development activities	R87-35	81,800		1-5-87		81,800	3-16-87	
Fossil energy research and development	R87-36	44,464		1-5-87		44,464	3-16-87	
Energy conservation	R87-37	87,433		1-5-87				
	R87-37A	-3500		1-28-87		83,933	3-16-87	
DEPARTMENT OF HEALTH AND HUMAN SERVICES								
Food and Drug Administration								
Buildings and facilities	R87-38	500		1-5-87		500	3-16-87	
Health Resources and Services Administration								
Health resources and services	R87-39	161,210		1-5-87		161,210	3-16-87	
	R87-39A			1-28-87				
Indian health facilities	R87-40	57,100		1-5-87		57,100	3-16-87	
	R87-40A			1-28-87				
National Institutes of Health								
National Library of Medicine	R87-41	5,405		1-5-87		5,405	3-16-87	
Office of the Assistant Secretary of Health								
Public health service management	R87-42	5,000		1-5-87		5,000	3-16-87	
Departmental Management								
Policy research	R87-43	2,200		1-5-87		2,200	3-16-87	
DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT								
Housing Programs								
Annual contributions for assisted housing	R87-44	473,313		1-5-87		473,313	3-16-87	
Housing counseling assistance	R87-45	3,500		1-5-87		3,500	3-16-87	
Community Planning and Development								
Community development grants	R87-46	375,200		1-5-87		375,200	3-16-87	
Urban development action grants	R87-47	237,500		1-5-87		237,500	3-16-87	
Management and Administration								
Salaries and expenses	R87-48	19,042		1-5-87		19,042	3-16-87	

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Rescission Number	Amount Previously Considered by Congress	Amount Currently before Congress	Date of Message	Amount Rescinded	Amount Made Available	Date Made Available	Congressional Action
DEPARTMENT OF THE INTERIOR								
Bureau of Land Management								
Management of lands and resources	R87-49	6,500		1-5-87		6,500	3-16-87	
Construction and access	R87-50	1,600		1-5-87		1,600	3-16-87	
Land acquisition	R87-51	2,700		1-5-87		2,700	3-16-87	
Bureau of Mines								
Mines and minerals	R87-52	16,594		1-5-87		16,594	3-16-87	
United States Fish and Wildlife Service								
Resource management	R87-53	20,500		1-5-87		20,500	3-16-87	
	R87-53A			1-28-87				
Construction	R87-54	23,200		1-5-87		23,200	3-16-87	
Land acquisition	R87-55	26,762		1-5-87		26,762	3-16-87	
National Park Service								
Operation of the national park system	R87-56	7,950		1-5-87		7,950	3-16-87	
Construction	R87-57	58,981		1-5-87		58,981	3-16-87	
Land acquisition	R87-58	97,638		1-5-87		97,638	3-16-87	
Historic preservation fund	R87-59	15,000		1-5-87		15,000	3-16-87	
Bureau of Indian Affairs								
Construction	R87-60	22,811		1-5-87		22,811	3-16-87	
Territorial and International Affairs								
Administration of territories	R87-61	2,500		1-5-87		2,500	3-16-87	
DEPARTMENT OF JUSTICE								
Immigration and Naturalization Service								
Salaries and expenses	R87-62	24,598		1-5-87		24,598	3-16-87	
DEPARTMENT OF LABOR								
Employment and Training Administration								
Training and employment services	R87-63	332,000		1-5-87		332,000	3-16-87	

Attachment A - Status of Rescissions - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Rescission Number	Amount Previously Considered by Congress	Amount Currently before Congress	Date of Message	Amount Rescinded	Amount Made Available	Date Made Available	Congressional Action
DEPARTMENT OF THE TREASURY								
Federal Law Enforcement Training Center Salaries and expenses.....	R87-64	8,450		1-5-87		8,450	3-16-87	
Bureau of Alcohol, Tobacco, and Firearms Salaries and expenses.....	R87-65	15,000		1-5-87		15,000	3-16-87	
United States Customs Service Salaries and expenses.....	R87-66	38,945		1-5-87		38,945	3-16-87	
ENVIRONMENTAL PROTECTION AGENCY								
Abatement, control, and compliance.....	R87-67	47,500		1-5-87		47,500	3-16-87	
Buildings and facilities.....	R87-68	2,500		1-5-87		2,500	3-16-87	
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION								
Research and development.....	R87-69	25,796		1-5-87		25,796	3-16-87	
VETERANS ADMINISTRATION								
Medical care.....	R87-70	75,000		1-5-87		(See Note Below)		
OTHER INDEPENDENT AGENCIES								
Appalachian Regional Commission Appalachian regional development programs	R87-71	31,059		1-5-87		31,059	3-16-87	
National Endowment for the Humanities National capital arts and cultural affairs	R87-72	4,000		1-5-87		4,000	3-16-87	
Selective Service System Salaries and expenses.....	R87-73	409		1-5-87		409	3-17-87	
Total, rescissions.....		5,835,751	0			5,760,751		

NOTE. - The $75 million proposed for rescission in Rescission Proposal No. R87-70 was never withheld from obligation. Therefore, there was no need to release the funds on March 16.

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Deferral Number	Amount Transmitted Original Request	Amount Transmitted Subsequent Change	Date of Message	Cumulative OMB/Agency Releases	Congressionally Required Releases	Congressional Action	Cumulative Adjustments	Amount Deferred as of 5-1-87
FUNDS APPROPRIATED TO THE PRESIDENT									
International Security Assistance									
Foreign military sales credit	D87-22	4,040,441		12-15-86	1,855,000				2,185,441
Economic support fund	D87-1	95,000		9-26-86					
	D87-1A		2,351,470	12-15-86	1,413,633				1,032,837
Military assistance	D87-23	847,000		12-15-86	381,750				465,250
International military education and training	D87-24	2,000		12-15-86	2,000				0
Agency for International Development									
Functional development assistance	D87-32	2,278		1-28-87					2,278
International disaster assistance	D87-25	57,000		12-15-86	47,566				9,434
Special Assistance for Central America									
Assistance for the Nicaraguan Democratic Resistance	D87-26	60,000		12-15-86	60,000				0
Promotion of stability and security in Central America	D87-27	1,000		12-15-86					1,000
DEPARTMENT OF AGRICULTURE									
Commodity Credit Corporation									
Temporary emergency food assistance	D87-33	28,559		1-28-87		28,559	P.L. 100-6		0
Rural Electrification Administration									
Reimbursement to the Rural electrification and telephone and revolving fund for interest subsidies and losses	D87-34	20,000		1-28-87					20,000
Forest Service									
State and private forestry	D87-35	797		1-28-87					797
Land acquisition	D87-36	27,070		1-28-87					27,070
Expenses, brush disposal	D87-2	111,202		9-26-86					
	D87-2A		1,534	3-4-87					112,736
Timber roads, purchaser election	D87-37	11,900		1-28-87					11,900
Timber salvage sales	D87-3	29,731		9-26-86	6,113			3	23,621
Cooperative work	D87-4	526,938		9-26-86					
	D87-4A		8,336	3-4-87					535,275
Gifts, donations, and bequests for forest and rangeland research	D87-5	200		9-26-86	25				175

Attachment B - Status of Deferrals - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Deferral Number	Amount Transmitted Original Request	Amount Transmitted Subsequent Change	Date of Message	Cumulative OMB/Agency Releases	Congressionally Required Releases	Congressional Action	Cumulative Adjustments	Amount Deferred as of 5-1-87
DEPARTMENT OF DEFENSE - MILITARY									
Military Construction									
Military construction, Defense	D87-6	2,350		9-26-86					
	D87-6A		1,316,152	12-15-86	779,649				538,853
Family Housing									
Family housing, Defense	D87-7	76,943		9-26-86					
	D87-7A		190,022	12-15-86	121,758				145,207
DEPARTMENT OF DEFENSE - CIVIL									
Soldiers' and Airmen's Home									
Capital outlays	D87-38	1,132		1-28-87					1,132
Wildlife Conservation, Military Reservations									
Wildlife conservation	D87-8	1,065		9-26-86					
	D87-8A		25	1-5-87					
	D87-8B		46	3-4-87	40				1,096
DEPARTMENT OF ENERGY									
Power Marketing Administration									
Alaska Power Administration, Operation and maintenance	D87-9	165		9-26-86					165
Southwestern Power Administration, Operation and maintenance	D87-10	7,554		9-26-86					
	D87-10A		6,106	1-5-87					13,660
Western Area Power Administration, Construction, rehabilitation, operation and maintenance	D87-29	4,485		1-5-87					4,485
Departmental Administration									
Departmental administration	D87-30	24,182		1-5-87					24,182

Attachment B - Status of Deferrals - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Deferral Number	Amount Transmitted Original Request	Amount Transmitted Subsequent Change	Date of Message	Cumulative OMB/Agency Releases	Congressionally Required Releases	Congressional Action	Cumulative Adjustments	Amount Deferred as of 5-1-87
DEPARTMENT OF HEALTH AND HUMAN SERVICES									
Health Resources and Services Administration									
Indian catastrophic health emergency fund..	D87-28	10,000		12-15-86					10,000
Centers for Disease Control									
Disease control, research, and training....	D87-39	2,428		1-28-87					2,428
Alcohol, Drug Abuse, and Mental Health Administration									
Alcohol, drug abuse, and mental health...	D87-40	10,000		1-28-87					10,000
Office of Assistant Secretary for Health									
Scientific activities overseas (special foreign currency program).....	D87-11	2,900		9-26-86					2,900
Social Security Administration									
Limitation on administrative expenses (construction).....	D87-12	7,073		9-26-86					
	D87-12A		89	1-28-87		12			7,151
Limitation on administrative expenses (information technology systems).....	D87-57	134,437		3-4-87					134,437
DEPARTMENT OF THE INTERIOR									
Bureau of Land Management									
Payments for proceeds, sale of Mineral Leasing Act of 1920, Section 40(d).....	D87-31	49		1-5-87					49
DEPARTMENT OF JUSTICE									
Office of Justice Programs									
Crime victims fund.....	D87-13	70,000		9-26-86					70,000
DEPARTMENT OF LABOR									
Employment Standards Administration									
Salaries and expenses.....	D87-41	9,659		1-28-87					9,659

Attachment B - Status of Deferrals - Fiscal Year 1987

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Deferral Number	Amount Transmitted Original Request	Amount Transmitted Subsequent Change	Date of Message	Cumulative OMB/Agency Releases	Congressionally Required Releases	Congressional Action	Cumulative Adjustments	Amount Deferred as of 5-1-87
DEPARTMENT OF STATE									
Bureau for Refugee Programs									
United States emergency refugee and migration assistance fund, executive.....	D87-14	6,100		9-26-86					
	D87-14A		14,000	1-5-87					20,100
Other									
Assistance for implementation of a Contadora agreement.....	D87-15	2,000		9-26-86	2,000				0
DEPARTMENT OF TRANSPORTATION									
Federal Railroad Administration									
Rail service assistance.....	D87-42	462		1-28-87					462
Railroad safety.....	D87-43	1,101		1-28-87	1,101				0
Conrail labor protection.....	D87-44	646		1-28-87					646
Northeast corridor improvement program.....	D87-45	16,962		1-28-87					16,962
Conrail commuter transition assistance.....	D87-46	10,000		1-28-87					10,000
Urban Mass Transportation Administration									
Research, training and human resources.....	D87-47	4,336		1-28-87					4,336
Interstate transfer grants - transit.....	D87-48	51,800		1-28-87					51,800
Federal Aviation Administration									
Operation and maintenance, Metropolitan Washington Airports.....	D87-49	12,214		1-28-87					12,214
Facilities and equipment (Airport and airway trust fund).....	D87-16	803,877		9-26-86					
	D87-16A		295,611	12-15-86	19,996				1,079,492
Coast Guard									
Research, development, test, and evaluation.....	D87-50	5,000		1-28-87					5,000
Offshore oil pollution compensation fund...	D87-51	2,154		1-28-87					2,154
Deepwater port liability fund.....	D87-52	5,176		1-28-87					5,176
Office of the Secretary									
Payments to air carriers.....	D87-53	10,748		1-28-87					10,748

As of May 1, 1987 Amounts in Thousands of Dollars Agency/Bureau/Account	Deferral Number	Amount Transmitted Original Request	Amount Transmitted Subsequent Change	Date of Message	Cumulative OMB/Agency Releases	Congressionally Required Releases	Congressional Action	Cumulative Adjustments	Amount Deferred as of 5-1-87
DEPARTMENT OF THE TREASURY									
Office of Revenue Sharing									
Local government fiscal assistance trust fund	D87-17	74,149		9-26-86	71,144				3,005
Local government fiscal assistance trust fund	D87-21	5,981		9-26-86	3,714			723	2,990
ENVIRONMENTAL PROTECTION AGENCY									
Research and development	D87-54	11,000		1-28-87					11,000
Abatement, control, and compliance	D87-55	11,400		1-28-87					11,400
OTHER INDEPENDENT AGENCIES									
Commission on the Ukraine Famine Salaries and expenses	D87-18	100		9-26-86					100
Office of the Federal Inspector for the Alaska Natural Gas Transportation System, Salaries and expenses	D87-19	411		9-26-86	411				0
Pennsylvania Avenue Development Corporation Land acquisition and development fund	D87-20	11,873		9-26-86					11,873
United States Railway Association Administrative expenses	D87-56	1,155		1-28-87					1,155
TOTAL, DEFERRALS		7,274,185	4,183,392		4,765,912	28,559		726	6,663,832

Note: All of the above amounts represent budget authority except the Local Government Fiscal Assistance Trust Fund (D87-21) of outlays only.

[FR Doc. 87-11552 Filed 5-19-87; 8:45 am]
BILLING CODE 3110-01-C

federal register

Wednesday
May 20, 1987

Part V

Environmental Protection Agency

40 CFR Part 795 et al.

Toxic Substances; Testing Requirements; Final Rules and Proposed Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 796, 797, and 798

[OPTS-42079A; FRL 3202-1]

Revision of TSCA Test Guidelines

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This final rule amends the Toxic Substances Control Act (TSCA) test guidelines to provide more explicit guidance on the necessary minimum elements for each study.

DATE: This rule shall become effective on June 19, 1987.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Environmental Protection Agency, Rm. E-543, 401 M St., SW., Washington, DC 20460, (202-554-1404).

SUPPLEMENTARY INFORMATION: EPA is revising certain provisions in 40 CFR Parts 796, 797 and 798 of the TSCA test guidelines.

I. Background

Section 4(b)(1) of TSCA requires that test rules include standards for the development of test data. In the **Federal Register** of September 27, 1985 (50 FR 39252), EPA issued guidelines to be used in developing test standards in TSCA section 4 test rules. The TSCA guidelines, which are at Parts 796 (chemical fate), 797 (environmental effects), and 798 (health effects) of Part 40 of the Code of Federal Regulations (CFR), had been previously prepared for publication by EPA as OTS test guidelines or were published as Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals. The TSCA guidelines were developed to serve as a consensus document, reflecting expert scientific opinion, subject to peer and public review. They present generally formulated procedures for laboratory testing of an effect or characteristic deemed important for the evaluation of health and environmental hazards of a chemical. It is the intent of the Agency that when these guidelines are promulgated as standards in chemical-specific test rules they will become mandatory and enforceable.

The Agency reviewed the TSCA test guidelines of September 27, 1985, and found that certain of the suggested or recommended elements of these guidelines were often made minimum requirements for individual test rules. As a result, EPA proposed in the **Federal Register** of January 14, 1986 (51 FR 1522), that these elements be incorporated into the guidelines on a generic basis. The Agency believed that these revised guidelines would provide more explicit guidance on the minimum requirements for each study and would avoid repetitive chemical-by-chemical changes to the guidelines in their adoption as test standards for chemical-specific test rules under TSCA.

Elements for which the Agency proposed revisions pertained to minimum requirements for test procedures, analytical measurements, test conditions, animal selection (number of species to be tested, age of animals, sex, number of animals per dose level), use of controls, number of dose levels, facilities (test apparatus, dilution water, test substance delivery system, test parameters), observation period, administration of the substance, observation of animals, clinical exams, gross necropsy, histopathology, and reporting of data.

II. Provisions of Final Rule

The codified portion of this rule indicates the specific sections and paragraphs where changes in the TSCA test guidelines in 40 CFR Parts 796, 797, and 798 are made. An annotated copy of the TSCA test guidelines marking these changes is in the docket for this rulemaking. The guidelines for which revisions are made in this document are the following:

§ 796.1550 Partition coefficient (n-octanol/water).
§ 796.1840 Water solubility.
§ 796.1860 Water solubility (generator column method).
§ 796.2750 Sediment and soil adsorption isotherm.
§ 796.3100 Aerobic aquatic biodegradation.
§ 796.3140 Anaerobic biodegradability of organic chemicals.
§ 797.1050 Algal acute toxicity test.
§ 797.1300 Daphnid acute toxicity test.
§ 797.1330 Daphnid chronic toxicity test.
§ 797.1400 Fish acute toxicity test.
§ 797.1520 Fish bioconcentration test.
§ 797.1600 Fish early life stage toxicity test.
§ 797.1800 Oyster acute toxicity test.
§ 797.1830 Oyster bioconcentration test.
§ 797.1930 Mysid shrimp acute toxicity test.
§ 797.1950 Mysid shrimp chronic toxicity test.
§ 797.2150 Mallard reproduction test.
§ 798.2250 Dermal toxicity.
§ 798.2450 Inhalation toxicity.
§ 798.2650 Oral toxicity.
§ 798.3300 Oncogenicity.
§ 798.4350 Inhalation developmental toxicity.
§ 798.4420 Preliminary developmental toxicity screen.
§ 798.4700 Reproduction and fertility effects.
§ 798.4900 Developmental toxicity study.
§ 798.5200 Mouse visible specific locus test.
§ 798.5265 *Salmonella typhimurium* reverse mutation assay.
§ 798.5275 Sex-linked recessive lethal test in *Drosophila melanogaster.*
§ 798.5300 Detection of gene mutations in somatic cells in culture.
§ 798.5375 *In vivo* mammalian cytogenetics.
§ 798.5385 *In vivo* mammalian bone marrow cytogenetics tests: Chromosomal analysis.
§ 798.5395 *In vivo* mammalian bone marrow cytogenetics test: Micronucleus assay.
§ 798.5450 Rodent dominant lethal assay.
§ 798.5460 Rodent heritable translocation assays.
§ 798.6050 Functional observational battery.
§ 798.6200 Motor activity.
§ 798.6400 Neuropathology.

To conserve publication costs, EPA is not including a detailed discussion of public comments on the proposal in this preamble. EPA considered the public comments made by the 17 commenters and modified the proposal when warranted. A summary of the comments with the EPA response to these comments is contained in a separate document, which has been placed in the public record and which may be inspected at EPA in Rm. NE-G004, 401 M St., SW., Washington, DC 20460, from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays. Copies of the response to public comments may be obtained by writing the Freedom of Information Officer, U.S. Environmental Protection Agency, Mail Stop (A-101), 401 M St., SW., Washington, DC 20460.

Several comments addressed issues not raised in the January 14, 1986 proposal. These comments will be retained for consideration in the update of the guidelines.

Codification of these guidelines does not impose any regulatory obligation on any person who may be subject to a test rule under section 4 of TSCA. Specific guidelines will not become mandatory test standards until they are promulgated as such in individual section 4 rulemakings. When promulgated in such test rules, the relevent TSCA test guidelines will

become test standards for only that particular section 4 rule and will not serve as generic test standards. EPA may propose modifications to the various guidelines as they are utilized for chemical-specific test rules. In each chemical-specific rule, the proposed test standards and any modifications will be subject to public comment.

III. Rulemaking Record

EPA has established a record for this rulemaking [Docket number OPTS-42079], which is available for inspection in Rm. NE-G004 at 401 M St., SW., Washington, DC 20460 from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.

The record includes information EPA considered in developing this rule. The record includes:

1. **Federal Register** documents pertaining to this rule.
2. Public comments.
3. The document responding to public comments.

List of Subjects in 40 CFR Parts 796, 797, and 798

Chemicals, Chemical fate, Environmental effects, Environmental protection, Health effects, Testing.

Dated: May 4, 1987.

John A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, 40 CFR Chapter I is amended as follows:

PART 796—[AMENDED]

I. In Part 796:

1. The authority citation for Part 796 continues to read as follows:

Authority: 15 U.S.C. 2603.

2. The section heading "796.3100 Anaerobic Aquatic Biodegradation" in the Part 796 table of contents is revised to read "796.3100 Aerobic aquatic biodegradation."

§ 796.1550 [Amended]

3. Section 796.1550 *Partition coefficient (n-octanol/water)* is amended in paragraph (b) as follows:

a. By removing the phrase "it is recommended that" or "it is extremely important that" or "it is important that" and changing the word "be" to "shall be" wherever they appear in paragraphs (b)(1) (ii), (iii) first sentence, (iv), (vi), (ix)(A) second sentence, and (xi), and (b)(2)(i)(A) except for the first and fourth sentences.

b. By changing the word "should" to "shall" wherever it appears in paragraph (b)(3)(vii) and by revising paragraph (b)(1)(vii), to read as follows:

(vii) *Chemical analysis of the octanol and water phases.* In determining the K_{ow} value for any given solute, both the octanol and water phases shall be analyzed unless analysis of the octanol phase indicates a $\log_{10} K_{ow} > 6$ for the chemical. An analytical method shall be selected that is most applicable to the analysis of the specific chemical. Chromatographic methods are preferable because of their compound specificity in analyzing the parent chemical without interference from impurities. Whenever practicable, the chosen analytical method shall have a precision with ± 5 percent.

c. By changing the word "can" to "shall" wherever it appears in paragraphs (b)(2)(i) (A) and (C) and by revising paragraph (b)(1)(viii) to read as follows:

(viii) *Emulsion and ultracentrifugation.* Gentle shaking shall be used to minimize the formation of emulsions. Ultracentrifugation is necessary to separate troublesome emulsions and to separate the octanol and water phases unless turbidometric measurements are used to demonstrate that the emulsion is broken. Ultracentrifugation shall be carried out at 25 °C for 20 minutes in a temperature-controlled ultracentrifuge. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases shall be determined by trial-and-error experimentation.

d. By changing the word "can" to "shall" in certain sentences in paragraph (b)(3)(iv), which is revised to read as follows:

(iv) Centrifuge the samples at 25 °C for 20 minutes to break any emulsion and to separate the octanol and water phases. Evidence for breaking the emulsion and separation of the water and octanol phases can be obtained using a turbidimeter. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases shall be determined by trial-and-error experimentation.

e. By revising paragraph (b)(3)(ix) to read as follows:

(ix) For materials that reversibly ionize or protonate, determine K_{ow} at pH 5.0, 7.0, and 9.0 as described in paragraph (b)(1)(x) of this section. Follow the steps in paragraph (b)(1) (i) through (vii) of this section using the buffered aqueous solutions described in paragraph (b)(2)(i)(B) of this section. Using the acid dissociation constant and the concentration of the chemical in the aqueous phase [C_{water}], the term [ALPHA] can be calculated. The concentration of undissociated chemical can be determined from [ALPHA] and C_{water}.

f. By removing the phrase "It is extremely important that" and "it is recommended that", and changing the word "be" to "shall be" and "can" to "shall" and changing the number "0.0" to "9.0", wherever they appear in paragraph (b)(1)(x)(D) which is revised to read as follows:

(D) If a molecule dissociates or associates in octanol and water, then equation 1 under paragraph (a)(2)(i) of this section shall be modified to take into account such speciation changes as ionization, aggregation, and hydration. For the special case where no association takes place in water, equation 3 under paragraph (a)(2)(ii) of this section shall be used. For chemicals that reversibly ionize or protonate (e.g., carboxylic acids, phenols, or anilines), use equation 3 under paragraph (a)(2)(ii) of this section with water buffered at pH 5.0, 7.0, 9.0. Buffers described in paragraph (b)(2)(i)(B) of this section shall be used.

§ 796.1840 [Amended]

4. Section 796.1840 *Water solubility* is amended as follows:

a. Paragraph (b) is amended as follows:

i. By removing the phrase "it is recommended that" or "it is important that" and changing the word "be" to "shall be" wherever they appear in paragraphs (b)(1) (iii), (v), (vi)(A) first and third sentences, and (vii) first sentence and (b)(2)(ii).

ii. By changing the word "should" to "shall" wherever it appears in paragraphs (b)(1)(ix) and (b)(3)(i)(B).

iii. By changing the word "will" to "shall" wherever it appears in paragraph (b)(3)(ii)(B).

iv. By removing the phrase "it is extremely important that" and adding the word "shall" in paragraph (b)(1)(vi)(B), which is revised to read as follows:

(B) Centrifugation shall be carried out at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25 °C until concentration changes are small. For hydrophobic compounds (solubility <10 ppm), the acceleration G values shall differ by 10,000 G and include a determination of 39,000 or higher.

v. By changing the word "should" to "shall" in certain sentences in paragraph (b)(3)(i)(A), which is revised to read as follows:

(A) Dissolve a sufficient amount of the solid compound in suitable volatile organic solvent and coat on the walls of

a vessel. Viscous liquids may be coated on vessels in a similar fashion: Nonviscous liquids do not require solvents. Remove the solvent under reduced pressure or with a pure nitrogen gas stream. When all the solvent is removed, add reagent-grade water or, for compounds which reversibly ionize or protonate, the appropriate buffer solution and slowly stir or agitate the mixture under temperature control. Mixing may be accomplished by use of a Teflon-coated stirring bar and shall be continued for a minimum of 24 hours before aliquots are withdrawn. Prior to taking aliquots, the mixture shall be left to stand at constant temperature for at least 1 hour to permit separation of any small particles. To determine the concentration of the compound in the aqueous phase, aliquots shall be centrifuged at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25 °C until concentration changes are small. The concentration value so obtained is plotted against the time of mixing. At a later time, aliquots are again taken and analyzed in the same fashion to produce another data point on a concentration versus time plot. When the concentration reaches a plateau, equilibrium is assumed. For hydrophobic compounds (solubility <10 ppm) it is extremely important that the acceleration (G) values differ by 10,000 G and include a determination at 39,000 G or higher.

§ 796.1860 [Amended]

5. Section 796.1860 *Water solubility (generator column method)* is amended as follows:

a. Paragraph (b) is amended as follows:

i. By changing the word "are" to "shall be" wherever it appears in paragraph (b)(1)(i)(A).

ii. By changing the words "is recommended" to "shall be used" wherever they appear in paragraph (b)(1)(ii).

iii. By revising paragraph (b)(1)(iii) to read as follows:

(iii) *Purity of solvents.* It is important that all solvents used in this method be reagent grade or better. Solvents shall contain no impurities which could interfere with the determination of the test compound.

iv. By changing the word "should" to "shall" wherever it appears in paragraph (b)(1)(iv) and by revising paragraph (b)(1)(v) to read as follows:

(v) *Effect of pH on solubility.* For chemicals that reversibly ionize or protonate with a pK_a or pK_b between 3 and 11, experiments shall be performed at pH 5.0, 7.0, and 9.0 using appropriate buffers.

v. By removing the phrase "It is important that" and by changing the words "be dissolved" to "shall be dissolved" wherever they appear in paragraph (b)(2)(ii).

§ 796.2750 [Amended]

6. Section 796.2750 *Sediment and soil adsorption isotherm* is amended in paragraph (b) as follows:

a. The proposed change to paragraph (b)(1)(i)(A) is not being made based on comments received.

b. By changing the words "that are" to "shall be" wherever they appear in paragraph (b)(1)(i)(B).

c. By removing the phrase "It is recommended that" and by changing the word "be" to "shall be" wherever they appear in paragraphs (b)(1) (ii), (iii), and (vii) and by revising paragraph (b)(1)(vi) to read as follows:

(vi) *Solid/solution ratio.* The solid/solution ratio shall be equal to or greater than 1/10. If possible, the ratios should be equal to or greater than 1/5. The sediment or soil dry weight after drying for a 24-hour minimum at 90 °C is recommended for use as the weight of the solid for ratio and data calculations. If an insufficient amount of chemical remains in the water phase for quantification, the solid/solution ratio should be adjusted so that measurable amounts of the test chemical remain in solution.

d. By changing the phrase "It is extremely important that these" to "The following" and by changing the word "be" to "shall be" wherever they appear in paragraphs (b)(1) (iv) and (v) introductory text.

e. By revising paragraph (b)(1)(x), to read as follows:

(x) *Solvents for extraction.* It is important that the solvent used to extract the chemical from the sediment or soil is reagent grade or better. Solvents shall contain no impurities which could interfere with the determination of the test compound.

§ 796.3100 [Amended]

7. Section 796.3100 *Aerobic aquatic biodegradation* is amended as follows:

a. By changing the word "should" to "shall" wherever it appears in paragraphs (a) (4) and (6), (b)(1)(iii) and (2)(ii), and (c)(1)(iv) and (2)(i).

b. By changing the word "should" to "shall" in certain sentences in paragraph (b)(2)(vi), which is revised to read as follows:

(vi) Flasks shall be incubated in the dark to minimize both photochemical reactions and algal growth. Appropriate sterile controls or controls containing a metabolic inhibitor, such as 50 mg/l $HgCl_2$, are needed to correct for interferences due to nonbiological degradation. With volatile organic materials, sparging with CO_2-free air is performed only once, just prior to addition of the test chemical. Analyses for CO_2 evolution and DOC removal are conducted within 2 to 3 hours of sampling to minimize interferences which may occur in storage. All glassware should be free of organic carbon contaminants.

c. By changing the words "in the range of 80 to 100 percent" to "of at least 60 percent" wherever they appear in paragraph (c)(1)(v).

§ 796.3140 [Amended]

8. Section 796.3140 *Anaerobic biodegradability of organic chemicals* is amended as follows:

a. Paragraph (a) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (a) (3) and (5).

b. Paragraph (b) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (b)(1)(iii)(C), (2)(iii)(B) and (G), and (3)(i).

c. Paragraph (c)(2) is amended by adding an introductory phrase, as follows:

(2) *Test report.* The following shall be reported:

PART 797—[AMENDED]

II. In Part 797:

1. The authority citation for Part 797 continues to read as follows:

Authority: 15 U.S.C. 2603.

§ 797.1050 [Amended]

2. Section 797.1050 *Algal acute toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4)(iii) and (v) and (6)(i)(A) and by revising paragraph (c)(4)(v)(B) introductory text to read as follows:

(B) In test concentrations where growth is maximally inhibited, algistatic effects may be differentiated from algicidal effects by the following two methods for *Skeletonema* and by the second method for *Selenastrum.*

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii), which is revised to read as follows:

(ii) Algae should be exposed to five or more concentrations of the test chemical in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, and 64 mg/l). Algae shall be placed in a

minimum of three replicate test containers for each concentration of test chemical and control. More than three replicates may be required to provide sufficient quantities of test solution for determination of test substance concentration at the end of the test. Each test chamber should contain equal volumes of test solution and approximately 1×10^4 *Selenastrum* cells/ml or 7.7×10^4 *Skeletonema* cells/ml of test solution. The chemical concentrations should result in greater than 90 percent of algal growth being inhibited or stimulated at the highest concentrations of test substance compared to controls.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(iv), which is revised to read as follows: .

(iv) The test begins when algae from 5- to 10-day-old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. Algal growth in controls should reach the logarithmic growth phase by 96 hours. If logarithmic growth cannot be demonstrated, the test shall be repeated. At the end of 24, 48, 72, and 96 hours the algal growth response (number or weight of algal cells/ml) in all test containers and controls shall be determined by an indirect (spectrophotometry, electronic cell counters, dry weight, etc.) or a direct (actual microscopic cell count) method. Indirect methods shall be calibrated by a direct microscopic count. The percentage inhibition or stimulation of growth for each concentration, EC_{10}, EC_{50}, EC_{90} and the concentration-response curves are determined from these counts.

iv. The proposed change to paragraph (c)(6)(ii) is not being made based on comments received.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1), (2) (iii) and (vi), and (3) (ii) and (v).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(v)(A), which is revised to read as follows:

(A) Formulation and sterilization of nutrient medium used for algal culture and preparation of test solutions should conform to those currently recommended by the U.S. EPA for freshwater and marine algal bioassays. No chelating agents are to be included in the nutrient medium used for test solution preparation. Nutrient medium should be freshly prepared for algal testing and may be dispensed in appropriate volumes in test containers and sterilized by autoclaving or filtration. The pH of the nutrient medium shall be 7.5 (±0.1) for *Selenastrum* and 8.1 (±0.1) for *Skeletonema* at the start of the test and may be adjusted prior to test chemical addition with 0.1N NaOH or HCl.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(i), which is revised to read as follows:

(i) The test temperature shall be 24 °C for *Selenastrum* and 20 °C for *Skeletonema*. Excursions from the test temperature shall be no greater than ±2 °C. Temperature should be recorded hourly during the test.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iii), which is revised to read as follows:

(iii) Stock algal cultures should be shaken twice daily by hand. Test containers shall be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/minute for *Selenastrum* and at approximately 60 cycles/minute for *Skeletonema* during the test. The rate of oscillation should be determined at least once daily during testing.

v. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iv) which is revised to read as follows:

(iv) The pH of nutrient medium in which algae are subcultured shall be 7.5 (±0.1) for *Selenastrum* and 8.1 (±0.1) for *Skeletonema*, and is not adjusted after the addition of the algae. The pH of all test solutions shall be measured at the beginning and end of the test.

c. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in the introductory text of paragraph (e).

ii. By revising paragraph (e)(4), to read as follows:

(4) The number of algal cells per milliliter in each treatment and control and the method used to derive these values at the beginning, 24, 48, and 72 hours, and end of the test; the percentage of inhibition or stimulation of growth relative to controls; and other adverse effect in the control and in each treatment.

iii. By revising paragraph (e)(5), to read as follows:

(5) The 96-hour EC_{10}, EC_{50}, and EC_{90} values, and when sufficient data have been generated, the 24, 48, and 72 hour LC_{50}'s and 95 percent confidence limits, the methods used to derive these values, the data used to define the shape of the concentration-response curve and the goodness-of-fit determination.

§ 797.1300 [Amended]

3. Section 797.1300 *Daphnid acute toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4) (iii) and (vi).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(viii), which is revised to read as follows:

(viii) The concentration of the test chemical in the chambers should be measured as often as is feasible during the test. In the static test the concentration of test chemical shall be measured, at a minimum, at the beginning of the test and at the end of the test in each test chamber. In the flow-through test the concentration of test chemical shall be measured at a minimum:

(A) In each chamber at the beginning of the test and at 48 hours after the start of the test;

(B) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

Among replicate test chambers of a treatment concentration, the measured concentration of the test chemical shall not vary more than ±20 percent.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(i), which is revised to read as follows:

(i) *Test chemical.* Deionized water should be used in making stock solutions of the test chemical. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test chemical in a sample shall be validated before beginning the test by appropriate laboratory practices. Any analytical method is not acceptable if likely degradation products of the test chemical, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

iv. By changing the word "should" to "shall" wherever it appears in paragraph (c)(4)(iv) which is revised to read as follows:

(iv) The dissolved oxygen concentration, temperature and pH shall be measured at the beginning and end of the test in each chamber.

v. By changing the word "should" to "shall" wherever it appears in paragraph (c)(4)(v), which is revised to read as follows:

(v) The test duration is 48 hours. The test is unacceptable if more than 10 percent of the control organisms are immobilized during the 48-hour test period. Each test chamber shall be checked for immobilized daphnids at 24 and 48 hours after the beginning of the test. Concentration-response curves and 24-hour and 48-hour EC_{50} values for immobilization shall be determined along with their 95 percent confidence limits.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vii), which is revised to read as follows:

(vii) Test organisms shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. In addition, test chambers within the testing area shall be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(ii), which is revised to read as follows:

(ii) *Numerical.* The number of immobilized daphnids shall be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration-response curves. A 24- and 48-hour EC_{50} and corresponding 95 percent interval shall be calculated.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii), which is revised to read as follows:

(ii) A minimum of 20 daphnids per concentration shall be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, and 64 mg/l). An equal number of daphnids shall be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they shall be commonly used carriers and shall not possess a synergistic or antagonistic effect on the toxicity of the test chemical. The concentration of solvent should not exceed 0.1 mg/l. The concentration ranges shall be selected to determine the concentration-response curves and EC_{50} values at 24 and 48 hours. Concentration of test chemical in test solutions should be analyzed prior to use.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(i)(C) and (iv), (d)(2)(i)(A) and (C), (iii) (B) and (C), and (d)(3).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(i)(B), which is revised to read as follows:

(B) Daphnids to be used in acute toxicity tests should be cultured at the test facility. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test shall have originated from the same culture population.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(A), which is revised to read as follows:

(A) Brood daphnids shall be maintained in 100-percent dilution water at the test temperature for at least 48 hours prior to the start of the test. This is easily accomplished by culturing them in the dilution water at the test temperature. During production of neonates, daphnids should not be fed.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(iii)(A), which is revised to read as follows:

(A) Daphnids should be cultured in dilution water under similar environmental conditions to those used in the test. Organisms should be handled as little as possible. When handling is necessary it should be done as gently, carefully, and quickly as possible. During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage and mortality. Dead and abnormal individuals shall be discarded. Organisms that touch dry surfaces or are dropped or injured in handling shall be discarded.

v. The proposed change to paragraph (d)(2)(ii)(A) is not being made based on comments received.

vi. The proposed change to paragraph (d)(2)(iii)(A) is not being made based on comments received.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(v), which is revised to read as follows:

(v) *Test substance delivery system.* In flow-through tests, proportional diluters, metering pump systems, or other suitable devices should be used to deliver test chemical to the test chambers. The system shall be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test chemical in each chamber. The general operation of the test substance delivery system should be checked twice during a test. The 24-hour flow through a test chamber shall be equal to at least 5 times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from any one test chamber to another.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iii), which is revised to read as follows:

(iii) The number of daphnids placed in a test chamber shall not affect test results. Loading shall not exceed 40 daphnids per liter test solution in the static system. In the flow-through test, loading limits will vary depending on the flow rate of dilution water. Loading shall not cause the dissolved oxygen concentration to fall below the recommended levels.

ix. By changing the word "should" to "shall" wherever it appears in paragraph (d)(2)(iv), which is revised to read as follows:

(iv) *Cleaning.* All test equipment and test chambers shall be cleaned before each use using standard laboratory procedures.

x. By revising paragraph (d)(3)(i), to read as follows:

(i) The test temperature shall be 20 °C. Excursions from the test temperature shall be no greater than ±2 °C.

xi. By revising paragraph (d)(3)(iv), to read as follows:

(iv) Photoperiod of 16 hours light and 8 hours darkness.

c. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears in the introductory text of paragraph (e) and by revising paragraph (e)(4) as follows:

(4) Detailed information about the daphnids used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, acclimation procedures, and culture method. The age of the daphnids used in the test shall be reported.

§ 797.1330 [Amended]

4. Section 797.1330 *Daphnid chronic toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraph (c)(4) and by revising paragraph (c)(4)(vii) to read as follows:

(vii) Test organisms shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. In addition, test chambers within the testing area shall be positioned in a random manner as in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6) (i) and (ii), which are revised to read as follows:

(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample shall be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical.* The number of immobilized adults, total offspring per adult, and immobilized offspring per adult shall be counted during each test. Appropriate statistical analyses should provide a goodness-of-fit determination for the adult immobilization concentration-response curves calculated on day 21. A 21-day EC_{50} based on adult immobilization and corresponding 95 percent confidence intervals shall also be calculated. Appropriate statistical tests (e.g., analysis of variance, mean separation test) should be used to test for significant chemical effects on chronic test criteria (cumulative number of immobilized adults, cumulative number of offspring per adult and cumulative number of immobilized offspring per adult) on day 21. An MATC shall be calculated using these chronic test criteria.

iii. By revising paragraph (c)(4)(v), to read as follows:

(v) The number of immobilized daphnids in each chamber shall be recorded on day 21 of the test. After offspring are produced, they shall be counted and removed from the test chambers every 2 or 3 days. Concentration-response curves, EC_{50} values and associated 95 percent confidence limits for adult immobilization shall be determined for day 21. An MATC shall be determined for the most sensitive test criteria measured (number of adult animals immobilized, number of young per adult, and number of immobilized young per adult).

iv. By revising paragraph (c)(4)(ii), to read as follows:

(ii) A minimum of 20 daphnids per concentration shall be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/l). An equal number of daphnids shall be placed in two or more replicates. The concentration ranges shall be selected to determine the concentration-response curves, EC_{50} values and MATC. Solutions shall be analyzed for chemical concentration at designated times during the test.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(ii)(B), (iii)(A), (iv), and (vi)(A), (2)(i)(A), (ii) (C) and (D), (iv) (B) and (C), and (3)(i)(B) and by revising paragraph (d)(1)(vi)(A) to read as follows:

(A) Brood daphnids shall be maintained in 100 percent dilution water at the test temperature for at least 48 hours prior to the start of the test. This is easily accomplished by culturing them in dilution water at the test temperature. During acclimation, daphnids shall be fed the same food as will be used for the definitive test.

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(A), which is revised to read as follows:

(A) Daphnids to be used in chronic toxicity tests should be cultured at the test facility. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test shall have originated from the same culture population.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(v)(B), which is revised to read as follows:

(B) Organisms should be handled as little as possible. When handling is necessary it should be done as gently, carefully, and quickly as possible. During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage, and mortality. Dead and abnormal individuals shall be discarded. Organisms that touch dry surfaces or are dropped or injured during handling shall be discarded.

iv. The proposed change to paragraph (d)(2)(ii)(A) is not being made based on comments received.

v. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(iii)(B), which is revised to read as follows:

(B) The test substance delivery system shall be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow rate through a test chamber shall be equal to at least five times the volume of the test chamber. During a test, the flow rates shall not vary more than 10 percent from any one test chamber to another. For the renewal test, test substance dilution water shall be completely replaced at least once every 3 days.

vi. The proposed change to paragraph (d)(2)(iv)(A) is not being made based on comments received.

vii. By changing the "should" to "shall" wherever it appears in paragraph (d)(2)(v), which is revised to read as follows:

(v) *Cleaning of test system.* All test equipment and test chambers shall be cleaned before each use following standard laboratory procedures. Cleaning of test chambers may be necessary during the testing period.

viii. By revising paragraph (d)(3)(i)(A) to read as follows:

(A) The test temperature shall be 20 °C. Excursions from the test temperature shall be no greater than ±2 °C.

ix. By revising paragraph (d)(3)(ii)(A), to read as follows:

(A) The concentration of the test substance in the chambers shall be measured during the test.

x. By revising paragraph (d)(3)(ii)(B)(*3*) to read as follows:

(*3*) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

xi. By revising paragraph (d)(3)(ii)(C), to read as follows:

(C) The dissolved oxygen concentration, temperature and pH shall be measured at the beginning of the test and on days 7, 14, and 21 in at least two chambers of the high, middle, low, and control test concentrations.

xii. By revising paragraph (d)(2)(i)(A)(*4*), to read as follows:

(*4*) An apparatus for providing a 16-hour light and 8-hour dark photoperiod.

xiii. By revising (d)(3)(i)(C), to read as follows:

(C) Photoperiod of 16 hours light and 8 hours darkness.

c. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraph (e).

ii. By revising paragraph (e)(4), to read as follows:

(4) Detailed information about the daphnids used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, acclimation procedures, and culture methods. The age of the daphnids used in the test shall be reported.

iii. By removing the phrase "7, 14 or" wherever it appears in paragraph (e)(12).

iv. By removing the phrase "7, 14 and" wherever it appears in paragraph (e)(13).

v. By amending the phrase "days 7, 14 and 21" to read "day 21" wherever it appears in paragraph (e)(14).

§ 797.1400 [Amended]

5. Section 797.1400 *Fish acute toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c) (4)(iii) and (5) and by revising paragraph (c)(6)(iii)(A) to read as follows:

(iii) *Measurement of test substance.* (A) For static tests, the concentration of the test substance shall be measured at a minimum in each test chamber at each test concentration at the beginning (0-hour, before fish are added) and at the end of the test. During flow-through tests, the concentration of test substance shall be measured as follows:

(*1*) In at least the chamber of each test concentration at 0-hour.

(*2*) In at least the chamber of each test concentration at 96-hours and every 4 days thereafter, as long as the test is continued.

(*3*) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

(*4*) Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis.

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii), which is revised to read as follows:

(ii) For exposure to each concentration of a test substance, an equal number of test fish shall be placed in two or more replicate test chambers. Test fish shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(iv), which is revised to read as follows:

(iv) Mortality data collected during the test are used to calculate a 96-hour LC_{50}. The 24-, 48-, and 72-hour values should be calculated whenever there is sufficient mortality data to determine such values. If the 96-hour LC_{50} is less than 50 percent of the estimated 48-hour LC_{50} in a flow-through test, the test shall be continued until the mean increase in mortality at any test concentration does not exceed 10 percent over a 24-hour period or until 14 days.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(v), which is revised to read as follows:

(v) Test fish shall not be fed while they are being exposed to the test substance under static conditions or during the first 96 hours of flow-through testing. If the test continues past 96 hours, the fish should be fed a suitable food at a maintenance level every other day beginning on test day 5. Any excess food and the fecal material should be removed when observed.

v. The proposed change to paragraph (c)(6)(i)(A) is not being made based on comments received.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(i)(B), which is revised to read as follows:

(B) During static tests, the dissolved oxygen concentration, temperature, and pH shall be measured in each test chamber at the beginning and end of the test. The test solution volume shall not be reduced by more than 10 percent as a result of these measurements.

vii. By changing the word "should" to "shall" wherever it appears in paragraph (c)(6)(i)(C), which is revised to read as follows:

(C) During flow-through tests, dissolved oxygen, temperature and pH measurements shall be made in each chamber at the beginning and end of the test.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(iii)(C), which is revised to read as follows:

(C) The analytical methods used to measure the amount of test substance in a sample shall be validated before beginning the test. The accuracy of a method should be verified by a method such as using known additions. This involves adding a known amount of the test substance to three water samples taken from a chamber containing dilution water and the same number and species of fish as are used in the test. The nominal concentration of the test substance in those samples should span the concentration range to be used in the test.

ix. By changing the word "should" to "shall" in certain sentences of paragraph (c)(6)(iii)(G), which is revised to read as follows:

(G) Among replicate test chambers, the measured concentrations shall not vary more than 20 percent. The measured concentration of the test substance in any chamber during the test should not vary more than 30 percent from the measured concentration at time 0.

x. By changing the word "should" to "shall" wherever it appears in paragraph (c)(6)(iii)(H), which is revised to read as follows:

(H) The mean measured concentration of test substance shall be used to calculate all LC_{50}'s and to plot all concentration-response curves.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d) (1)(ii) (A), (C), and (3)(ii).

ii. By revising paragraph (d)(2)(iii) to read as follows:

(iii) *Test substance delivery system.* In flow-through tests, diluters, metering pump systems, or other suitable devices should be used to deliver the test substance to the test chambers. The system used should be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance delivered to each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow rate through a test chamber should be a minimum of 6 tank volumes. During a test, the flow rates should not vary more than 10 percent from one test chamber to another.

iii. The proposed change to paragraph (d)(2)(vi)(A) is not being made based on comments received.

iv. The proposed change to paragraph (d)(3)(i) is not being made based on comments received.

v. By revising paragraphs (d)(3) (iii) and (iv), to read as follows:

(iii) *Temperature.* The test temperature shall be 22 °C for rainbow trout. Excursions from the test temperature shall be no greater than ±2 °C. The temperature shall be measured at least hourly in one test chamber.

(iv) *Light.* A 16-hour light and 8-hour dark photoperiod should be maintained.

c. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears and by revising paragraph (e)(7) to read as follows:

(7) The concentrations of the test substance at each test concentration just before the start of the test and at all subsequent sampling periods.

§ 797.1520 [Amended]

6. Section 797.1520 *Fish bioconcentration test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4) (i), (ii)(B), (iii)(C), (v)(B); (vi), (vii), and (viii)(C) and (5)(i) and (ii)(C).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii)(A), which is revised to read as follows:

(A) At least one concentration shall be tested to assess the propensity of the compound to bioconcentrate. The concentration selected should not stress or adversely affect the fish and should be less than one-tenth the 96-hour or incipient LC_{50} determined from a flow-through test with fathead minnows. The test concentration shall be less than the solubility limit of the compound in water. The test concentration should be close to the potential or expected environmental concentration. The limiting factor of how low one can test is based on the detection limit of the analytical methods. The concentration of the test material in the test solution should be at least 3 times greater than the detection limit in water.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(iii)(A), which is revised to read as follows:

(A) An estimate of the length of the uptake and depuration phases should be made prior to testing. This will allow the most effective sampling schedule to be determined. The uptake phase shall continue until steady-state has been reached, but need not be longer than 28 days. The test shall continue for at least 4 days.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(iv)(A), which is revised to read as follows:

(A) The test shall not be started until the test substance delivery system has been observed to be functioning properly for at least 48 hours. This time should be sufficient to allow the test substance concentration to become equilibrated with the test exposure system. Analyses of two sets of test solution samples taken prior to test initiation should document this equilibrium (i.e., the concentrations do not vary more than 20 percent from each other). At initiation (time 0), test solution samples shall be collected immediately prior to the addition of fish to the test chambers.

v. By changing the word "should" to "shall" in certain sentences in paragraph (c)(5)(ii)(A), which is revised to read as follows:

(A) All samples shall be analyzed using EPA methods and guidelines whenever feasible. The specific methodology used shall be validated before the test is initiated. The accuracy of the method should be measured by the method of known additions. This involves adding a known amount of the test substance to three water samples taken from an aquarium containing dilution water and a number of fish equal to that to be used in the test. The nominal concentration of these samples should be the same as the concentration to be used in the test. Samples taken on 2 separate days should be analyzed. The accuracy and precision of the analytical method should be checked using reference or split samples or suitable corroborative methods of analysis. The accuracy of standard solutions should be checked against other standard solutions whenever possible.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(v)(A), which is revised to read as follows:

(A) Fish shall be fed once a day throughout the uptake and depuration phases. Feeding shall be done just after sampling to minimize the effects of the test substance present in the gut when sampling. Fish should be fed the same food at a similar quantity as they received during holding and acclimation.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(5)(ii)(D), which is revised to read as follows:

(D) When radiolabelled test compounds are used, total radioactivity shall be measured in all samples. At the end of the uptake phase, water and tissue samples should be analyzed using appropriate methodology to identify and estimate the amount of any major (>10 percent of the parent compound) degradation products or metabolites that may be present.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(i) (A), (C), and (D), and (iv), and (d)(2)(i)(A) and (3)(i).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(i)(B), which is revised to read as follows:

(B) Immature fish should be used. They shall be young enough so as not to mature during the test. Fish used in the same test should be as similar in size as possible to reduce variability. The standard deviation of the weight should be less than 20 percent of the mean (N=30).

iii. By changing the word "should" to "shall" in certain sentences in paragraphs (d)(1)(ii) (B) and (C), which are revised to read as follows:

(B) During holding, the fish should not be crowded and the dissolved oxygen concentration shall be above 60 percent saturation. Holding tanks should be kept clean and free of debris. Fish should be fed at least once a day with a food which will support their survival and growth.

(C) Fish shall be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible using dip nets made of small mesh nylon, silk, bolting cloth, plankton netting, or other similar knotless materials. Handling equipment should be sterilized between uses by autoclaving, treating with an iodophor or with 200 mg hypochlorite/liter.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(iii), which is revised to read as follows:

(iii) *Acclimation.* If the holding water is not from the same source as the test dilution water, acclimation to the dilution water should be done over a period of 2 or more days. The fish should then be held an additional 14 days in the dilution water prior to testing. Any changes in water temperature should not exceed 3 °C per day over 72 hours. Fish shall be held for at least 2 days at the test temperature prior to testing.

v. The proposed change to paragraph (d)(2)(i)(E), is not being made based on comments received.

vi. The proposed change to paragraph (d)(3)(v) is not being made based on comments received.

vii. By changing the word "should" to "shall" in certain sentences in paragraphs (d)(2)(i) (B) and (C), which are revised to read as follows:

(B) The total hardness, alkalinity, pH, specific conductance, temperature and dissolved oxygen concentration of the dilution water shall be determined weekly. The pH should not vary more than 0.4 unit and the other parameters more than 10 percent on a monthly basis.

(C) Reconstituted soft water, if used, should be prepared by adding 4.8 g $NaHCO_3$, 3.0 g $CaSO_4$ $2H_20$, 3.0 g $MgSO_4$ and 200 mg KCl to each 100 L of deionized or glass-distilled water, or to dechlorinated tap water with a total residual chlorine concentration less than 1 μg/l. In all cases the specific conductance at 25 °C of the water source shall be less than 1 micromho/cm.

viii. By revising paragraph (d)(3)(ii) to read as follows:

(ii) *Temperature.* The test temperature shall be 22 °C. Excursions from the test

temperature shall be no greater than ± 2 °C.

c. Paragraph (e) is amended by changing the word "should" to "shall" whenever it appears.

§ 797.1600 [Amended]

7. Section 797.1600 *Fish early life stage toxicitv test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(1)(iii) introductory text and (A); (4) (i)(A), (iii), (iv), (vii), (ix), and (x), (D), (E), and (G); (5) (i), (ii) introductory text, and (iii)(D); and (6)(iii).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(i)(D), which is revised to read as follows:

(D) When embryos are received from an outside culture source (i.e., rainbow and brook trout) at a temperature at variance with the recommended test temperature they shall be acclimated to the test temperature. When eggs are received, they should be immediately unpacked and the temperature of the surrounding water determined. Sudden temperature changes should be avoided. Acclimation to the appropriate test temperature should be accomplished within a period of 6 hours, and should incorporate the use of dilution water.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(i)(E) which is revised to read as follows:

(E) Embryos should be visually inspected prior to placement in the embryo cups or screen trays. All dead embryos shall be discarded. Dead embryos can be discerned by a change in coloration from that of living embryos (e.g., trout embryos turn white when dead). During visual inspection, empty shells, opaque embryos, and embryos with fungus or partial shells attached shall be removed and discarded. If less than 50 percent of the eggs to be used appear to be healthy, all embryos in such a lot shall be discarded.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii)(B), which is revised to read as follows:

(B) Each day until hatch the embryos are visually examined. Minnow embryos may be examined with the aid of a magnifying viewer. Trout embryos should not be touched. Trout embryos should be maintained in low intensity light or in darkness until 1-week post hatch, and are usually examined with the aid of a flashlight or under low intensity light. Dead embryos should be removed and discarded. Any embryos which are heavily infected with fungus shall be discarded and shall be subtracted from the initial number of embryos used as a basis for the calculations of percentage hatch.

v. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(v)(A), which is revised to read as follows:

(A) The fathead and sheepshead minnow fry shall be fed newly hatched brine shrimp nauplii for the duration of the test at approximately 4-hour intervals three times a day during the week and twice on the weekend after the first week. Trout fry shall be fed at similar intervals and may receive live brine shrimp nauplii in addition to the trout starter food after the first week. Between days 1 and 8 after first hatching, silverside fry are fed the rotifer, *Brachionus plicatilis,* three times daily at a concentration of 5,000 to 10,000 organisms per egg cup (based on 15 fish/cup). From days 9 to 11, the fry shall be fed approximately 2,500 newly hatched brine shrimp (*Artemia*) nauplii and 5,000 to 10,000 rotifers twice daily. For the remainder of the test, the fish will be fed brine shrimp exclusively. The number of organisms used should be gradually increased to approximately 5,000 nauplii by test day 28.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(viii), which is revised to read as follows:

(viii) *Randomization.* The location of all test chambers within the test system shall be randomized. A representative sample of the test embryos should be impartially distributed by adding to each cup or screen tray no more than 20 percent of the number of embryos to be placed in each cup or screen tray and repeating the process until each cup or screen tray contains the specified number of embryos. Alternatively, the embryos can be assigned by random assignment of a small group (e.g., 1 to 5) of embryos to each embryo cup or screen tray, followed by random assignment of a second group of equal number to each cup or tray, which is continued until the appropriate number of embryos are contained in each embryo cup or screen tray. The method of randomization used shall be reported.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(x)(F), which is revised to read as follows:

(F) At termination, all surviving fish shall be measured for growth. Standard length measurements should be made directly with a caliper, but may be measured photographically. Measurements shall be made to the nearest millimeter (0.1 mm is desirable). Weight measurements shall also be made for each fish alive at termination (wet, blotted dry, and to the nearest 0.01 g for the minnows and 0.1 g for the trout). If the fish exposed to the toxicant appear to be edematous compared to control fish, determination of dry, rather than wet, weight is recommended.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(iv)(A), which is revised to read as follows:

(A) Prior to the addition of the test substance to the dilution water, it is recommended that the test substance stock solution be analyzed to verify the concentration. After addition of the test substance, the concentration of test substance should be measured at the beginning of the test in each test concentration and control(s), and at least once a week thereafter. Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. If a malfunction in the delivery system is discovered, water samples shall be taken from the affected test chambers immediately and analyzed.

ix. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(v)(B), which is revised to read as follows:

(B) For measurement of the test substance, water samples shall be taken midway between the top, bottom, and sides of the test chamber and should not include any surface scum or material stirred up from the bottom or sides. Samples of test solutions shall be handled and stored appropriately to minimize loss of test substance by microbial degradation, photodegradation, chemical reaction, volatilization, or sorption.

x. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(v)(C), which is revised to read as follows:

(C) Chemical and physical analyses shall be performed using standardized methods whenever possible. The analytical method used to measure the concentration of the test substance in the test solution shall be validated before the beginning of the test. At a minimum, a measure of the accuracy of the method should be obtained on each of two separate days by using the method of known additions, and using dilution water from a tank containing test organisms. Three samples should be analyzed at the next-to-lowest test substance concentration. It is also desirable to study the accuracy and precision of the analytical method for test guideline determination by use of

reference (split) samples, or interlaboratory studies, and by comparison with alternative, reference, or corroborative methods of analysis.

xi. By revising paragraph (c)(1)(iii)(B) to read as follows:

(B) At least 60 embryos divided equally in such a manner that test results show no significant bias from the distributions, between the embryo incubation trays or cups for each test concentration and control (i.e., 30 per embryo cup with 2 replicates);

xii. By changing the word "should" to "shall" in certain sentences of paragraphs (c)(4)(x) (A) and (B), which are revised to read as follows:

(A) Death of embryos shall be recorded daily.

(B) When hatching commences, daily records of the number of embryos remaining in each embryo cup are required. This information is necessary to quantify the hatching success. A record of all deformed larvae shall be kept throughout the entire post-hatch exposure. Time to swim-up shall be recorded for the trout. Upon transfer of fry from the embryo cups to the test chambers, daily counts of the number of live fish should be made. At a minimum, live fish shall be counted on days 4, 11, 18, 25 and (weekly thereafter for the trout species) finally on termination of the test.

xiii. By changing the word "should" to "shall" in certain sentences of paragraphs (c)(6) (i) and (ii), which are revised to read as follows:

(i) *Analysis of water quality.* Measurement of certain dilution water quality parameters shall be performed every 6 months, to determine the consistency of the dilution water quality. In addition, if data in 30-day increments are not available to show that freshwater dilution water is constant, measurements of hardness, alkalinity, pH, acidity, conductivity, TOC or COD and particulate matter should be conducted once a week in the highest test substance concentration. Measurement of calcium, magnesium, sodium, potassium, chloride, and sulfate is desirable.

(ii) *Dissolved oxygen measurement.* The dissolved oxygen concentration shall be measured in each test chamber at the beginning of the test and at least once weekly thereafter (as long as live organisms are present) in two replicates of the control and the high, medium, and low test substance concentrations.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(ii) introductory text and (ii)(B); (2)(ii)(C), (iii)(A), (iv)(C), (vii)(C)(*1*); and (3) (i) and (iv) (A), (B), and (C).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(A), which is revised to read as follows:

(A) All embryos used in the test shall be from the same source. Embryos shall be obtained from a stock cultured in-house when possible, and maintained under the same parameters as specified for the test conditions. When it is necessary to obtain embryos from an external source, caution should be exercised to ensure embryo viability and to minimize the possibility of fungal growth. A description of the brood stock history or embryo source shall be made available to EPA upon request.

iii. The proposed change to paragraph (d)(1)(ii)(C) is not being made based on comments received.

iv. The proposed change to paragraph (d)(1)(ii)(E) is not being made based on comments received.

v. The proposed change to paragraph (d)(2)(i) is not being made based on comments received.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(iv)(A), which is revised to read as follows:

(A) The choice of a specific delivery system depends upon the specific properties and requirements of the test substance. The apparatus used should accurately and precisely deliver the appropriate amount of stock solution and dilution water to the test chambers. The system selected shall be calibrated before each test. Calibration includes determining the flow rate through each chamber, and the proportion of stock solution to dilution water delivered to each chamber. The general operation of the test substance delivery system shall be checked at least twice daily for normal operation throughout the test. A minimum of five test substance concentrations and one control shall be used for each test.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(vii)(A)(*1*), which is revised to read as follows:

(*1*) A constant supply of acceptable dilution water should be available for use throughout the test. Dilution water shall be of a minimum quality such that the test species selected will survive in it for the duration of testing without showing signs of stress (e.g., loss of pigmentation, disorientation, poor response to external stimuli, excessive mucous secretion, lethargy, lack of feeding, or other unusual behavior). A better criterion for an acceptable dilution water for tests on early life stages should be such that the species selected for testing will survive, grow, and reproduce satisfactorily in it.

viii. By changing the word "should" to "shall" in certain sentences in paragraphs (d)(2)(vii)(A) (*2*), and (*3*), which are revised to read as follows:

(*2*) The concentration of dissolved oxygen in the dilution water (fresh or salt) shall be between 90 percent and 100 percent saturation. When necessary, dilution water should be aerated by means of airstones, surface aerators, or screen tubes before the introduction of the test substance.

(*3*) Water that is contaminated with undesirable microoganisms (e.g., fish pathogens) shall not be used. If such contamination is suspected, the water should be passed through a properly maintained ultraviolet sterilizer equipped with an intensity meter before use. Efficacy of the sterilizer can be determined by using standard plate count methods.

ix. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iii)(B), which is revised to read as follows:

(B) Excursions from the test temperature shall be no greater than ±2.0° C. It is recommended that the test system be equipped with an automatic alarm system to alert staff of instantaneous temperature changes in excess of 2 °C. If the water is heated (i.e., for minnow species), precautions should be taken to ensure that supersaturation of dissolved gases is avoided. Temperatures shall be recorded in all test chambers at the beginning of the test and weekly thereafter. The temperature shall be recorded at least hourly in one test chamber throughout the test.

x. Paragraph (d)(3)(iv)(E) is revised to read as follows:

(E) Light intensities ranging from 30 to 100 lumens at the water surface shall be provided; the intensity selected should be duplicated as closely as possible for all test chambers.

xi. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(ii)(B), which is revised to read as follows:

(B) A lower loading or higher flow rate or both shall be used if necessary to meet the following three criteria at all times during the test in each chamber containing live test organisms:

(1) The concentration of dissolved oxygen shall not fall below 75 percent saturation for the fathead and sheepshead minnows and 90 percent for the rainbow and brook trout;

(2) The concentration of un-ionized ammonia should not exceed 1 μg/1; and

(3) The concentration of toxicant should not be lowered (i.e., caused by uptake by the test organisms and/or materials on the sides and bottoms of the chambers) more than 20 percent of the mean measured concentration.

xii. Paragraph (e) introductory text is revised as follows:

(e) *Reporting.* A report of the results of an early life stage toxicity test shall include the following:

§ 797.1800 [Amended]

8. Section 797.1800 *Oyster acute toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4) (iv), (viii), and (ix) (A), (B).

ii. By changing the word "should" to "shall" or "are" in certain sentences in paragraph (c)(4)(i), which is revised to read as follows:

(i) Oysters which meet condition criteria (age, size, reproductive status, health) and which have been acclimated to test conditions should have approximately 3 to 5 mm of the shell periphery, at the rounded (ventral) end, ground away with a small electric disc grinder or other appropriate device, taking care to uniformly remove the shell rim to produce a smooth, rounded, blunt profile. The oyster's valves should be held together tightly during grinding to avoid vibrating the shell and injuring the adductor muscle. Oysters of which so much of the shell rim has been removed that an opening into the shell cavity is visible shall not be used.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vii), which is revised to read as follows:

(vii) Shell growth is the primary criterion used in this test guideline to evaluate the toxicity of the test chemical. Shell growth increments in all oysters shall be measured after 96 hours exposure. Record the length of the longest "finger" of new shell growth to the nearest 0.5 mm. Oysters should be handled very gently at this stage to prevent damage to the new shell growth.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (c)(5)(i), which is revised to read as follows:

(i) At the end of the test, appropriate statistical analysis should be conducted on the oyster shell deposition test data. The probit transformation should then be applied to the response variable and then regressed, using least squares regression, on dose or log-dose. An F Test for linearity should be conducted to determine whether the chosen regression technique adequately describes the experimental data.

v. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(v), which is revised to read as follows:

(v) Test oysters shall be impartially distubuted among test chambers in such a manner that test results show no significant bias from the distributions. The oysters should be spread out equidistantly from one another so that the entire test chamber is used. The oysters should also be placed with the left (cupped) valve down and the open, unhinged ends all oriented in the same direction facing the incoming flow of test solution.

vi. By changing the word "should" to "shall" in certain sentences in paragraphs (c)(4)(ix) (C), (D), and (E), which are revised to read as follows:

(C) If evidence of spawning is observed, the test shall be repeated.

(D) There shall be evidence that the concentration of the substance being tested has been satisfactorily maintained over the test period. The concentration of the test substance should be measured:

(*1*) In each chamber at 0-hour;

(*2*) In each chamber at 96-hours; and

(*3*) In at least one appropriate chamber whenever a malfunction is detected in any part of the test chemical delivery system.

(E) Dissolved oxygen, temperature, salinity, and pH measurements shall be made at the beginning and end of the test in each chamber.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(i) (A), (C), and (D) and (d)(3)(ii).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(iii)(A), which is revised to read as follows:

(A) Oysters should be attended to immediately upon arrival. Oyster shells should be brushed clean of fouling organisms and the transfer of the oysters to the holding water should be gradual to reduce stress caused by differences in water quality characteristics and temperature. Oysters shall be held for at least 12 to 15 days before testing. All oysters shall be maintained in dilution water at the test temperature for at least 2 days before they are used.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(iii)(B), which is revised to read as follows:

(B) During holding, the oysters should not be crowded, and the dissolved oxygen concentration shall be above 60-percent saturation. The temperature of the holding water shall be the same as that used for testing. Holding tanks should be kept clean and free of debris. Cultured algae may be added to dilution water sparingly, as necessary to support life and growth and such that test results are not affected as confirmed by previous testing.

iv. The proposed change to paragraph (d)(2)(i)(B) is not being made based on comments received.

v. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(ii), which is revised to read as follows:

(ii) *Dissolved oxygen.* The dissolved oxygen concentrations shall be at least 60 percent of the saturation value and should be recorded daily.

vi. By revising paragraph (d)(3)(iv) to read as follows:

(iv) *Temperature.* The test temperature shall be 20 °C. Temporary fluctuations (less than 8 hours) within ±5 °C are permissible. Temperature should be recorded continuously.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(i)(B), which is revised to read as follows:

(B) Oysters used in the same test shall be 30 to 50 mm in valve height and should be as similar in age and/or size as possible to reduce variability. The standard deviation of the valve height shouid be less than 20 percent of the mean.

viii. By revising paragraph (d)(1)(iii)(D) to read as follows:

(D) A batch of oysters is acceptable for testing if the percentage mortality over the 7-day period prior to testing is less than 5 percent. If the mortality is between 5 and 10 percent, acclimation should continue for 7 additional days. If the mortality is greater than 10 percent, the entire batch of oysters shall be rejected. Oysters which appear diseased or otherwise stressed or which have cracked, chipped, bored, or gaping shells shall not be used. Oysters infested with mudworms (*Poly dora sp.*) or boring sponges (*Cilona cellata*) should not be used.

ix. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(ii), which is revised to read as follows:

(ii) *Dilution water.* A constant supply of good quality unfiltered seawater should be available throughout the holding, acclimation and testing periods. Natural seawater is recommended, although artificial seawater with food added may be used. In either case, to ensure each oyster is provided equal

amounts of food, the water should come from a thoroughly mixed common source and shall be delivered at a flowrate of at least 1 and preferably 5 liters per hour per oyster. The flowrate should be ±10 percent of the nominal flow. A dilution water is acceptable if oysters will survive and grow normally for 14 days without exhibiting signs of stress; i.e., excessive mucus production (stringy material floating suspended from oysters), lack of feeding, shell gaping, poor shell closing in response to prodding, or excessive mortality. The dilution water shall have a salinity in excess of 12 parts per thousand, and should be similar to that in the environment from which the test oysters originated. A natural seawater should have a weekly range in salinity of less than 10 parts per thousand and a monthly range in pH of less than 0.8 unit. Artificial seawater salinity should not vary more than 2 parts per thousand nor more than 0.5 pH unit. Oysters shall be tested in dilution water from the same origin.

x. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(i), which is revised to read as follows:

(3) *Test parameters*—(i) *Carriers.* Stock solutions of substances of low aqueous solubility may be prepared by ultrasonic dispersion or, if necessary, by use of organic solvents, emulsifiers or dispersants of low toxicity to oysters. When such carriers are used the control oysters shall be exposed to the same concentration of the carrier as that used in the highest concentration of the test substance. The concentration of such carriers should not exceed 0.1 ml/l.

xi. By revising paragraph (d)(3)(v) to read as follows:

(v) *pH.* The pH shall be measured at the beginning and end of the test in each test chamber.

c. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears.

§ 797.1830 [Amended]

9. Section 797.1830 *Oyster bioconcentration test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4)(i), (iv), (v) introductory text, (v)(B), and (C), (vi) introductory text, (vi)(C) and (F), and (vii)(A) and (B)(*1*) and (*3*); and (6)(iii) and (iv).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii), which is revised to read as follows:

(ii) At least one or more concentrations shall be tested to assess the propensity of the compound to bioconcentrate. The concentrations selected should not stress or adversely affect the oysters and should be less than one-tenth the EC_{50} determined in either the rangefinding or 96-hour definitive test under § 797.1800 of this chapter. The test concentration shall be less than the solubility limit of the test substance in water and should be close to the potential or expected environmental concentration. The limiting factor of how low one can test is based on the detection limits of the analytical methods. The concentration of the test material in the test solution should be at least 10 times greater than the detection limit in water.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(iii), which is revised to read as follows:

(iii) If it is desirable to document that the potential to bioconcentrate is independent of the test chemical concentration, at least two concentrations shall be tested which are at least a factor of 10 apart.

iv. By revising paragraph (c)(4)(v)(A), to read as follows:

(A) If it is observed that the stability or homogeneity of the test chemical cannot be maintained, then care should be taken in the interpretation of the results and a note shall be made that these results may not be reproducible.

v. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vi)(B), which is revised to read as follows:

(B) The appropriate number of oysters should be brushed clean and shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. The oysters should be spread out equidistant from one another and placed with the left (cupped) valve down and the unhinged ends (opposite from umbo) all oriented in the same direction facing the incoming flow.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vi)(D), which is revised to read as follows:

(D) Oysters shall be observed (and data recorded) at least daily for feeding activity (deposition of feces) or any unusual conditions such as excessive mucus production (stringy material floating suspended from oysters), spawning, or appearance of shell (closure or gaping). If gaping is noted, the oyster(s) should be prodded. Oysters which fail to make any shell movements when prodded are to be considered dead, and shall be removed promptly with as little disturbance as possible to the test chamber(s) and remaining live oysters.

vii. The proposed change to paragraph (c)(4)(vii)(B)(*2*) is not being made based on comments received.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(i), which is revised to read as follows:

(i) All samples should be analyzed using USEPA methods and guidelines whenever feasible. The specific methodology used shall be validated before the test is initiated. The accuracy of the method should be measured by the method of known additions. This involves adding a known amount of the test chemical to three water samples taken from an aquarium containing dilution water and a number of oysters equal to that to be used in the test. The nominal concentration of these samples shall be the same as the concentration to be used in the test. Samples taken on two separate days shall be analyzed. The accuracy and precision of the analytical method shall be checked using reference or split samples or suitable corroborative methods of analysis. The accuracy of standard solutions should be checked against other standard solutions whenever possible.

ix. By revising paragraph (c)(4)(v)(E) to read as follows:

(E) If evidence of spawning is observed, the test shall be repeated.

x. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vii)(C), which is revised to read as follows:

(C) If a radiolabeled test compound is used, a sufficient number of oysters shall also be sampled at termination to permit identification and quantitation of any major (greater than 10 percent of parent) metabolites present. It is crucial to determine how much of the activity present in the oyster is directly attributable to the parent compound, and to correct the bioconcentration factor appropriately.

xi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(5)(ii), which is revised to read as follows:

(ii) If 95 percent elimination has not been observed after 14 days depuration then a depuration rate constant shall also be calculated. This rate constant should be based on the elimination of the parent compound.

xii. By revising paragraph (c)(4)(v)(D), to read as follows:

(D) There shall be evidence that the concentration of the chemical being

tested has been satisfactorily maintained over the test period.

xiii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vi)(A), which is revised to read as follows:

(A) The test shall not be started until the test chemical delivery system has been observed to be functioning properly and the test chemical concentrations have equilibrated (i.e., the concentration does not vary more than 20 percent). Analyses of two sets of test solution samples taken prior to test initiation should document this equilibrium. At initiation (time 0), test solution samples shall be collected immediately prior to the addition of oysters to the test chambers.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(i), (iii), (iv), and (3)(i) and (iii).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii), which is revised to read as follows:

(ii) Oysters used in the same test should be 30 to 50 mm in valve height and should be as similar in age and/or size as possible to reduce variability. The standard deviation of the valve height shall be less than 20 percent of the mean.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(vi)(A), which is revised to read as follows:

(A) Oysters should be attended to immediately upon arrival. Oyster shells should be brushed clean of fouling organisms, and the transfer of the oysters to the holding water should be gradual to reduce stress caused by differences in water quality characteristics and temperature. Oysters shall be held for at least 12 to 15 days before testing. All oysters shall be maintained in dilution water at the test temperature for at least 2 days before they are used.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(vi)(B), which is revised to read as follows:

(B) During holding, the oysters should not be crowded, and the dissolved oxygen concentration shall be above 60-percent saturation. The temperature of the holding waters shall be the same as that used for testing. Holding tanks should be kept clean and free of debris. Cultured algae may be added to dilution water sparingly, as necessary to support life and growth, such that test results are not affected, as confirmed by previous testing. Oysters should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible.

v. The proposed change to paragraph (d)(2)(i)(B) is not being made based on comments received.

vi. The proposed change to paragraph (d)(2)(ii) is not being made based on comments received.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iv), which is revised to read as follows:

(iv) *Temperature.* The test temperature shall be 20 °C. Temporary excursions (less than 8 hours) within ± 5 °C are permissible. Temperature should be recorded continually.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(vi)(C), which is revised to read as follows:

(C) A batch of oysters is acceptable for testing if the percentage mortality over the 7-day period prior to testing is less than 5 percent. If the mortality is between 5 and 10 percent, acclimation shall continue for 7 additional days. If the mortality is greater than 10 percent, the entire batch of oysters shall be rejected. Oysters which appear diseased or otherwise stressed or which have cracked, chipped, bared, or gaping shells shall not be used. Oysters infested with mudworms (*Polydora sp.*) or boring sponges (*Cilona cellata*) should not be used.

ix. By changing the word "should" to "shall" wherever it appears in paragraph (d)(3)(ii), which is revised to read as follows:

(ii) *Dissolved oxygen.* This dissolved oxygen concentration shall be at least 60 percent of the air saturation value and should be measured weekly in each chamber.

x. By changing the word "should" to "shall" wherever it appears in paragraph (d)(3)(v), which is revised to read as follows:

(v) *pH.* The pH shall be measured weekly in each test chamber.

c. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears.

§ 797.1930 [Amended]

10. Section 797.1930 *Mysid shrimp acute toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4)(ii), (iv), and (vii) and by revising paragraph (c)(4)(iii), to read as follows:

(iii) A minimum of 20 mysids per concentration shall be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, and 64 mg/l). An equal number of mysids shall be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they shall be commonly used carriers and shall not possess a synergistic or antagonistic effect on the toxicity of the test substance. The concentration of solvent shall not exceed 0.1 ml/1. The concentration ranges shall be selected to determine the concentration-response curves and LC_{50} values at 48 and 96 hours.

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(3)(iii), which is revised to read as follows:

(iii) This test should be conducted with both newly hatched juvenile (< 24 hours old) and young adult (5 to 6 days old) mysids. For each age class (juvenile or young adult), a minimum of 10 mysids should be exposed to each concentration of test substance for up to 96 hours. The exposure period may be shortened if data suitable for the purpose of the range-finding test can be obtained in less time. The age class which is most sensitive to the test substance in the range-finding test shall be utilized in the definitive test. When no apparent difference in sensitivity of the two life stages is found, juveniles shall be utilized in the definitive test. No replicates are required, and nominal concentrations of the chemical are acceptable.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ix), which is revised to read as follows:

(ix) The concentration of the test substance in the chambers should be measured as often as is feasible during the test. At a minimum, during static tests the concentration of test substance shall be measured at each concentration at the beginning and at the end of the test. During the flow-through test, the concentration of test substance should be measured at the beginning and end of the test and in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

iv. By changing the word "should" to "shall" in certain sentences in

paragraph (c)(6)(i), which is revised to read as follows:

(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample shall be validated before beginning the test by appropriate laboratory practices. Any analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

v. By revising paragraphs (c)(4) (v) and (vi), to read as follows:

(v) The dissolved oxygen concentration temperature, salinity, and pH shall be measured at the beginning and end of the test in each chamber.

(vi) The test duration is 96 hours. The test is unacceptable if more than 10 percent of the control organisms die or exhibit abnormal behavior during the 96 hour test period. Each test chamber should be checked for dead mysids at 24, 48, 72, and 96 hours after the beginning of the test. Concentration-response curves and 24-, 48-, 72- and 96-hour LC_{50} values should be determined along with their 95 percent confidence limits.

vi. By revising paragraph (c)(4)(viii), to read as follows:

(viii) Test organisms shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. In addition, test chambers within the testing area shall be positioned in a random manner or in a way in which appropriated statistical analyses can be used to determine the variation due to placement.

vii. By revising paragraph (c)(6)(ii), to read as follows:

(ii) *Numerical.* The number of dead mysids shall be counted during each definitive test. Appropriated statistical analyses should provide a goodness-of-fit determination for the concentration-response curves. A 48- and 96-hour LC_{50} and corresponding 95 percent interval shall be calculated.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (i)(C) and (ii)(A); and (d)(2) (i)(B), (ii), and (iv) and (d)(3) introductory text and (ii).

ii. The proposed change to paragraph (d)(2)(iii)(A) is not being made based on comments received.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(v), which is revised to read as follows:

(v) *Test substance delivery system.* In flow-through tests, proportional diluters, metering pumps, or other suitable systems should be used to deliver test substance to the test chambers. The system used shall be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow through a test chamber shall be equal to at least 5 times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent among test chambers or across time.

iv. By revising paragraph (d)(3)(iii), to read as follows:

(iii) The number of mysids placed in a test solution shall not be so great as to affect results of the test. Loading shall not exceed 30 mysids per liter for a static test. Loading requirements for the flow-through test will vary depending on the flow rate of dilution water. The loading shall not cause the dissolved oxygen concentration to fall below the recommended levels.

v. By revising paragraph (d)(1)(i)(B), to read as follows:

(B) Mysids to be used in chronic toxicity tests should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. Because of similarities with other mysids species, taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.

vi. By revising paragraph (d)(3)(i), to read as follows:

(i) The test temperature shall be 25°C. Excursions from the test temperature shall be not greater than ± 2°C.

c. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in the introductory text of paragraph (e) and in paragraph (e)(6).

ii. By revising paragraph (e)(7), to read as follows:

(7) The 96-hour LC_{50} and when sufficient data have been generated, the 24-, 48-, and 72-hour LC_{50}'s and the corresponding 95-percent confidence limits and the methods used to calculate the values. These calculations shall be made using the average measured concentration of the test substance.

§ 797.1950 [Amended]

11. Section 797.1950 *Mysid shrimp chronic toxicity test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4)(v), and (vi).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(viii), which is revised to read as follows:

(viii) The concentration of the test substance in the chambers should be measured as often as is feasible during the test. The concentration of test substance shall be measured:

(A) At each test concentration at the beginning of the test and on days 7, 14, 21, and 28; and

(B) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

Equal aliquots of test solutions may be removed from each test chamber and pooled for analysis. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(i), which is revised to read as follows:

(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be employed whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample shall be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

iv. By changing the word "should" to "shall" wherever it appears in paragraph (c)(4)(iv), which is revised to read as follows:

(iv) The dissolved oxygen concentration, temperature, salinity, and pH shall be measured weekly in each chamber.

v. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vii), which is revised to read as follows:

(vii) Test organisms shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. In addition, test chambers within the testing area shall be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determined the variation due to placement.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(ii), which is revised to read as follows:

(ii) *Numerical.* (A) The number of dead mysids, cumulative young per female, and body lengths of male and female mysids shall be recorded during each definitive test. Appropriate statistical analyses shall provide a goodness-of-fit determination for the day 7, 14, 21 and 28 adult (Gl) death concentration-response curves.

(B) A 7-, 14-, 21- and 28-day LC_{50}, based on adult (Gl) death, and corresponding 95 percent confidence intervals shall be calculated. Appropriate statistical tests (e.g., analysis of variance, mean separation test) should be used to test for significant chemical effects on chronic test criteria (cumulative mortality of adults, cumulative number of young per female and body lengths of adult male and females) on designated days. An MATC shall be calculated using these chronic tests criteria.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii), which is revised to read as follows:

(ii) A minimum of 40 mysids per concentration shall be exposed to four or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32 and 64 mg/1). An equal number of mysids shall be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they shall be commonly used carriers and shall not possess a synergistic or antagonistic effect on the toxicity of the test substance. The concentration of solvent should not exceed 0.1 ml/1. The concentration ranges should be selected to determine the concentration response curves, LC_{50} values and MATC. Concentration of test substance in test solutions should be analyzed prior to use.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1)(i)(C) and (D), (2)(i)(C) and (iv), and (3) introductory text, (ii) and (iii).

ii. By revising paragraph (d)(1)(i)(B) to read as follows:

(B) Mysids to be used in chronic toxicity tests should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. Because of similarities with other mysid species, taxonomic verification should be obtained from the commercial supplier, by experienced laboratory personnel, or by an outside expert.

iii. The proposed change to paragraph (d)(1)(ii)(A) is not being made based on comments received.

iv. The proposed change to paragraph (d)(2)(iii)(A) is not being made based on comments received.

v. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(v), which is revised to read as follows:

(v) *Test substance delivery system.* Proportional diluters, metering pumps, or other suitable systems should be used to deliver test substance to the test chambers. The system used shall be calibrated before each test. Calibration includes determining the flow rate and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow rate through a chamber shall be equal to at least 5 times the volume of the chamber. The flow rates should not vary more than 10 percent among chambers or across time.

vi. By changing the word "should" to "shall" wherever it appears in paragraph (d)(2)(ii), which is revised to read as follows:

(ii) *Cleaning.* Test substance delivery systems and test chambers shall be cleaned before each use following standard laboratory practices.

vii. By revising paragraph (d)(3)(i) to read as follows:

(i) The test temperature shall be 25°C. Excursions from the test temperature shall be no greater than ± 2°C.

c. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears in the introductory text and in paragraphs (e)(9) and (10) and in (e)(8), which is revised to read as follows:

(8) The MATC is calculated as the geometric mean between the lowest measured test substance concentration that had a significant ($P<0.05$) effect and the highest measured test substance concentration that had no significant ($P<0.05$) effect in the chronic test. The most sensitive of the test criteria for adult (Gl) mysids (cumulative number of dead mysids, body lengths of males and females or the number of young per female) is used to calculate the MATC. The criterion selected for MATC computation is the one which exhibits an effect (a statistically significant difference between treatment and control groups; $P<0.05$) at the lowest test substance concentration for the shortest period of exposure. Appropriate statistical tests (analysis of variance, mean separation test) should be used to test for significant chemical effects. The statistical tests employed and the results of these tests shall be reported.

§ 797.2150 [Amended]

12. Section 797.2150 *Mallard reproduction test* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the words "should be" to "are" wherever they appear in paragraph (c)(1)(i), (ii), (iv), and (vi).

ii. By changing the words "should be" to "is" wherever they appear in paragraph (c)(1)(v) and (ix).

iii. By changing the word "should" to "shall" wherever it appears in paragraphs (c)(4) (i)(B), (ii), (iii)(A), (iv), (v)(A), (vi), (ix), and (x) and (6)(ii)(B).

iv. By changing the words "should be" to "is" or "are" in certain sentences in paragraph (c)(1)(iii), which is revised to read as follows:

(iii) The test substance is thoroughly and evenly mixed into the diet at concentrations specified in the test rule. All treatment levels are analyzed for test substance concentrations at the beginning and midway through the test.

v. By changing the words "should be" to "are" in certain sentences in paragraph (c)(1)(vii), which is revised to read as follows:

(vii) Eggs are removed daily and stored until there is a sufficient quantity for incubation. All eggs are candled for cracks, and cracked eggs are removed. Once every 2 weeks, all eggs produced that day are analyzed for eggshell thickness. Incubated eggs are candled on day 14 and day 21. Hatching should be completed by day 27.

vi. By changing the words "should be" to "is" or "are" in certain sentences in paragraph (c)(1)(viii), which is revised to read as follows:

(viii) Hatchlings are maintained in pens until they are 14 days old. Abnormal behavior or death is reported. Ducklings are weighed on day 14.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(1)(x), which is revised to read as follows:

(x) The report shall include all conditions, procedures, and results.

Data should be sufficiently detailed for an independent statistical analysis.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(i)(A), which is revised to read as follows:

(A) The concentrations of test substance in the diet will be specified in the test rule. At least three treatment groups and a control group shall be used. The higher two treatment levels shall be multiples (often 5x, 10x, or 20x) of the lowest treatment level. The highest treatment levels shall usually be below lethal levels, unless predicted exposure levels are high enough to approximate lethal levels.

ix. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(vii), which is revised to read as follows:

(vii) *Egg collection, storage, and incubation.* All eggs shall be collected daily, marked according to the date collected and the parental pen from which collected, and should be stored at 16°C and 55 to 80 percent relative humidity. Storage in plastic bags may improve uniformity of hatching. Stored eggs should be turned daily. At biweekly intervals, eggs shall be removed for storage and be candled to detect eggshell cracks. Except for eggs with cracked shells and those eggs removed for eggshell thickness measurements, all eggs should be set after candling for incubation in a commercial incubator. If incubators are not equipped to automatically turn eggs, they should be turned daily by hand. During the incubation period, eggs should be maintained at 37.5°C and approximately 70-percent relative humidity. Eggs shall be candled again on day 14 of incubation to determine fertility and early death of embryos. A final candling shall be done on day 21 to measure embryo survival. On day 23, eggs shall be removed to a separate incubator or hatcher. Hatching will normally be complete by the end of day 27.

x. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(viii), which is revised to read as follows:

(viii) *Duckling maintenance.* By day 27 of incubation, the hatched mallard ducklings should be removed from the hatcher or incubator. Ducklings shall be either housed according to the appropriate parental pen group or individually marked (such as by leg bands) as to parental group and housed together. Ducklings should be maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal or stainless steel. Temperature in the pens should be controlled, preferably by a thermostatic control device. A temperature gradient in the pen from approximately 35°C to approximately 22°C will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Ducklings shall be provided a standard commercial duck starter ration, or its nutritional equivalent. No test substance may be added to the diets of ducklings. Ducklings shall be maintained until they are 14 days old.

xi. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(i)(A), which is revised to read as follows:

(A) Experimental groups should be individually compared to the control group by analysis of variance. Other accepted statistical methods may be used as long as they are documented and described. In particular, regression analysis is highly desirable if the data and number of dose levels allow the use of this technique. Sample units are the individual pens within each treatment level or control. Analysis shall include:

(*1*) Body weights of adults.

(*2*) Food consumption of adults.

(*3*) Percentage of hens laying eggs. This should always be determined when pens contain a single pair; if feasible, it should be determined when pens contain groups.

(*4*) Number of eggs laid per pen.

(*5*) Percentage of cracked eggs.

(*6*) Percent viable embryos of eggs set.

(*7*) Percent live 21-day embryos of viable embryos.

(*8*) Percent hatching of viable embryos.

(*9*) Percentage of hatchlings that are normal.

(*10*) Percent 14-day-old survivors of normal hatchlings.

(*11*) Number of 14-day-old survivors per hen.

(*12*) Body weights of 14-day-old survivors.

(*13*) Eggshell thickness.

xii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(ii)(A), which is revised to read as follows:

(A) Samples of treated diets shall be analyzed to confirm proper dietary concentrations of the test substance. If samples cannot be analyzed immediately, they should be stored appropriately (e.g., frozen at a temperature of −15°C or lower) until analysis can be performed. Analyses shall be conducted on all test substance concentrations at the beginning of the test period and again 10 to 12 weeks later. If not otherwise available, data shall be generated to indicate whether or not the test substance degrades or volatilizes. If the test substance is known or found to be volatile or labile to the extent that 25 percent or more loss occurs within 1 week, then test substance diets shall be prepared (freshly or from frozen concentrate) at a frequency that will prevent more than 25 percent loss of test substance.

xiii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(ii)(C), which is revised to read as follows:

(C) *Analysis of basal diet.* A nutrient analysis of the basal diet shall be included in the test report. For commercially prepared basal diets, the list of ingredients supplied by the manufacturer is normally sufficient, if it is detailed. The composition of any vitamin or other supplements should also be reported.

xiv. By changing the word "should" to "shall" wherever it appears in paragraph (c)(4)(iii)(B), which is revised to read as follows:

(B) Test birds shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions.

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraph (d)(1)(i) (C), (D), and (E) and (ii)(A)(*1*).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(i)(A), which is revised to read as follows:

(A) The mallard, *Anas platyrhynchos* L., is the test species. Test birds should be pen-reared. They may be reared in the laboratory or purchased from commercial breeders. Rearing stock and/or test birds shall be obtained only from sources that have met the requirements for "U.S. Pullorum-Typhoid Clean" classification under paragraph (f)(1) of this section. Birds shall be obtained only from sources whose colonies have known breeding histories. If possible, a history of rearing practices for test birds should be obtained and made available upon request. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and exact age. Test birds shall be phenotypically indistinguishable (except for size) from wild stock. Conscientious breeders of such birds will periodically outbreed their flocks with genetically wild stock in order to maintain a genetic composition that approximates the

heterogeneity of naturally occurring birds.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(i)(B), which is revised to read as follows:

(B) All control and experimental birds used in a test shall be from the same source and strain. If shipped, all birds shall be examined following shipment for possible physical injury that may have occurred in transit. All birds should have a health observation period of at least 2 weeks prior to selection for treatment. Birds should be in apparent good health. Deformed, abnormal, sick, or injured birds shall not be used. A population of birds shall not be used if more than 3 percent of either sex die during the health observation period. Birds shall not have been selected in any way for resistance to toxic substances. Birds shall not have been used in a previous test, either in control or treatment group. Offspring of birds used in a treatment group in a previous test shall not be used, but offspring of birds used as controls in a previous test are acceptable.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(A)(*2*), which is revised to read as follows:

(*2*) The test substance shall be mixed into the diet in a manner that will ensure even distribution of the test substance throughout the diet. If possible, the test substance should be added to the diet without the use of a carrier or diluent. If a diluent is needed, the preferred diluent is distilled water; but water shall not be used for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent-grade evaporative diluent (e.g., acetone, methylene chloride) and then mixed with the test diet. The solvent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table-grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it shall constitute no more than 2 percent by weight of the treated diet, and an equivalent amount of diluent shall be added to control diets.

v. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(A)(*3*), which is revised to read as follows:

(*3*) Diets may be mixed by commercial or mechanical food mixers. Other means are acceptable as long as they result in even distribution of the test substance throughout the diet. Screening of the basal diet before mixing is suggested to remove large particles. For many test substances, it is recommended that diets be mixed under a hood. Frequently, the test substance is added to an aliquot of the basal diet to form a premix concentrate. The premix concentrate should be stored so as to maintain the chemical concentration. For final preparation of test diets, the premix is mixed with additional basal diet to form the proper concentrations. The frequency with which final treated diets are prepared will depend upon the stability and other characteristics of the test substance. Unless otherwise specified in the test rule or determined by degradation or volatility studies, it is recommended that final diets be prepared weekly, either fresh or from a concentrate. For volatile or labile test substances, test diets shall be mixed frequently enough so that the concentrations are not reduced from initial concentrations by more than 25 percent. Analysis of diets for test substance concentrations is required as specified in paragraph (c)(6)(ii) of this section.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(A)(*4*), which is revised to read as follows:

(*4*) Clean water shall be available *ad libitum*. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water shall be changed daily or more often.

vii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(1)(ii)(B), which is revised to read as follows:

(B) *Young birds.* Young birds produced during the test should be fed a commercial duck starter ration, or its nutritional equivalent. No test substance shall be added to the diets of young birds. No antibiotics or medication shall be used in the diet.

viii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(iii), which is revised to read as follows:

(iii) Pens should be kept indoors in order to better control lighting, temperature, humidity, and other factors. Outdoor pens may be used only during the normal breeding season. The photoperiod should be carefully controlled, preferably by automatic timers. A 15 to 30 minute transition period is desirable. The photoperiod regime is described under paragraph (c)(4)(v) of this section. Lights shall emit a spectrum simulating that of daylight. The use of shorter wave-length "cool-white" fluorescent lights that do not emit the daylight spectrum should be avoided. Illumination intensity should be about 6 foot-candles at the level of the birds.

ix. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(iv), which is revised to read as follows:

(iv) Temperature and humidity should be controlled during the study. Recommended levels are 21°C and 55 percent relative humidity. Temperature for indoor tests shall be recorded at least weekly at the same time of day and shall be reported. For tests conducted without temperature control, temperature minimums and maximums should be recorded daily. Continuous temperature monitoring is desirable. Temperature recording shall be made at levels of 2.5 to 4 cm above the floor of the cage. Recording of approximate humidity levels is also desirable. Good ventilation should be maintained. Suggested ventilation rates are 4 changes per hour in winter and 15 changes per hour in the summer.

b. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears.

PART 798—[AMENDED]

III. In Part 798:

1. The authority citation for Part 798 continues to read as follows:

Authority: 15 U.S.C. 2603.

§ 798.2250 [Amended]

2. Section 798.2250 *Dermal toxicity* is amended as follows:

a. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(1) (ii), (iii), and (iv), (2), (6)(i), (8), (9) (ii), (iii), (iv), (v), and (vi), (10)(i) introductory text, (10)(ii)(A), (11), and (12).

ii. By changing the word "recommended" to "required" wherever it appears in paragraph (e)(2).

iii. By revising paragraph (e)(1)(i), to read as follows:

(i) *Species and strain.* A mammalian species shall be used for testing. The rat, rabbit, or guinea pig may be used, although the albino rabbit is preferred. The albino rabbit is preferred because of its size, skin permeability, and extensive data base. Commonly used laboratory strains shall be employed. If another mammalian species is used, the tester shall provide justification/reasoning for its selection.

iv. By changing the word "should" to "shall" in certain sentences in paragraph (e)(4)(i), which is revised to read as follows:

(i) In subchronic toxicity tests, it is desirable to have a dose-response relationship as well as a no-observed-

toxic-effect level. Therefore, at least 3 dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) shall be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data shall be sufficient to produce a dose-response curve.

v. By revising paragraph (e)(6)(ii), to read as follows:

(ii) Animals in the satellite group scheduled for followup observations should be kept for at least 28 days further without treatment to detect recovery from, or persistence of, toxic effects.

vi. By changing the word "should" to "shall" in certain sentences in paragraph (e)(7)(i), which is revised to read as follows:

(i) Shortly before testing, fur shall be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out approximately 24 hours before the test. Repeat clipping or shaving is usually needed at approximately weekly intervals. When clipping or shaving the fur, care should be taken to avoid abrading the skin, which could alter its permeability.

vii. By changing the words "at least five" to "all" wherever they appear in the introductory text of paragraph (e)(10)(i), and by changing the words "should be" to "shall be" or "are" in certain sentences in paragraph (e)(10)(i) (A) and (B), which are revised to read as follows:

(A) Certain hematology determinations shall be carried out at least two times during the test period: Just prior to initiation of dosing (baseline data), and just prior to terminal sacrifice at the end of the test period. Hematology determinations which are appropriate to all studies: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations on blood shall be carried out at least two times: Just prior to initiation of dosing (if adequate historical baseline data are not available), and just prior to terminal sacrifice at the end of the test period. Test areas which are considered appropriate to all studies: Electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: Calcium, phosphorus, chloride, sodium, potssium, fasting glucose (with the period of fasting appropriate to the species), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include: Analyses of lipids hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

viii. By changing the word "should" to "shall" in paragraph (e)(9)(i), which is revised to read as follows:

(i) Each animal shall be observed daily, and if necessary handled to appraise its physical condition.

ix. By revising the introductory text of paragraph (e)(10)(ii) to read as follows:

(ii) The following examinations shall be made on high dose and control groups. If changes in the eyes are detected all animals should be examined.

x. By revising paragraph (e)(11)(iii), to read as follows:

(iii) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: All gross lesions; lungs—which should be removed intact, weighed, and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure); nasopharyngeal tissues; brain—including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; (skin); gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur—including articular surface); (spinal cord at three levels—cervical, midthoracic, and lumbar); and (zymbal and exorbital lachrymal glands).

b. Paragraph (f) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (f)(1)(i) and (3) and by changing the word "time" to "date" wherever it appears in paragraph (f)(3)(ii) (A) and (B).

§ 798.2450 [Amended]

3. Section 798.2450 *Inhalation toxicity* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (ii), (iii), and (iv)(B), (2), (6)(i), (8) introductory text, and (iv), (10) (iii), (v), and (vi), (11)(i) introductory text, (11)(i)(B), (12) (i) and (ii), and (13).

ii. By revising paragraph (d)(1)(i), to read as follows:

(i) *Species and strain.* A mammalian species shall be used for testing. A variety of rodent species may be used, although the rat is the preferred species. Commonly used laboratory strains shall be employed. If another mammalian species is used, the tester shall provide justification/ reasoning for its selection.

iii. By changing the word "recommended" to "required" wherever it appears in paragraph (d)(2).

iv. By changing the word "should" to "shall" in certain sentences in paragraph (d)(4)(i), which is revised to read as follows:

(i) In subchronic toxicity tests, it is desirable to have a concentration-response relationship as well as a no-observed-toxic-effect level. Therefore, at least 3 concentration levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) shall be used. Concentrations should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a concentration-response curve.

v. By revising paragraph (d)(5), to read as follows:

(5) *Exposure conditions.* The animals should be exposed to the test substance, ideally for 6 hours per day on a 7-day per week basis, for a period of 90 days. However, based primarily on practical considerations, exposure on a 5-day-per-week basis for 6 hours per day is the minimum acceptable exposure period.

vi. By revising paragraph (d)(6)(ii), to read as follows:

(ii) Animals in a satellite group scheduled for followup observations should be kept for at least 28 days further without treatment to detect recovery from, or persistence of, toxic effects.

vii. By changing the word "should" to "shall" in certain sentences in

paragraphs (d)(7) (i) and (ii), which are revised to read as follows:

(i) The animals shall be tested in inhalation equipment designed to sustain a minimum dynamic air flow of 12 to 15 air changes per hour and ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. To ensure stability of a chamber atmosphere, the total "volume" of the test animals shall not exceed 5 percent of the volume of the test chamber. Oronasal or head-only exposure may be used if it is desirable to avoid concurrent exposure by the dermal or oral routes.

(ii) A dynamic inhalation system with a suitable flow control system shall be used. The rate of air flow shall be adjusted to ensure that conditions throughout the exposure chamber are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into surrounding areas.

viii. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(8) (i), (ii), and (iii), which are revised to read as follows:

(i) The rate of air flow shall be monitored continuously and recorded at least every 30 minutes.

(ii) The actual concentrations of the test substance shall be measured in the breathing zone. During the exposure period the actual concentrations of the test substance shall be held as constant as practicable, monitored continuously or intermittently depending on the method of analysis, and recorded at least at the beginning, at an intermediate time, and at the end of the exposure period.

(iii) During the development of the generating system, particle size analysis shall be performed to establish the stability of aerosol concentrations with respect to particle size. During exposure, analysis shall be conducted as often as necessary to determine the consistency of particle size distribution.

ix. By revising paragraphs (d)(9) and (10)(i) to read as follows:

(9) *Food and water during exposure period.* Food shall be withheld during exposure. Water may also be withheld during exposure.

(10) *Observation of animals.* (i) Each animal shall be observed daily and, if necessary, handled to appraise its physical condition.

x. By changing the words "should be" to "shall be" or "are" in certain sentences in paragraph (d)(11)(i)(A), which is revised to read as follows:

(A) Certain hematology determinations shall be carried out at least two times during the test period: just prior to initiation of dosing (if adequate historical baseline data are not available), and just prior to terminal sacrifice at the end of the test period. Hematology determinations which are appropriate to all studies: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

xi. By changing the word "three" to "two" and deleting the phrase "after approximately 30 days on test" in the first sentence in paragraph (d)(11)(i)(B).

xii. By changing the word "should" to "shall" in certain sentences in the introductory text of paragraph (d)(11)(ii) and in (d)(11)(ii)(A), which are revised to read as follows:

(ii) The following examinations shall be made on high dose and control groups. If changes in the eyes are detected, all animals shall be examined:

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, shall be made prior to exposure to the test substance and at the termination of the study.

xiii. By revising paragraph (d)(12)(iii), to read as follows:

(iii) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: All gross lesions; lungs—which should be removed intact, weighed, and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure); nasopharyngeal tissues; brain—including sections of medulla/pons cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; (skin); gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur—including articular surface); (spinal cord at three levels—cervical, midthoracic, and lumbar); and (zymbal and exorbital lachrymal glands).

xiv. By revising paragraph (d)(1)(iv)(A) to read as follows:

(iv) *Numbers.* (A) At least 20 rodents (10 females and 10 males) shall be used for each test group. If another mammalian species selected (e.g. dog, rabbit, or non-human primate), at least 8 animals (4 males and 4 females) shall be used.

xv. By revising paragraph (d)(4)(iv) to read as follows:

(iv) Ideally, the intermediate concentration level(s) should produce minimal observable toxic effects. If more than one intermediate concentration level is used, the concentrations should be spaced to produce a gradation of toxic effects.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(1)(i) and (3).

ii. By changing the word "time" to "date" wherever it appears in paragraph (e)(3)(iv).

§ 798.2650 [Amended]

4. Section 798.2650 *Oral toxicity* is amended as follows:

a. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(1) (ii), (iii) and (iv), (2), (6)(i), (7) (ii) and (iv), (8), (9)(i) introductory text and (ii), (10), and (11).

ii. By revising paragraph (e)(1)(i), to read as follows:

(i) *Species and strain.* A mammalian species shall be used for testing. A variety of rodent species may be used, although the rat is the preferred species. Commonly used laboratory strains shall be employed. The commonly used nonrodent species is the dog, preferably of a defined breed; the beagle is frequently used. If other mammalian species are used, the tester shall provide justification/reasoning for his or her selection.

iii. By changing the word "recommended" to "required" wherever it appears in paragraph (e)(2).

iv. By changing the word "should" to "shall" in certain sentences in paragraph (e)(4)(i), which is revised to read as follows:

(i) In subchronic toxicity tests, it is desirable to have a dose response relationship as well as a no-observed-toxic-effect level. Therefore, at least 3 dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) shall be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data

should be sufficient to produce a dose-response curve.

v. By revising paragraph (e)(6)(ii), to read as follows:

(ii) Animals in the satellite group scheduled for followup observations should be kept for at least 28 days further without treatment to detect recovery from, or persistence of, toxic effects.

vi. By changing the word "may" to "shall" wherever it appears in paragraph (e)(7)(iv) and by revising paragraph (e)(7)(v) to read as follows:

(v) For a substance administered by gavage or capsule, the dose shall be given at approximately the same time each day, and adjusted at intervals (weekly or bi-weekly) to maintain a constant dose level in terms of animal body weight.

vii. By changing the words "at least five" to "all" wherever they appear in the introductory text of paragraph (e)(9)(i), and by changing the words "should be" to "shall be" or "are" in certain sentences in paragraph (e)(9)(i) (A) and (B), which are revised to read as follows:

(A) Certain hematology determinations shall be carried out at least two times during the test period: Just prior to initiation of dosing (baseline data) and just prior to terminal sacrifice at the end of the test period. Hematology determinations which are appropriate to all studies: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations on blood shall be carried out at least two times: Just prior to initiation of dosing (if adequate historical baseline data are not available) and just prior to terminal sacrifice at the end of the test period. Test areas which are considered appropriate to all studies: Electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: Calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with the period of fasting appropriate to the species), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include: analyses of lipids, hormones, acid/base balance, methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

viii. By revising paragraph (e)(8)(i) to read as follows:

(i) Each animal shall be observed daily and, if necessary, handled to appraise its physical condition.

ix. By revising the introductory text of paragraph (e)(9)(ii) to read as follows:

(ii) The following examinations shall be made on high dose and control groups. If changes in the eyes are detected, all animals should be examined.

x. By removing the sentence "If changes in the eyes are detected, all animals should be examined" in paragraph (e)(9)(ii)(A).

xi. By revising paragraph (e)(10)(iii), to read as follows:

(iii) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: All gross lesions; lungs—which should be removed intact, weighed, and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure); nasopharyngeal tissues; brain— including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; (skin); gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur—including articular surface); (spinal cord at three levels—cervical, midthoracic, and lumbar); and (zymbal and exorbital lachrymal glands).

b. Paragraphs (f)(1)(i) and (3) are amended by changing the word "should" to "shall" wherever it appears and changing the word "time" to "date" wherever it appears in paragraph (f)(3)(ii).

§ 798.3300 [Amended]

5. Section 798.3300 *Oncogenicity* is amended as follows:

a. Paragraph (b) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (b)(1) (i), (ii), (iii) and (iv) (A) and (B), (2), (5), (6)(i)(B), (ii)(C), (iii)(C), (7)(ii), (iv), (v), and (vi), (8) (ii) and (iv), (9), (10), and (11).

ii. By removing the phrase "It is recommended that" in the first sentence of paragraph (b)(1)(i) and by removing the phrase "Equal numbers of" in the first sentence of paragraph (b)(1)(iii)(A).

iii. By changing the word "recommended" to "required" wherever it appears in paragraph (b)(2)(i).

iv. By removing the word "only" in the fourth sentence of paragraph (b)(9).

v. By changing the phrase "female mammary gland" to "mammary gland" and by removing the phrase "special studies such as" in paragraph (b)(10)(ii).

vi. By changing the phrase "a necessary requirement" to "required" in paragraph (b)(10)(iii).

vii. By changing the word "should" to "shall" in certain sentences in paragraph (b)(3)(i), which is revised to read as follows:

(i) For risk assessment purposes, at least 3 dose levels shall be used, in addition to the concurrent control group. Dose levels should be spaced to produce a gradation of chronic effects.

viii. The proposed change to paragraph (b)(6)(ii)(B) is not being made based on comments received.

ix. By changing the word "should" to "shall" in certain sentences in paragraph (b)(6)(iii)(A), which is revised to read as follows:

(A) The animals shall be tested with inhalation equipment designed to sustain a minimum dynamic air flow of 12 to 15 air changes per hour, ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. To ensure stability of a chamber atmosphere, the total "volume" of the test animals shall not exceed 5 percent of the volume of the test chamber. Alternatively, oro-nasal, head-only, or whole-body individual chamber exposure may be used.

x. The proposed change to paragraph (b)(6)(iii)(B) is not being made based on comments received.

xi. By changing the word "should" to "shall" in certain sentences in paragraph (b)(7)(iii), which is revised to read as follows:

(iii) Clinical signs and mortality shall be recorded for all animals. Special

attention should be paid to tumor development. The day of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumor shall be recorded.

xii. By revising paragraph (b)(8)(i), to read as follows:

(i) The rate of air flow shall be monitored continuously and recorded at intervals of at least once every 30 minutes.

xiii. By changing the word "should" to "shall" in certain sentences in paragraph (b)(6)(i)(A), which is revised to read as follows:

(A) The animals shall receive the test substance in their diet, dissolved in drinking water at levels that do not exceed the maximum solubility of the test chemical under testing condition.

xiv. By changing the word "should" to "shall" wherever it appears in paragraph (b)(6)(iii)(D), which is revised to read as follows:

(D) A dynamic inhalation system with a suitable flow control system shall be used. The rate of air flow shall be adjusted to ensure that conditions throughout the equipment are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

xv. By changing the word "should" to "shall" wherever it appears in paragraph (b)(7)(i), which is revised to read as follows:

(7) *Observations of animals.* (i) Each animal shall be observed daily and if necessary should be handled to appraise its physical condition.

xvi. By changing the word "should" to "shall" wherever it appears in paragraph (b)(8)(iii), which is revised to read as follows:

(iii) During the development of the generating system, particle size analysis shall be performed to establish the stability of aerosol concentrations with respect to particle size. During exposure, analyses shall be conducted as often as necessary to determine the consistency of particle size, distribution, and homogeneity of the exposure stream.

b. Paragraph (c) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (c)(1), (2) (i) and (iii), and (3) and by changing the words "is no less than" to "should be no less than" wherever they appear in paragraph (c)(2)(iii).

§ 798.4350 [Amended]

6. Section 798.4350 *Inhalation developmental toxicity study* is amended as follows:

a. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e) (1), (2), (3) (i), (ii), and (vi), (4), (5), (7) (i), (ii) and (iii), (9) (i), (iii), (vi), (vii) and (10).

ii. By changing the word "recommended" to "required" in paragraph (e)(1)(iv).

iii. By changing the word "should" to "shall" in certain sentences in paragraphs (e)(6)(i) (A) and (B), which are revised to read as follows:

(A) The animals shall be tested in inhalation equipment designed to substain a minimum dynamic air flow of 12 to 15 air changes per hour and ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. To ensure stability of a chamber atmosphere, the total "volume" of the test animals shall not exceed 5 percent of the volume of the test chamber.

(B) Pregnant animals shall not be subjected to beyond the minimum amount of stress. Since whole-body exposure appears to be the least stressful mode of exposure, it is the method preferred. In general oro-nasal or head-only exposure, which is sometimes used to avoid concurrent exposure by the dermal or oral routes, is not recommended because of the associated stress accompanying the restraining of the animals. However, there may be specific instances where it may be more appropriate than whole-body exposure. The tester shall provide justification/reasoning for its selection.

iv. By revising paragraph (e)(7)(iv), to read as follows:

(iv) Temperature and humidity shall be monitored continuously and be recorded at least every 30 minutes.

v. By changing the word "should" to "shall" in certain sentences in paragraph (e)(9)(iv), which is revised to read as follows:

(iv) Cage-side observations shall include, but not be limited to: Changes in skin and fur, eye and mucous membranes, as well as respiratory, autonomic and central nervous systems, somatomotor activity and behavioral pattern. Particular attention should be directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

vi. By changing the word "should" to "shall" wherever it appears in paragraph (e)(3)(iv), which is revised to read as follows:

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the highest concentration level shall induce some overt maternal toxicity such as reduced body weight or body weight gain, but not more than 10 percent maternal deaths.

vii. By changing the word "should" to "shall" wherever it appears in paragraph (e)(6)(ii), which is revised to read as follows:

(ii) A dynamic inhalation system with a suitable flow control system shall be used. The rate of air flow shall be adjusted to ensure that conditions throughout the exposure chamber are essentially the same. Test material distribution should be established before animals are committed to dosing. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

viii. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(7) (ii) and (iii), which are revised to read as follows:

(ii) The actual concentration of the test substance shall be measured in the breathing zone. During the exposure period the actual concentrations of the test substance shall be held as constant as practicable, monitored continously or intermittently depending on the method of analysis and measured at least at the beginning, at an intermediate time and at the end of the exposure period.

(iii) During the development of the generating system, particle size analysis shall be performed to establish the stability of aerosol concentrations with respect to particle size. During exposure, analysis shall be conducted as often as necessary to determine the consistency of particle size distribution.

ix. By changing the word "should" to "shall" wherever it appears in paragraph (e)(10)(ii), which is revised to read as follows:

(ii) Immediately after sacrifice or death, the uterus shall be removed, weighed, and the contents examined for embryonic or fetal deaths and the number of viable fetuses. Gravid uterine weights should not be obtained from dead animals if autolysis or where decomposition has occurred. The degree of resorption shall be described in order to help estimate the relative time of death.

b. Paragraph (f) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (f) (1) and (3).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (f)(2), which is revised to read as follows:

(2) *Evaluation of results.* The findings of a developmental toxicity study shall be evaluated in terms of the observed effects and the exposure levels producing effects. It is necessary to consider the historical developmental toxicity data on the species/strain tested. A properly conducted developmental toxicity study should provide a satisfactory estimation of a no-effect level.

iii. By changing the word "time" to "date" wherever it appears in paragraphs (f)(3)(iii) (C) and (D).

§ 798.4420 [Amended]

7. Section 798.4420 *Preliminary developmental toxicity screen* is amended as follows:

a. Paragraph (c) is amended by changing the word "should" to "shall" wherever it appears in paragraph (c).

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (ii), (iii) and (iv), (2), (3) (ii) and (iv), (4), (5), (7) (i), (ii), (iii) and (iv) and (8).

ii. By changing the word "pregnant" to "bred" wherever it appears in paragraph (d)(1)(iv).

iii. By replacing the word "neurotoxic" with "other adverse clinical" wherever it appears in paragraph (d)(3)(iv)(A).

iv. By changing the figure "10" to "1" wherever it appears in paragraph (d)(3)(iv)(B).

v. By changing the words "at the same time" to "at approximately the same time" wherever they appear in paragraph (d)(5).

vi. By removing the phrase "During the treatment and observation periods," wherever it appears in paragraph (d)(7)(iii).

vii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(7)(v), which is revised to read:

(v) During the dosing period females that die or are sacrificed because they are moribund shall be examined for signs of pregnancy and details of the conditions of the uterus and/or its contents recorded. Animals that have not delivered two days after expected date of parturition should be sacrificed and similar examinations made.

viii. By revising paragraph (d)(9)(ii) to read as follows:

(ii) Dead pups shall be subjected to a thorough external examination and gross soft tissue abnormalities noted.

c. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears and by changing the word "time" to "date" wherever it appears in paragraphs (e)(3) (iii) and (iv).

§ 798.4700 [Amended]

8. Section 798.4700 *Reproduction and fertility effects* is amended as follows:

a. Paragraph (c) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (c) (1), (2), (3)(i), (4)(i), (5)(i)(B), (6)(i)(C), (7)(i), (ii), (iv), and (v), (8) and (9) and by revising paragraph (c)(2)(iii) to read as follows:

(iii) If a vehicle or other additive is used to facilitate dosing, it shall not interfere significantly with absorption of the test substance or produce toxic effects.

ii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(4)(ii)(A), which is revised to read as follows:

(A) All P males should be sacrificed at the end of the 3-week mating period, or may be retained for possible production of a second litter. If these animals are retained for a second litter, dosing shall be continued.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (c)(6)(i)(A), which is revised to read as follows:

(A) For each mating, each female shall be placed with a single male from the same dose level until pregnancy occurs or 1 week has elapsed. If mating has not occurred after 1 week, the female shall be placed with a different male. Paired matings should be clearly identified.

iv. By changing the word "should" to "shall" in paragraph (c)(6)(iii) which is revised to read as follows:

(iii) *Special housing.* After evidence of copulation, pregnant animals shall be caged separately in delivery or maternity cages. Pregnant animals shall be provided with nesting materials when parturition is near.

v. By changing the word "weekly" to "weekly except during the mating period" in the last sentence of paragraph (c)(7)(i).

vi. By revising paragraph (c)(7)(iii) to read as follows:

(iii) Each litter should be examined as soon as possible after delivery for the number of pups, stillbirths, live births, sex, and the presence of gross anomalies. Live pups should be counted and litters weighed at birth or soon thereafter, and on days 4, 7, 14, and 21 after parturition.

vii. By changing the word "animals" to "adult animals" wherever it appears in paragraph (c)(8)(i).

viii. By changing the words "target organ(s)" to "target organ(s) when previously identified" wherever they appear in paragraph (c)(8)(iii).

ix. By changing the word "should" to "shall" wherever it appears in the paragraph (c)(9) introductory text, which is revised to read as follows:

(9) *Histopathology.* Except if carried out in other studies of comparable duration and dose levels the following histopathology shall be performed:

b. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d) (1) and (3).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2)(i), which is revised to read as follows:

(i) An evaluation of test results, including the statistical analysis, based on the clinical findings, the gross necropsy findings, and the microscopic results shall be made and supplied. This should include an evaluation of the relationship, or lack thereof, between the animals' exposure to the test substance and the incidence and severity of all abnormalities.

iii. By changing the word "time" to "date" wherever it appears in paragraphs (d)(3) (iii) and (v).

§ 798.4900 [Amended]

9. Section 798.4900 *Developmental toxicity study* is amended as follows:

a. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(1), (2), (3)(i), (ii), and (iii), (4), (5), (7)(i), (ii), (iii), (iv), (vi), and (vii), and (8)(i), (iii), (iv), (v), (vi) and (vii).

ii. By changing the word "recommended" to "required" wherever it appears in paragraph (e)(1)(iv).

iii. By revising (e)(3)(iv), to read as follows:

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the highest dose level shall induce some overt maternal toxicity such as reduced body weight or body weight gain, but not more than 10 percent maternal deaths.

iv. By changing the words "at the same time" to "approximately the same time" wherever they appear in paragraph (e)(5).

v. By removing the phrase "During the treatment and observation period," wherever it appears in paragraph (e)(7)(iv).

vi. By revising paragraph (e)(8)(ii) as follows:

(ii) Immediately after sacrifice or as soon as possible after death, the uterus shall be removed and the contents

examined for embryonic or fetal deaths and the number of viable fetuses. The degree of resorption shall be described in order to help estimate the relative time of death of the conceptus. The weight of the gravid uterus should be recorded for dams that are sacrificed. Gravid uterine weights should not be obtained from dead animals if autolysis or decomposition has occurred.

b. Paragraph (f) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (f) (1) and (3).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (f)(2), which is revised to read as follows:

(2) *Evaluation of results.* The findings of a developmental toxicity study shall be evaluated in terms of the observed effects and the exposure levels producing effects. It is necessary to consider the historical developmental toxicity data on the species/strain tested. A properly conducted developmental toxicity study should provide a satisfactory estimation of a no-effect level.

iii. By changing the word "time" to "date" wherever it appears in paragraphs (f)(3) (iii) and (iv).

§ 798.5200 [Amended]

10. Section 798.5200 *Mouse visible specific locus test* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the words "are recommended" to "shall be used" in paragraph (d)(3)(i).

ii. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(3) (i), (ii) and (iv), and (4)(i).

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(5)(i), which is revised to read as follows:

(i) *Vehicle.* When possible, test chemicals should be dissolved or suspended in distilled water or isotonic saline buffered appropriately, if needed, for stability. Water-insoluble chemicals shall be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test compound nor produce major toxic effects. Fresh preparations of the test chemical should be employed.

iv. By revising paragraph (d)(5)(ii), to read as follows:

(ii) *Dose levels.* Usually, only one dose level need be tested. This should be the highest dose tolerated without toxic effects, provided that any temporary sterility induced due to elimination of spermatagonia is of only moderate duration, as determined by a return of males to fertility within 80 days after treatment. For evaluation of dose-response, it is recommended that at least two dose levels be tested.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(1) and (2)(i) and by adding the following sentence to the end of paragraph (e)(1): "Repeated mating cycles should be conducted until the entire spermatogonial cycle has been evaluated and enough offspring have been obtained to meet the power criterion of the assay."

ii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(2)(ii), which is revised to read as follows:

(ii) Nonmutant progeny should be discarded. Mutant progeny shall be subjected to genetic tests for verification.

c. Paragraph (f) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (f) (2) and (5).

ii. By changing the word "should" to "shall" wherever it appears in paragraph (f)(1), which is revised to read as follows:

(1) *Treatment of results.* Data shall be presented in tabular form and shall permit independent analysis of cell stage specific effects and dose dependent phenomena. The data shall be recorded and analyzed in such a way that clusters of identical mutations are clearly identified. The individual mutants detected shall be thoroughly described. In addition, concurrent positive and negative control data, if they are available, shall be tabulated so that it is possible to differentiate between concurrent (when available) and long-term accumulated mutation frequencies.

§ 798.5265 [Amended]

11. Section 798.5265 *Salmonella typhimurium reverse mutation assay* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(5) (i) and (ii) and (6)(ii)(B).

ii. By revising paragraph (d)(6)(i) to read as follows:

(6) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive control reference substances should be dissolved or suspended in an appropriate vehicle and then further diluted in vehicle for use in the assay.

iii. By changing the word "should" to "shall" wherever it appears in paragraph (d)(6)(ii)(C), which is revised to read as follows:

(C) When appropriate, a single positive response shall be confirmed by testing over a narrow range of concentrations.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e) (4) and (5).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(1), which is revised to read as follows:

(1) *Direct plate incorporation method.* For this test without metabolic activation, test chemica1 and 0.1 m1 of a fresh bacterial culture should be added to 2.0 ml of overlay agar. For tests with metabolic activation, 0.5 ml of activation mixture containing an adequate amount of postmitochondrial fraction should be added to the agar overlay after the addition of test chemical and bacteria. Contents of each tube shall be mixed and poured over the surface of a selective agar plate. Overlay agar shall be allowed to solidify before incubation. At the end of the incubation period, revertant colonies per plate shall be counted.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(2)(i), which is revised to read as follows:

(i) For this test with metabolic activation, 0.5 ml of S–9 mix containing 150 ul of S–9 and 0.1 ml of bacterial culture should be added to a test tube kept on ice. One-tenth milliliter of chemical should be added, and the tubes should be incubated with shaking at 30° C for 30 min. At the end of the incubation period, 2.0 ml of agar should be added to each tube, the contents mixed and poured over the surface of a selective agar plate. Overlay agar shall be allowed to solidify before incubation. At the end of the incubation period, revertant colonies per plate shall be counted.

iv. By changing the word "should" to "shall" in certain sentences in paraqraph (e)(2)(ii), which is revised to read as follows:

(ii) For tests without metabolic activation, 0.5 ml of buffer should be used in place of the 0.5 ml of S–9 mix. All other procedures shall be the same as those used for the test with metabolic activation.

v. By revising paragraph (e)(6), to read as follows:

(6) *Number of cultures.* All plating should be done at least in triplicate.

c. Paragraph (f) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (f)(1) and (5).

ii. By changing the words "revertants of a statistically" to "revertants or a statistically" in paragraph (f)(3)(ii).

iii. By revising paragraph (f)(5)(ii) to read as follows:

(ii) Metabolic activation system used (source, amount and cofactor); details of preparations of S–9 mix.

§ 798.5275 [Amended]

12. Section 798.5275 *Sex-linked recessive lethal test in Drosophila melanogaster* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraph (d)(4).

ii. By removing the last sentence of paragraph (d)(4)(iv), which is revised to read as follows:

(iv) *Negative controls.* Negative (vehicle) controls shall be included. The size of the negative (vehicle) control group shall be determined by the availability of appropriate laboratory historical control data.

iii. By revising paragraph (d)(5)(ii), to read as follows:

(ii) *Dose levels.* For the initial assessment of mutagenicity, it is sufficient to test a single dose of the test substance for screening purposes. This dose should be the maximum tolerated dose, or that which produces some indication of toxicity, or shall be the highest dose attainable. For dose-response purposes, at least three additional dose levels should be used.

b. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (e) (1) and (2) and by changing the word "excess" to "appropriate number" wherever it appears in paragraph (e)(1).

c. Paragraph (f) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (f) (1), (2), and (5).

§ 798.5300 [Amended]

13. Section 798.5300 *Detection of gene mutations in somatic cells in culture* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d) (3)(ii), (4), and (5).

ii. By revising paragraph (d)(6)(i), to read as follows:

(i) *Vehicle.* Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. The final concentration of the vehicle shall not interfere with cell viability or growth rate. Treatment vessels should be chosen to ensure that there is no visible interaction, such as etching, between the solvent, the test chemical, and the vessel.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(6)(ii)(B), which is revised to read as follows:

(B) Several concentrations (usually at least 4) of the test substance shall be used. Generally, these shall yield a concentration-related toxic effect. The highest concentration shall produce a low level of survival (approximately 10 percent), and the survival in the lowest concentration shall approximate the negative control. Cytotoxicity shall be determined after treatment with the test substance both in the presence and in the absence of an exogenous metabolic activation system. Relatively insoluble substances should be tested up to their limit of solubility under culture conditions. For freely-soluble nontoxic substances the highest concentration used should be determined on a case-by-case basis.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e) (1) and (2), and by changing the words "without metabolic" to "without exogenous metabolic" wherever it appears in paragraph (e)(1).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(3), which is revised to read as follows:

(3) At the end of the expression period, which shall be sufficient to allow near optimal phenotypic expression of induced mutants, cells should be grown in medium with and without selective agent(s) for determination of number of mutants and cloning efficiency, respectively.

iii. By revising paragraph (e)(4) to read as follows:

(4) Results shall be confirmed in an independent experiment. When appropriate, a single positive response should be confirmed by testing over a narrow range of concentrations.

c. Paragraph (f) is amended by changing the word "should" to "shall" wherever it appears in paragraph (f) (1) and (5).

§ 798.5375 [Amended]

14. Section 798.5375 *In vitro mammalian cytogenetics* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d) (3)(ii), (4), and (5).

ii. By revising paragraph (d)(6)(i), to read as follows:

(i) *Vehicle.* Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. Final concentration of the vehicle shall not interfere with cell viability or growth rate. Treatment vessels should be chosen to ensure that there is no visible interaction, such as etching, between the solvent, the test chemical, and the vessel.

iii. By replacing the phrase "The highest test substance concentration" with "Generally the highest test substance concentrations" and by changing the words "or cytotoxicity" to "of cytotoxicity" wherever they appear in paragraph (d)(6)(ii).

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e) (3) and (4), and by changing the phrase "absence of a metabolic" to "absence of an exogenous metabolic" wherever it appears in paragraph (e)(3).

ii. By revising paragraph (e)(5)(i) to read as follows:

(i) For established cell lines and strains, multiple harvest times are recommended. However, for screening purposes, a single harvest time may be appropriate. If the test chemical changes the cell cycle length, the fixation intervals should be changed accordingly. If a single harvest time is selected, supporting data for the harvest time should be presented in such a study.

iii. The proposed change to paragraph (e)(5)(ii) is not being made based on comments received.

iv. By revising paragraph (e)(5)(iii), to read as follows:

(iii) Cell cultures shall be treated with a spindle inhibitor, (e.g., colchicine or Colcemid®), 1 or 2 hours prior to harvesting. Each culture shall be harvested and processed separately for the preparation of chromosomes.

v. By revising paragraph (e)(7), to read as follows:

(7) *Analysis.* Slides shall be coded before analysis. In human lymphocytes, only cells containing 46 centromeres shall be analyzed. In established cell

lines and strains, only metaphases containing ±2 centromeres of the modal number shall be analyzed. Uniform criteria for scoring aberrations shall be used.

vi. By adding a new paragraph (e)(8) to read as follows:

(8) *Confirmatory tests.* When appropriate, a single positive response shall be confirmed by testing over a narrow range of concentrations.

c. Paragraph (f) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraph (f)(5) and by changing the word "time" to "date" wherever it appears in paragraph (f)(5)(vi).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (f)(1), which is revised to read as follows:

(1) *Treatment of results.* Data shall be presented in a tabular form. Different types of structural chromosomal aberrations shall be listed with their numbers and frequencies for experimental and control groups. Data should be evaluated by appropriate statistical methods. Gaps or achromatic lesions are recorded separately and not included in the total aberration frequency.

§ 798.5385 [Amended]

15. Section 798.5385 *In vivo mammalian bone marrow cytocenetics tests: Chromosomal analysis* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(3) (ii) and (iv) and (4).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iii), which is revised to read as follows:

(iii) *Number and sex.* At least five female and five male animals per experimental and control group shall be used. Thus, 10 animals would be sacrificed per time per group treated with the test compound if several test times after treatment are included in the experimental schedule. The use of a single sex or smaller number of animals should be justified.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(5)(i), which is revised to read as follows:

(i) *Vehicle.* When possible, test chemicals shall be dissolved in isotonic saline or distilled water. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicles used shall neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test compound should be employed.

iv. By revising paragraph (d)(5)(ii), to read as follows:

(ii) *Dose levels.* For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose (to a maximum of 5,000 mg/kg) or that producing some indication of cytotoxicity (e.g., partial inhibition of mitosis) or shall be the highest dose attainable (to a maximum of 5,000 mg/kg). Additional dose levels may be used. For determination of dose-response, at least three dose levels should be used.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e) (1)(ii), (2), and (3) and by changing the word "should" to "shall" in certain sentences in paragraph (e)(1)(i), which is revised to read as follows:

(i) Animals should be treated with the test substance once at the selected dose(s). Samples should be taken at three times after treatment. For rodents, the central sampling interval is 24 hours. Since cell cycle kinetics can be influenced by the test substance, one earlier and one later sampling interval adequately spaced within the range of 6 to 48 hours shall be applied. Where the additional dose levels are tested in a subsequent experiment, samples shall be taken at the predetermined most sensitive interval or, if this is not established, at the central sampling time. If the most sensitive interval is known and documented with data, only this one time point shall be sampled.

ii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(4), which is revised to read as follows:

(4) *Analysis.* The number of cells to be analyzed per animal should be based upon the number of animals used, the negative control frequency, the predetermined sensitivity, and the power chosen for the test. Slides shall be coded before microscopic analysis.

c. Paragraph (f) is amended by changing the word "should" to "shall" wherever it appears in paragraph (f)(5).

§ 798.5395 [Amended]

16. Section 798.5395 *In vivo mammalian bone marrow cytogenetics tests: Micronucleus assay* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(3) (iv) and (d)(4).

ii. By revising paragraph (d)(3)(ii), to read as follows:

(ii) *Age.* Young adult animals shall be used.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iii), which is revised to read as follows:

(iii) *Number and sex.* At least five female and five male animals per experimental and control group shall be used. Thus, 10 animals would be sacrificed per time per group if several test times after treatment were included in the experimental schedule. The use of a single sex or a smaller number of animals should be justified.

iv. By changing the word "should" to "shall" in certain sentences in paragraphs (d)(5) (i) and (ii), which are revised to read as follows:

(i) *Vehicle.* When appropriate for the route of administration, solid and liquid test substances should be dissolved or suspended in distilled water or isotonic saline. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test compound nor produce toxic effects. Fresh preparations of the test compound should be employed.

(ii) *Dose levels.* For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose (to a maximum of 5,000 mg/kg) or that producing some indication of cytotoxicity, e.g., a change in the ratio of polychromatic to normochromatic erythrocytes. Additional dose levels may be used. For determination of dose response, at least three dose levels shall be used.

v. By redesignating the text of paragraph (d)(1) as (d)(1)(i) and adding new paragraphs (d)(1) (ii) and (iii) to read as follows:

(d) *Test method*—(1) *Principle*—

(i) * * *

(ii) Micronuclei may also be detected in other test systems:

(A) Tissue culture.

(B) Plants.

(C) Blood smears.

(D) Fetal tissues.

(E) Meiotic cells.

(F) Hepatic cells.

(iii) The present guideline is based on the mammalian bone marrow assay.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (e)(1) (ii) and (iii).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(1)(i), which is revised to read as follows:

(i) Animals shall be treated with the test substance once at the highest tolerated dose. Sampling times should coincide with the maximum responses of the assay which 149 varies with the test substance. Therefore, using the highest dose, bone marrow samples should be taken at least three times, starting not earlier than 12 hours after treatment, with appropriate intervals following the first sample but not extending beyond 72 hours. When other doses are used sampling shall be at the maximum sensitive period, or, if that is not known, approximately 24 hours after treatment. Other appropriate sampling times may be used in addition. If the most sensitive interval is known and documented with data, only this one time point need be sampled.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (e)(2), which is revised to read as follows:

(2) *Analysis.* Slides shall be coded before microscopic analysis. At least 200 polychromatic erythrocytes per animal shall be scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes should be determined for each animal by counting a total of 200 erythrocytes. To ensure consistency with OECD and other guidelines, 1,000 polychromatic erythrocytes are recommended. Additional information may be obtained by scoring normochromatic erythrocytes for micronuclei.

c. Paragraph (f) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (f) (1) and (5).

d. Paragraph (g)(7) is added to read as follows:

(7) Heddle, J.A., Hite, M., Kurkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., Salamone, M.F. "The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program," *Mutation Research*, 123: 61–118 (1983).

§ 798.5450 [Amended]

17. Section 798.5450 *Rodent dominant lethal assay*, is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(3) (ii) and (iv) and (4).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(3)(iii), which is revised to read as follows:

(iii) *Number.* An adequate number of animals shall be used taking into account the spontaneous variation of the biological characteristics being evaluated. The number chosen should be based on the predetermined sensitivity of detection and power of significance. For example, in a typical experiment, the number of males in each group shall be sufficient to provide between 30 and 50 pregnant females per mating interval.

iii. By changing the word "should" to "shall" in certain sentences in paragraphs (d)(5) (i) and (ii), which are revised to read as follows:

(i) *Vehicle.* When possible, test substances shall be dissolved or suspended in isotonic saline or distilled water. Water-insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels.* Normally, three dose levels shall be used. The highest dose shall produce signs of toxicity (e.g., slightly reduced fertility and slightly reduced body weight). However, in an initial assessment of dominant lethality a single high dose may be sufficient. Nontoxic substances shall be tested at 5g/kg or, if this is not practicable, then as the highest dose attainable.

b. Paragraph (f) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (f) (2) and (5).

ii. By changing the word "should" to "shall" wherever it appears in paragraph (f)(1), which is revised to read as follows:

(1) *Treatment of results.* Data shall be tabulated to show the number of males, the number of pregnant females, and the number of nonpregnant females. Results of each mating, including the identity of each male and female, shall be reported individually. For each female, the dose level and week of mating and the frequencies of live implants and of dead implants shall be enumerated. If the data are recorded as early and late deaths, the tables shall make that clear. If preimplantation loss is estimated, it shall be reported. Preimplantation loss can be calculated as the difference between the number of corpora lutea and the number of implants or as a reduction in the average number of implants per female in comparison with control matings.

iii. By removing current paragraph (f)(5)(vi) and by revising paragraph (f)(5)(vii) to read as follows and by redesignating it as paragraph (f)(5)(vi):

(vi) Criteria for scoring dominant lethals including the number of early and late embryonic deaths.

iv. By redesignating paragraph (f)(5)(viii) as paragraph (f)(5)(vii).

§ 798.5460 [Amended]

18. Section 798.5460 *Rodent heritable translocation assays* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(3) (ii) and (iv) and (4).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(5)(i), which is revised to read as follows:

(i) *Vehicle.* When appropriate for the route of administration, solid and liquid test substances should be dissolved or suspended in distilled water or isotonic saline. Water-insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

iii. By revising paragraph (d)(5)(ii), to read as follows:

(ii) *Dose levels.* At least two dose levels shall be used. The highest dose level shall result in toxic effects (which shall not produce an incidence of fatalities which would prevent a meaningful evaluation) or shall be the highest dose attainable or 5g/kg body weight.

b. Paragraph (e) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in the introductory text to paragraph (e)(2).

ii. By changing the words "should be" and "are" to "shall be" in certain sentences in paragraph (e)(1), which is revised to read as follows:

(1) *Treatment and mating.* The animals shall be dosed with the test substances 7 days per week over a period of 35 days. After treatment, each male shall be caged with 2 untreated females for a period of 1 week. At the end of 1 week, females shall be separated from males and caged individually. When females give birth, the day of birth, litter size, and sex of progeny shall be recorded. All male progeny should be weaned, and all female progeny should be discarded.

c. Paragraph (f) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (f) (1), (2), and (5).

§ 798.6050 [Amended]

19. Section 798.6050 *Functional observational battery* is amended as follows:

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (ii) and (iii)(B), (3), and (8)(ii).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2), which is revised to read as follows:

(2) *Number of animals.* At least eight animals of each sex should be used at each dose level and should be designated for behavioral testing. If interim sacrifices are planned, the number should be increased by the number of animals scheduled to be sacrificed before the end of the study. Animals shall be randomly assigned to treatment and control groups.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(8)(i), which is revised to read as follows:

(i) All animals in a given study should be observed carefully by trained technicians who are blind with respect to the animals' treatments. Standard procedures to minimize observer variability shall be followed. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required. All animals should be observed prior to initiation of exposure. Subsequent observations should be made with sufficent frequency to ensure the detection of behavioral and/or neurologic abnormalities, if present. At minimum, observations at 1 hour, 6 hours, 24 hours, 7 days, and 14 days and monthly thereafter are recommended. In a subchronic study, subsequent to the first exposure all observations should be made before the daily exposure. The animals should be removed from the home cage to a standard arena for observation. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect behavior are sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions. Explicit, operationally defined scales for each function should be used. The development of objective quantitative measures of the observational endpoints specified is encouraged.

b. By changing the word "should" to "shall" in certain sentences in paragraph (e)(3), which is revised to read as follows:

(3) *Evaluation of data.* The findings of a functional observational battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlative histopathological findings. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any neurotoxic effects. The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

§ 798.6200 [Amended]

20. Section 798.6200 *Motor activity* is amended in paragraph (d)(1)(iii)(B) by changing the word "should" to "shall" wherever it appears.

§ 798.6400 [Amended]

21. Section 798.6400 *Neuropathology* is amended as follows:

a. Paragraph (d) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (i) and (iii) and (8) (ii)(C), (iii), and (iv)(B).

b. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears in the introductory text and in paragraph (e)(1).

[FR Doc. 87-11124 Filed 5-19-87; 8:45 am]
BILLING CODE 6560-50-M

---

40 CFR Parts 795 and 799

[OPTS-42033D; FRL 3202-3]

Cresols; Final Test Standards and Reporting Requirements

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is issuing a final rule under section 4(a) of the Toxic Substances Control Act (TSCA) that specifies test standards and reporting requirements for testing of ortho (o), meta (m) and para (p) cresols (CAS 95-48-7, 108-39-4, 107-44-5).

DATES: In accordance with 40 CFR 23.5 (50 FR 7271; February 21, 1985), this rule shall be promulgated for purposes of judicial review at 1 p.m. eastern ["daylight" or "standard" as appropriate] time on June 3, 1987. This rule shall become effective on July 6, 1987.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St. SW., Washington, DC 20460, (202) 554-1404.

SUPPLEMENTARY INFORMATION: In the **Federal Register** of April 28, 1986 (51 FR 15771), EPA issued a final Phase I rule under section 4(a) of TSCA to require testing of cresols for mutagenic effects, developmental toxicity, and reproductive effects. Also, in that issue of the **Federal Register**, EPA issued a proposed Phase II rule which named the test guidelines to be used for testing and proposed that tests be submitted within specific time frames (51 FR 15803; April 28, 1986). The Agency is now promulgating a final Phase II rule specifying these test standards and reporting requirements for this testing. This test rule for cresols is being promulgated under 40 CFR 799.1250.

I. Background

The Phase I final rule specified the testing requirements for cresols: (1) Mutagenic effects studies (including tests for chromosomal aberrations, gene mutations, and cellular transformations) on specified cresol isomers; (2) a developmental toxicity study with each cresol isomer; and (3) a two-generation reproductive effects study with each cresol isomer.

Sections 790.50 and 790.52 of Title 40 of the Code of Federal Regulations (CFR) discuss the test standard rule development procedure. In the case of the cresols test rule, which was initiated under the two-phase process, EPA decided to propose the relevant TSCA test guidelines as the test standards. In addition, EPA proposed that the data from the required studies be submitted within certain time periods. These time periods serve as the data submission deadlines required by TSCA section 4(b)(1). The reasons for this change in the test rule development process for cresols were discussed in the proposed rule.

II. Modifications to the Two-Phase Rulemaking Process

Because EPA proposed certain TSCA guidelines as the test standards and proposed data submission deadlines, persons subject to the Phase I final rule were not required to submit proposed study plans for the required testing or proposed dates for the initiation and completion of that testing. They were, however, still required to submit notices of intent to test or exemption applications in accordance with 40 CFR 790.45.

On July 8, 1986, the Cresols Program Panel (the Panel) of the Chemical Manufacturers Association (CMA) notified EPA of its intent to conduct the testing required in the Phase I test rule for cresols (Ref. 1). In addition, the