

1	Table of Contents	
2	4.0 Test Method Data And Results.....	2
3	4.1 Availability of Original Data Used to Evaluate Test Method Performance	2
4	4.2 BG1Luc ER TA Agonist and Antagonist Reference Standard and Control Data.....	2
5	4.2.1 Agonist E2 Reference Standard	2
6	4.2.2 Agonist DMSO Control Values	3
7	4.2.3 Maximum Fold-Induction	4
8	4.2.4 Weak Agonist Positive Control: Methoxychlor	5
9	4.2.5 Antagonist Ral Reference Standard	6
10	4.2.6 Antagonist DMSO Control Values	7
11	4.2.7 Antagonist E2 Reference Standard.....	8
12	4.2.8 Maximum Fold Reduction of E2 Response During Antagonist Testing.....	9
13	4.2.9 Weak Antagonist Positive Control: Flavone	10
14	4.3 Solubility Test Results	11
15	4.4 Test Results for Coded Test Substances	17
16	4.4.1 Cell Viability Assessment	17
17	4.4.2 BG1Luc ER TA Agonist and Antagonist Data	17
18		
19		

19 **4.0 Test Method Data And Results**

20 This section summarizes the results from testing up to 53 coded reference substances in the three
21 participating laboratories, as well as an additional 25 coded reference substances tested in the lead
22 laboratory, using the agonist and antagonist protocols for the BG1Luc ER TA.

23 **4.1 Availability of Original Data Used to Evaluate Test Method Performance**

24 All data were provided to the validation study project coordinator as electronic MS Excel and GraphPad
25 Prism files. Data files and laboratory reports are available upon request from NICEATM. Requests can be
26 made by mail, fax, or e-mail to Dr. William S. Stokes, NICEATM, NIEHS, P.O. Box 12233, MD K2-16,
27 Research Triangle Park, NC, 27709, (phone) 919-541-2384, (fax) 919-541-0947, (e-mail)
28 niceatm@niehs.nih.gov.

29 **4.2 BG1Luc ER TA Agonist and Antagonist Reference Standard and Control Data**

30 During Phase 1, each laboratory established a historical database for the control and reference substances.
31 The database was used to calculate acceptance criteria using reference standards and controls for use in
32 subsequent study phases. Although E2 reference standard EC₅₀, Ral reference standard IC₅₀,
33 methoxychlor RLU, and flavone RLU values were not used for plate acceptance following Phase 2a of
34 the validation study (see Sections 2.7.1 and 2.7.2), these values were collected throughout the study for
35 information purposes (see **Tables 4-1** through **4-8**). The RLU values for the agonist and antagonist
36 DMSO control and the antagonist E2 control were used for acceptance criteria throughout the study, so
37 were used in the evaluation of intra- and inter-laboratory reproducibility (See **Section 6**). The reported
38 data only represent plates that passed. The total number of plates that were run (combination of number of
39 acceptable plates and plates that failed one or more acceptance criteria) are also reported. Details of the
40 rationale for any plate failures along with their impact on intralaboratory reproducibility are discussed in
41 **Section 6.0**.

42 **4.2.1 Agonist E2 Reference Standard**

43 As indicated in **Table 4-1**, the historical E2 EC₅₀ data collected by each laboratory in Phase 1 ranged from
44 8.47×10^{-12} to 1.13×10^{-11} M on the 10 acceptable plates required to generate the historical database at
45 XDS and Hiyoshi. XDS successfully generated their historical database in 10 consecutive experiments.
46 Hiyoshi required two additional experiments because two plates failed the fold induction acceptance
47 criterion. ECVAM generated data on 18 consecutive experiments due to a concern that a portion of the
48 plates may not pass the acceptance criterion. However, none of these 18 plates failed acceptance and
49 therefore the ECVAM historical database is based on a total of 18 plates. E2 EC₅₀ values collected by

50 each laboratory in subsequent phases of the validation study ranged from 6.15×10^{-12} to 1.74×10^{-11} M
 51 (see **Table 4-1**).

52 **Table 4-1 Summary of Agonist E2 Reference Standard EC₅₀ Data by Study Phase**

Laboratory	Study Phase	Mean EC ₅₀ (M) ^a	SD	N
XDS	1	8.47×10^{-12}	1.66×10^{-12}	10/10
ECVAM	1	8.34×10^{-12}	3.10×10^{-12}	18/18
Hiyoshi	1	1.13×10^{-11}	2.91×10^{-12}	10/12
XDS	2a	9.95×10^{-12}	1.53×10^{-12}	7/15
ECVAM	2a	1.16×10^{-11}	4.07×10^{-12}	6/30
Hiyoshi	2a	8.54×10^{-12}	1.73×10^{-12}	8/9
XDS	2b	9.97×10^{-12}	2.88×10^{-12}	13/13
ECVAM	2b	7.82×10^{-12}	4.80×10^{-12}	12/16
Hiyoshi	2b	1.02×10^{-11}	1.94×10^{-12}	13/16
XDS	3	1.36×10^{-11}	1.28×10^{-11}	34/47
ECVAM	3	1.48×10^{-11}	3.02×10^{-11}	24/35
Hiyoshi	3	6.15×10^{-12}	1.31×10^{-12}	34/34
XDS	4	1.74×10^{-11}	2.66×10^{-11}	29/41

53 Abbreviations: EC₅₀=the half maximal effective concentration; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi =
 54 Hiyoshi Corporation; N = number of plates that passed acceptance criteria/total number of plates; SD = standard deviation; XDS = Xenobiotic
 55 Detection Systems, Inc.

56 ^aThis value was only used as a test plate acceptance criterion during Phase 2a of the validation study. Subsequent to Phase 2a, this value was
 57 monitored but was no longer used to determine whether test plates passed acceptance criteria.

58

59 **4.2.2 Agonist DMSO Control Values**

60 Because DMSO control RLU values are not normalized, they can vary considerably between test plates,
 61 therefore mean plate DMSO RLU values ranged from a low of 511 and a high of 9885, both at XDS
 62 during Phases 3 and 1 respectively, with a mean of 3749 for plates that passed acceptance criteria at all
 63 laboratories (see **Table 4-2**). However, within plate variability of DMSO RLU control values between
 64 replicate DMSO wells was low with associated coefficient of variation (CV) values ranging from 1% to
 65 43% with a mean of 8% (see **Table 4-2**). Of the 218 agonist test plates that passed acceptance criteria,
 66 only 6 plates had within plate CV values greater than 20% (see **Annex L** for individual test plate mean
 67 DMSO control RLU values and associated CV values).

68

69 **Table 4-2 Summary of Agonist Within Plate DMSO Control Data by Study Phase**

Laboratory	Study Phase	Mean and Range of DMSO Control RLU Values	Mean and Range of CV (%)	N
XDS	1	5362 (2031-9885)	7 (5-9)	10/10
ECVAM	1	3519 (1379-6342)	8 (2-14)	18/18
Hiyoshi	1	4213 (2323-6087)	7 (4-15)	10/12
XDS	2a	2271 (636-5114)	10 (3-21)	7/15
ECVAM	2a	2900 (828-5017)	8 (1-17)	6/30
Hiyoshi	2a	4199 (2023-6314)	5 (1-9)	8/9
XDS	2b	2084 (628-4094)	5 (2-10)	13/13
ECVAM	2b	4291 (3256-6209)	6 (3-11)	12/16
Hiyoshi	2b	6291 (4330-8078)	5 (1-10)	13/16
XDS	3	2314 (511-6826)	10 (1-43)	34/47
ECVAM	3	2938 (1097-7306)	10 (3-33)	24/35
Hiyoshi	3	5760 (1362-9383)	6 (1-24)	34/34
XDS	4	2943 (913-5987)	8 (1-17)	29/41
All Laboratories	All Phases	3749 (511-9885)	8 (1-43)	218/286

70 Abbreviations: CV = coefficient of variation; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi
 71 Corporation; N = number of plates that passed acceptance criteria/total number of plates; XDS = Xenobiotic Detection Systems, Inc.

72

73 **4.2.3 Maximum Fold-Induction**

74 As indicated in **Table 4-4**, mean fold induction across the three laboratories throughout the validation
 75 study was 5.72 ± 1.82 . With the exception of Phase 2b, ECVAM consistently reported the highest mean

76 fold induction, and their highest mean value (9.2) was observed during Phase 3. Hiyoshi reported the
77 lowest values in all study phases except Phase 3, where both XDS and Hiyoshi reported similar values
78 (4.3 and 4.9, respectively). The lowest mean fold induction reported during the validation study was 4.0,
79 which was observed at both Hiyoshi (Phase 3) and XDS (Phase 4).

80 **Table 4-4 Summary of Agonist Maximum Fold-Induction Data by Laboratory and Study**
81 **Phase**

Laboratory	Study Phase	Mean Fold Induction ^{a,b}	SD	N
XDS	1	4.7	0.7	10/10
ECVAM	1	8.1	0.9	18/18
Hiyoshi	1	4.5	0.9	10/12
XDS	2a	6.4	2.7	7/15
ECVAM	2a	8.0	1.9	6/30
Hiyoshi	2a	4.4	0.7	8/9
XDS	2b	7.3	2.0	13/13
ECVAM	2b	4.6	0.9	12/16
Hiyoshi	2b	4.0	0.7	13/16
XDS	3	4.3	1.0	34/47
ECVAM	3	9.2	3.0	24/35
Hiyoshi	3	4.9	1.0	34/34
XDS	4	4.0	1.3	29/41
All	all	5.72	1.82	13/13

82 Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; N = number of plates that
83 passed acceptance criteria/total number of plates; RLU = relative light units; SD = standard deviation; XDS = Xenobiotic Detection Systems,
84 Inc.

85 ^aFold induction is measured by dividing the test plate averaged highest E2 reference standard RLU value by the averaged DMSO control mean
86 RLU value (see Section 2.7.1.3)

87 ^bTest plate acceptance criteria for maximum-fold induction state that fold induction must be greater than 3

88

89 4.2.4 Weak Agonist Positive Control: Methoxychlor

90 During the development of the historical methoxychlor control databases, the normalized and adjusted
91 response was highest at Hiyoshi and lowest at ECVAM (Table 4-5) and variability was low in all three
92 laboratories ($CV \leq 17\%$). Variability remained low throughout subsequent phases of the validation study
93 ($CV \leq 23\%$).

94

94 **Table 4-5 Summary of Agonist Methoxychlor Control Data by Laboratory and Study Phase**

Laboratory	Study Phase	Mean Adjusted RLU ^{a,b}	SD	N
XDS	1	5709	974	10/10
ECVAM	1	4494	590	18/18
Hiyoshi	1	7917	430	10/12
XDS	2a	5494	981	7/15
ECVAM	2a	5199	508	6/30
Hiyoshi	2a	8500	424	8/9
XDS	2b	6126	941	13/13
ECVAM	2b	8117	789	12/16
Hiyoshi	2b	7861	854	13/16
XDS	3	6420	1475	35/47
ECVAM	3	6885	1043	24/35
Hiyoshi	3	8029	1579	34/34
XDS	4	5902	1275	29/41

95 Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; N = number of plates that
 96 passed acceptance criteria/total number of plates; RLU = relative light units; SD = standard deviation; XDS = Xenobiotic Detection Systems,
 97 Inc.

98 ^aAgonist test plate data is adjusted by subtracting the DMSO control RLU values from the RLU value for each agonist test plate well. The data is
 99 then normalized by setting the maximum E2 response to 10,000 RLU and adjusting all other RLU values relative to the maximum E2
 100 response.

101 ^bThis value was only used as a test plate acceptance criterion during Phase 2a of the validation study. Subsequent to Phase 2a, test plate
 102 acceptance criteria were modified to state that this value must be greater than the DMSO mean plus three times the standard deviation from
 103 that mean (i.e., the methoxychlor control must be positive).

104

105 **4.2.5 Antagonist Ral Reference Standard**

106 As indicated in **Table 4-6**, the historical Ral IC₅₀ values obtained by each laboratory ranged from
 107 8.43×10^{-10} to 1.23×10^{-9} M. Like the agonist testing, the laboratories were instructed to generate
 108 historical reference standard and control databases based on data generated from at least 10 acceptable
 109 test plates. All three laboratories generated data on more than 10 acceptable test plates due to concerns
 110 that a portion of the plates may not pass the acceptance criterion (i.e., fold induction ≥ 3) which required a
 111 > 3 -fold reduction in E2 control values. The historical databases at ECVAM, Hiyoshi, and XDS were
 112 based on 18, 12, and 14 plates, respectively. None of the runs at ECVAM or Hiyoshi failed the acceptance
 113 criterion, while XDS had a single plate failure. The calculated CV of the Ral IC₅₀ values was within 33%
 114 for all laboratories with the exception of XDS during Phase 3, where a CV value of 60% was observed.

115 **Table 4-6 Summary of Antagonist Ral Reference Standard IC₅₀ Data by Laboratory and**
 116 **Study Phase**

Laboratory	Study Phase	Mean IC ₅₀ (M) ^a	SD	N
XDS	1	8.35×10^{-10}	1.76×10^{-10}	14/15
ECVAM	1	8.43×10^{-10}	1.54×10^{-10}	18/18
Hiyoshi	1	1.23×10^{-9}	2.53×10^{-10}	12/12
XDS	2a	7.43×10^{-10}	2.44×10^{-10}	8/14
ECVAM	2a	8.39×10^{-10}	1.56×10^{-10}	7/14
Hiyoshi	2a	1.23×10^{-9}	3.31×10^{-10}	6/6
XDS	2b	1.06×10^{-9}	1.88×10^{-10}	12/12
ECVAM	2b	1.15×10^{-9}	2.32×10^{-10}	12/18
Hiyoshi	2b	1.48×10^{-9}	1.95×10^{-10}	14/14
XDS	3	1.25×10^{-9}	7.49×10^{-10}	30/59
ECVAM	3	1.84×10^{-9}	4.67×10^{-10}	25/36
Hiyoshi	3	9.94×10^{-10}	1.76×10^{-10}	21/24
XDS	4	5.76×10^{-10}	1.19×10^{-10}	15/23

117 Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; IC₅₀ = half maximal
 118 inhibitory concentration; N = number of plates that passed acceptance criteria/total number of plates; SD = standard deviation; XDS =
 119 Xenobiotic Detection Systems, Inc.

120 ^aThis value was only used as a test plate acceptance criterion during Phase 2a of the validation study. Subsequent to Phase 2a, this value was
 121 monitored but was no longer used to determine whether test plates passed acceptance criteria.

122
 123 **4.2.6 Antagonist DMSO Control Values**

124 Because DMSO control RLU values are not normalized, they can vary considerably between test plates,
 125 therefore mean plate DMSO RLU values ranged from a low of 132 at XDS during Phase 1 and a high of
 126 8451 at Hiyoshi during Phase 3, with a mean of 3299 for plates that passed acceptance criteria at all
 127 laboratories (see **Table 4-7**). However, within plate variability of DMSO RLU control values between
 128 replicate DMSO wells was low with associated CV values ranging from 1% to 52% with a mean of 8%
 129 (see **Table 4-7**). Of the 194 antagonist test plates that passed acceptance criteria, only 8 plates had within
 130 plate CV values greater than 20% (see **Annex L** for individual test plate mean DMSO control RLU values
 131 and associated CV values).

132

132 **Table 4-7 Summary of Antagonist Within Plate DMSO Control Data by Study Phase**

Laboratory	Study Phase	Mean and Range of DMSO Control RLU Values	Mean and Range of CV (%)	N
XDS	1	499 (132-1331)	9 (3-18)	14/15
ECVAM	1	3783 (1490-7333)	8 (3-17)	18/18
Hiyoshi	1	4048 (1625-6541)	5 (3-9)	12/12
XDS	2a	1378 (271-2073)	10 (2-14)	8/14
ECVAM	2a	2154 (1352-5102)	11 (1-23)	7/14
Hiyoshi	2a	4915 (2846-7221)	5 (1-12)	6/6
XDS	2b	1910 (930-2773)	4 (2-9)	12/12
ECVAM	2b	4128 (2522-5102)	7 (1-18)	12/18
Hiyoshi	2b	6280 (4633-7992)	7 (1-20)	14/14
XDS	3	2746 (415-6860)	8 (2-52)	30/59
ECVAM	3	3852 (2615-5498)	12 (4-37)	25/36
Hiyoshi	3	4030 (2018-8451)	7 (1-20)	21/24
XDS	4	3742 (2498-6482)	8 (1-15)	15/23
All Laboratories	All Phases	3299 (132-8451)	8 (1-52)	194/251

133 Abbreviations: CV = coefficient of variation; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi
 134 Corporation; N = number of plates that passed acceptance criteria/total number of plates; XDS = Xenobiotic Detection Systems, Inc.

135
 136

137 **4.2.7 Antagonist E2 Reference Standard**

138 Using the historical data developed by each laboratory during Phase 1, XDS and ECVAM reported
 139 similar normalized E2 responses (8284-8881 mean adjusted RLU), while Hiyoshi was considerably lower

140 (5728 mean adjusted RLU) (Table 4-8). With the exception of Phase 1 Hiyoshi testing (CV = 21%), the
141 calculated CV was no more than 14% at any of the laboratories throughout the study.

142 **Table 4-8 Summary of Antagonist E2 Control Data by Study Phase**

Laboratory	Study Phase	Mean Adjusted RLU ^{a,b}	SD	N
XDS	1	8284	744	14/15
ECVAM	1	8881	640	18/18
Hiyoshi	1	5728	1221	12/12
XDS	2a	8646	783	8/14
ECVAM	2a	9106	554	7/14
Hiyoshi	2a	5767	347	6/6
XDS	2b	8259	711	12/12
ECVAM	2b	9175	725	12/18
Hiyoshi	2b	5270	478	14/14
XDS	3	7851	1065	30/49
ECVAM	3	9584	901	25/36
Hiyoshi	3	6185	521	21/24
XDS	4	7428	662	15/23

143 Abbreviations: E2 = 17 β -estradiol; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; N =
144 number of plates that passed acceptance criteria/total number of plates; RLU = relative light units; SD = standard deviation; XDS = Xenobiotic
145 Detection Systems, Inc.

146 ^aAntagonist test plate data is adjusted by subtracting the DMSO control RLU values from the RLU value each antagonist test plate well. The data
147 is then normalized by setting the maximum Ral response to 10,000 RLU and adjusting all other RLU values relative to the maximum Ral
148 response.

149 ^bThe mean E2 control RLU value must be within the mean plus or minus 2.5 times the SD of the historical mean RLU value for the E2 control
150

151 **4.2.8 Maximum Fold Reduction of E2 Response During Antagonist Testing**

152 As indicated in Table 4-9, mean fold reduction of E2 response across the three laboratories throughout
153 the validation study was 9.56 ± 2.47 . Both the highest (14.2, Phase 1) and lowest (6.5, Phase 3) values
154 reported were from XDS. There was no consistency as to which laboratory reported the highest value in
155 each phase.

156

156 **Table 4-9 Summary of Antagonist Maximum Fold-Reduction Data by Study Phase**

Laboratory	Study Phase	Mean Fold Reduction ^{a,b}	SD	N
XDS	1	14.2	2.4	14/15
ECVAM	1	8.0	0.7	18/18
Hiyoshi	1	7.9	2.3	12/12
XDS	2a	11.1	2.7	8/14
ECVAM	2a	12.1	1.7	7/14
Hiyoshi	2a	11.4	3.2	6/6
XDS	2b	11.4	2.4	12/12
ECVAM	2b	6.6	0.6	12/18
Hiyoshi	2b	10.9	1.6	14/14
XDS	3	6.5	2.5	30/59
ECVAM	3	7.5	1.2	25/36
Hiyoshi	3	9.8	2.1	21/24
XDS	4	7.0	2.3	15/23
ALL	all	9.56	2.47	13/13

157 Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; N = number of plates that
 158 passed acceptance criteria/total number of plates; RLU = relative light units; SD = standard deviation; XDS = Xenobiotic Detection Systems,
 159 Inc.

160 ^aReduction for comprehensive test plates is measured by dividing the averaged highest Ral reference standard RLU value by the lowest averaged
 161 Ral reference standard RLU value (see Section 2.7.2.3).

162 ^bTest plate acceptance criteria for mean-fold reduction state that fold reduction must be greater than 3.

163

164 **4.2.9 Weak Antagonist Positive Control: Flavone**

165 During the development of the historical flavone control databases, the normalized response was highest
 166 at XDS, where the lowest CV (30%) was also observed; the response was lowest at ECVAM where the
 167 highest CV (71%) was also observed (Table 4-10). Variability was lowest at XDS, but high CVs were
 168 seen in all laboratories during the latter phases of the study (CV ranges from 40% to 217%).

169 **Table 4-10 Summary of Antagonist Flavone Control Data by Study Phase**

Laboratory	Study Phase	Mean Adjusted RLU ^{a,b}	SD	N
XDS	1	3583	1089	14/15
ECVAM	1	644	458	18/18
Hiyoshi	1	1226	723	12/12
XDS	2a	3620	753	8/14

Laboratory	Study Phase	Mean Adjusted RLU ^{a,b}	SD	N
ECVAM	2a	733	521	7/14
Hiyoshi	2a	497	203	6/6
XDS	2b	3164	1272	12/12
ECVAM	2b	801	580	12/18
Hiyoshi	2b	87	188	14/14
XDS	3	3081	1627	30/59
ECVAM	3	431	361	25/36
Hiyoshi	3	1302	697	21/24
XDS	4	1444	870	15/23

Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; N = number of plates that passed acceptance criteria/total number of plates; RLU = relative light units; SD = standard deviation; XDS = Xenobiotic Detection Systems, Inc.

^aAntagonist test plate data is adjusted by subtracting the DMSO control mean RLU values from the RLU value each antagonist test plate well. The data is then normalized by setting the maximum Ral response to 10,000 RLU and adjusting all other RLU values relative to the maximum Ral response.

^bThis value was only used as a test plate acceptance criterion during Phase 2a of the validation study. Subsequent to Phase 2a, test plate acceptance criteria were modified to state that this value must be less than the E2 control mean minus three times the standard deviation from that mean (i.e., the flavone control must be positive).

4.3 Solubility Test Results

As indicated in Section 2.5.1, starting concentrations for range finder testing during Phases 2a and 2b were established by determining the maximum soluble test substance concentration at log intervals up to the 1000 µg/mL (v/v in 1% DMSO/EFM) limit concentration. Following Phase 2b comprehensive testing, differences in ER TA antagonist activity were noted across laboratories for two substances (flavone and genistein) that were attributed to differences in solubility. At ECVAM and XDS, 100 µg/mL was considered the maximum soluble concentration for these two substances and therefore used as the starting concentration for range finder testing. Both ultimately tested positive for antagonist activity at concentrations above 10 µg/mL¹. In contrast, Hiyoshi considered 10 µg/mL to be the maximum soluble concentration for these two substances, which was then used as the starting concentration for range finder. Both substances were negative for antagonist activity and were subsequently retested at Hiyoshi up to 100 µg/mL, at which point both were positive. To maximize the likelihood of detecting weak agonists and antagonists, protocols were modified to determine test substance solubility in 100% DMSO as the starting concentration for range finder testing. This protocol modification was used for Phase 3 and 4 range finder

192

¹ ER TA antagonist activity classifications for Phase 2 did not limit the evaluation of concentrations above 1.0×10^{-5} M, see Section 2.4.5.

193 testing. Recognizing that this could result in range finder testing concentrations of substances that
194 precipitate out when added to EFM, the SMT concluded that there would be enough sufficiently soluble
195 concentrations within the seven point log serial dilution to effectively determine the starting
196 concentrations for comprehensive testing. However, differences in the maximum starting concentrations
197 in 100% DMSO were still observed across laboratories (see **Tables 4-11** and **4-12**).

198 Where these differences occurred, comprehensive test results were evaluated to determine if lower
199 starting concentrations were responsible for discordances among the laboratories. This occurred for only
200 three agonist substances (4-androstenedione, 2-*sec*-butylphenol, and flouranthene). 4-Androstendione was
201 negative at ECVAM when using a starting concentration of 10 µg/mL and positive at Hiyoshi when using
202 a starting concentration of 100 µg/mL. 2-*sec*-bultyphenol was negative at ECVAM when using a starting
203 concentration of 100 µg/mL but positive at Hiyoshi at this concentration and positive at XDS when using
204 a starting concentration of 1000 µg/mL. Flouranthene was negative at ECVAM when using a starting
205 concentration of 100 µg/mL but positive at Hiyoshi and XDS when using a starting concentration of 1000
206 µg/mL. (see **Table 4-13** for ER TA agonist testing results).

207

207 **Table 4-11 Agonist Range Finder Starting Concentrations Based on Solubility**

Chemical Name	Study Phase	FW	XDS Max Concentration Tested		ECVAM Max Concentration Tested		Hiyoshi Max Concentration Tested	
			µg/mL	M	µg/mL	M	µg/mL	M
Bisphenol A	2a	228.3	100	4.38 × 10 ⁻⁴	1000	4.38 × 10 ⁻³	1000	4.38 × 10 ⁻³
Bisphenol B	2a	242.3	1000	4.13 × 10 ⁻³	100	4.13 × 10 ⁻⁴	100	4.13 × 10 ⁻⁴
Corticosterone	2a	346.5	1000	2.89 × 10 ⁻³	1000	2.89 × 10 ⁻³	1000	2.89 × 10 ⁻³
Diethylstilbestrol	2a	268.4	100	3.73 × 10 ⁻⁴	100	3.73 × 10 ⁻⁴	10	3.73 × 10 ⁻⁵
17α-Ethinyl estradiol	2b	296.4	100	3.37 × 10 ⁻⁴	100	3.37 × 10 ⁻⁴	10	3.37 × 10 ⁻⁵
Atrazine	2b	215.7	100	4.64 × 10 ⁻⁴	100	4.64 × 10 ⁻⁴	100	4.64 × 10 ⁻⁴
Butylbenzyl phthalate	2b	312.4	100	3.20 × 10 ⁻⁴	10	3.20 × 10 ⁻⁵	10	3.20 × 10 ⁻⁵
Flavone	2b	222.2	100	4.50 × 10 ⁻⁴	100	4.50 × 10 ⁻⁴	100	4.50 × 10 ⁻⁴
Genistein	2b	270.2	100	3.70 × 10 ⁻⁴	100	3.70 × 10 ⁻⁴	100	3.70 × 10 ⁻⁴
<i>o,p'</i> -DDT	2b	354.5	100	2.82 × 10 ⁻⁴	100	2.82 × 10 ⁻⁴	10	2.82 × 10 ⁻⁵
<i>p</i> -n -Nonylphenol	2b	220.4	100	4.54 × 10 ⁻⁴	10	4.54 × 10 ⁻⁵	100	4.54 × 10 ⁻⁴
Vinclozolin	2b	286.1	100	3.50 × 10 ⁻⁴	10	3.50 × 10 ⁻⁵	100	3.50 × 10 ⁻⁴
12 - <i>O</i> -Tetradecanoylphorbol-13-acetate	3	616.8	1000	1.62 × 10 ⁻³	100	1.62 × 10 ⁻⁴	10	1.62 × 10 ⁻⁵
17α-Estradiol	3	272.4	1000	3.67 × 10 ⁻³	1000	3.67 × 10 ⁻³	10	3.67 × 10 ⁻⁵
17β-Estradiol	3	272.4	1000	3.67 × 10 ⁻³	1000	3.67 × 10 ⁻³	10	3.67 × 10 ⁻⁵
2- <i>sec</i> -Butylphenol	3	150.2	1000	6.66 × 10 ⁻³	100	6.66 × 10 ⁻⁴	100	6.66 × 10 ⁻⁴
2,4,5-Trichlorophenoxyacetic acid	3	255.5	1000	3.91 × 10 ⁻³	1000	3.91 × 10 ⁻³	1000	3.91 × 10 ⁻³
4-Androstenedione	3	286.4	100	3.49 × 10 ⁻⁴	10	3.49 × 10 ⁻⁵	100	3.49 × 10 ⁻⁴
4-Cumylphenol	3	212.3	1000	4.71 × 10 ⁻³	1000	4.71 × 10 ⁻³	100	4.71 × 10 ⁻⁴
4-Hydroxytamoxifen	3	387.5	1000	2.58 × 10 ⁻³	100	2.58 × 10 ⁻⁴	10	2.58 × 10 ⁻⁵
4- <i>tert</i> -Octylphenol	3	206.3	1000	4.85 × 10 ⁻³	100	4.85 × 10 ⁻⁴	10	4.85 × 10 ⁻⁵
5α-Dihydrotestosterone	3	290.4	1000	3.44 × 10 ⁻³	10	3.44 × 10 ⁻⁵	10	3.44 × 10 ⁻⁵
Actinomycin D	3	1255.4	1000	7.97 × 10 ⁻⁴	100	7.97 × 10 ⁻⁵	100	7.97 × 10 ⁻⁵
Apigenin	3	270.2	1000	3.70 × 10 ⁻³	1000	3.70 × 10 ⁻³	100	3.70 × 10 ⁻⁴
Clomiphene citrate	3	598.1	1000	1.67 × 10 ⁻³	100	1.67 × 10 ⁻⁴	10	1.67 × 10 ⁻⁵
Coumestrol	3	268.2	1000	3.73 × 10 ⁻³	100	3.73 × 10 ⁻⁴	10	3.73 × 10 ⁻⁵
Daidzein	3	254.2	1000	3.93 × 10 ⁻³	100	3.93 × 10 ⁻⁴	100	3.93 × 10 ⁻⁴
Dexamethasone	3	392.5	1000	2.55 × 10 ⁻³	1000	2.55 × 10 ⁻³	10	2.55 × 10 ⁻⁵
Di - <i>n</i> -butyl phthalate	3	278.3	1000	3.59 × 10 ⁻³	1000	3.59 × 10 ⁻³	100	3.59 × 10 ⁻⁴
Dibenzo[<i>a,h</i>] anthracene	3	278.4	10	3.59 × 10 ⁻⁵	1	3.59 × 10 ⁻⁶	10	3.59 × 10 ⁻⁵
Dicofol	3	370.5	1000	2.70 × 10 ⁻³	1000	2.70 × 10 ⁻³	10	2.70 × 10 ⁻⁵
Diethylhexyl phthalate	3	330.2	1000	3.03 × 10 ⁻³	1000	3.03 × 10 ⁻³	10	3.03 × 10 ⁻⁵
Estrone	3	270.4	1000	3.70 × 10 ⁻³	100	3.70 × 10 ⁻⁴	10	3.70 × 10 ⁻⁵
Ethyl paraben	3	166.2	1000	6.02 × 10 ⁻³	1000	6.02 × 10 ⁻³	100	6.02 × 10 ⁻⁴
Fluoranthene	3	202.3	1000	4.94 × 10 ⁻³	100	4.94 × 10 ⁻⁴	1000	4.94 × 10 ⁻³
Hydroxyflutamide	3	292.2	1000	3.42 × 10 ⁻³	100	3.42 × 10 ⁻⁴	100	3.42 × 10 ⁻⁴
Kaempferol	3	286.2	1000	3.49 × 10 ⁻³	100	3.49 × 10 ⁻⁴	100	3.49 × 10 ⁻⁴
Kepone	3	490.6	1000	2.04 × 10 ⁻³	1000	2.04 × 10 ⁻³	10	2.04 × 10 ⁻⁵
<i>meso</i> -Hexestrol	3	270.4	1000	3.70 × 10 ⁻³	1000	3.70 × 10 ⁻³	100	3.70 × 10 ⁻⁴

continued

209 **Table 4-11 Agonist Range Finder Starting Concentrations (continued)**

Chemical Name	Study Phase	FW	XDS Max Concentration Tested		ECVAM Max Concentration Tested		Hiyoshi Max Concentration Tested	
			µg/mL	M	µg/mL	M	µg/mL	M
Methyl testosterone	3	302.5	1000	3.31 × 10 ⁻³	100	3.31 × 10 ⁻⁴	100	3.31 × 10 ⁻⁴
Morin	3	302.2	1000	3.31 × 10 ⁻³	100	3.31 × 10 ⁻⁴	1000	3.31 × 10 ⁻³
Norethynodrel	3	298.4	1000	3.35 × 10 ⁻³	100	3.35 × 10 ⁻⁴	100	3.35 × 10 ⁻⁴
<i>p,p'</i> -DDE	3	318.0	1000	3.14 × 10 ⁻³	1000	3.14 × 10 ⁻³	10	3.14 × 10 ⁻⁵
<i>p,p'</i> -Methoxychlor	3	345.7	1000	2.89 × 10 ⁻³	1000	2.89 × 10 ⁻³	10	2.89 × 10 ⁻⁵
Phenobarbital	3	232.2	1000	4.31 × 10 ⁻³	100	4.31 × 10 ⁻⁴	NT	NT
Phenolphthalin	3	320.3	1000	3.12 × 10 ⁻³	1000	3.12 × 10 ⁻³	1000	3.12 × 10 ⁻³
Progesterone	3	314.5	100	3.18 × 10 ⁻⁴	100	3.18 × 10 ⁻⁴	10	3.18 × 10 ⁻⁵
Propylthiouracil	3	170.2	1000	5.87 × 10 ⁻³	1000	5.87 × 10 ⁻³	1000	5.87 × 10 ⁻³
Raloxifene HCl	3	510.1	1000	1.96 × 10 ⁻³	100	1.96 × 10 ⁻⁴	10	1.96 × 10 ⁻⁵
Resveratrol	3	228.2	1000	4.38 × 10 ⁻³	100	4.38 × 10 ⁻⁴	100	4.38 × 10 ⁻⁴
Sodium azide	3	65.0	100	1.54 × 10 ⁻³	100	1.54 × 10 ⁻³	100	1.54 × 10 ⁻³
Tamoxifen	3	371.5	100	2.69 × 10 ⁻⁴	100	2.69 × 10 ⁻⁴	10	2.69 × 10 ⁻⁵
Testosterone	3	288.4	1000	3.47 × 10 ⁻³	100	3.47 × 10 ⁻⁴	100	3.47 × 10 ⁻⁴
17β-Trenbolone	4	270.4	1000	3.70 × 10 ⁻³	NT	NT	NT	NT
19-Nortestosterone	4	274.4	1000	3.64 × 10 ⁻³	NT	NT	NT	NT
4-OH Androstenedione	4	302.4	1000	3.31 × 10 ⁻³	NT	NT	NT	NT
Ammonium perchlorate	4	117.5	1000	8.51 × 10 ⁻³	NT	NT	NT	NT
Apomorphine	4	267.3	1000	3.74 × 10 ⁻³	NT	NT	NT	NT
Bicalutamide	4	430.4	1000	2.32 × 10 ⁻³	NT	NT	NT	NT
Chrysin	4	254.2	1000	3.93 × 10 ⁻³	NT	NT	NT	NT
Cycloheximide	4	281.4	1000	3.55 × 10 ⁻³	NT	NT	NT	NT
Cyproterone acetate	4	416.9	1000	2.40 × 10 ⁻³	NT	NT	NT	NT
Fenarimol	4	331.2	1000	3.02 × 10 ⁻³	NT	NT	NT	NT
Finasteride	4	372.5	1000	2.68 × 10 ⁻³	NT	NT	NT	NT
Fluoxymestrone	4	336.4	1000	2.97 × 10 ⁻³	NT	NT	NT	NT
Flutamide	4	276.2	1000	3.62 × 10 ⁻³	NT	NT	NT	NT
Haloperidol	4	375.9	100	2.66 × 10 ⁻⁴	NT	NT	NT	NT
Ketoconazole	4	531.4	10	9.41 × 10 ⁻⁵	NT	NT	NT	NT
L-Thyroxine	4	776.9	1000	1.29 × 10 ⁻³	NT	NT	NT	NT
Linuron	4	249.1	1000	4.01 × 10 ⁻³	NT	NT	NT	NT
Medroxyprogesterone acetate	4	386.5	100	2.59 × 10 ⁻⁴	NT	NT	NT	NT
Mifepristone	4	429.6	1000	2.33 × 10 ⁻³	NT	NT	NT	NT
Nilutamide	4	317.2	1000	3.15 × 10 ⁻³	NT	NT	NT	NT
Oxazepam	4	286.7	1000	3.49 × 10 ⁻³	NT	NT	NT	NT
Pimozide	4	461.6	100	2.17 × 10 ⁻⁴	NT	NT	NT	NT
Procymidone	4	284.1	100	3.52 × 10 ⁻⁴	NT	NT	NT	NT
Reserpine	4	608.7	1000	1.64 × 10 ⁻³	NT	NT	NT	NT
Spironolactone	4	416.6	1000	2.40 × 10 ⁻³	NT	NT	NT	NT

Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; FW = formula weight; Hiyoshi = Hiyoshi Corporation; M = molar; Max = maximum; NT = not tested; XDS = Xenobiotic Detection Systems, Inc.

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213 **Table 4-12 Antagonist Range Finder Starting Concentrations Based on Solubility**

Chemical Name	Study Phase	FW	XDS Max Concentration Tested		ECVAM Max Concentration Tested		Hiyoshi Max Concentration Tested	
			µg/mL	M	µg/mL	M	µg/mL	M
Dibenzo[<i>a,h</i>] anthracene	2a	278.4	10	3.59 × 10 ⁻⁵	10	3.59 × 10 ⁻⁵	10	3.59 × 10 ⁻⁵
<i>p</i> -n -Nonylphenol	2a	220.4	1	4.54 × 10 ⁻⁶	100	4.54 × 10 ⁻⁴	10	4.54 × 10 ⁻⁵
Progesterone	2a	314.5	100	3.18 × 10 ⁻⁴	100	3.18 × 10 ⁻⁴	10	3.18 × 10 ⁻⁵
Tamoxifen	2a	371.5	10	2.69 × 10 ⁻⁵	100	2.69 × 10 ⁻⁴	10	2.69 × 10 ⁻⁵
Apigenin	2b	270.2	100	3.70 × 10 ⁻⁴	10	3.70 × 10 ⁻⁵	10	3.70 × 10 ⁻⁵
Atrazine	2b	215.7	100	4.64 × 10 ⁻⁴	100	4.64 × 10 ⁻⁴	100	4.64 × 10 ⁻⁴
Butylbenzyl phthalate	2b	312.4	100	3.20 × 10 ⁻⁴	10	3.20 × 10 ⁻⁵	10	3.20 × 10 ⁻⁵
Corticosterone	2b	346.5	1000	2.89 × 10 ⁻³	1000	2.89 × 10 ⁻³	100	2.89 × 10 ⁻⁴
Flavone	2b	222.2	100	4.50 × 10 ⁻⁴	100	4.50 × 10 ⁻⁴	10	4.50 × 10 ⁻⁵
Genistein	2b	270.2	100	3.70 × 10 ⁻⁴	100	3.70 × 10 ⁻⁴	10	3.70 × 10 ⁻⁵
<i>o,p'</i> -DDT	2b	354.5	100	2.82 × 10 ⁻⁴	NA	NA	10	2.82 × 10 ⁻⁵
Resveratrol	2b	228.2	100	4.38 × 10 ⁻⁴	100	4.38 × 10 ⁻⁴	100	4.38 × 10 ⁻⁴
12 - <i>O</i> -Tetradecanoylphorbol-13-acetate	3	616.8	1000	1.62 × 10 ⁻³	1000	1.62 × 10 ⁻³	10	1.62 × 10 ⁻⁵
17α-Estradiol	3	272.4	1000	3.67 × 10 ⁻³	100	3.67 × 10 ⁻⁴	10	3.67 × 10 ⁻⁵
17α-Ethinyl estradiol	3	296.4	100	3.37 × 10 ⁻⁴	10	3.37 × 10 ⁻⁵	10	3.37 × 10 ⁻⁵
17β-Estradiol	3	272.4	1000	3.67 × 10 ⁻³	100	3.67 × 10 ⁻⁴	10	3.67 × 10 ⁻⁵
2- <i>sec</i> -Butylphenol	3	150.2	1000	6.66 × 10 ⁻³	1000	6.66 × 10 ⁻³	100	6.66 × 10 ⁻⁴
2,4,5-Trichlorophenoxyacetic acid	3	255.5	1000	3.91 × 10 ⁻³	100	3.91 × 10 ⁻⁴	1000	3.91 × 10 ⁻³
4-Androstenedione	3	286.4	100	3.49 × 10 ⁻⁴	100	3.49 × 10 ⁻⁴	100	3.49 × 10 ⁻⁴
4-Cumylphenol	3	212.3	100	4.71 × 10 ⁻⁴	1000	4.71 × 10 ⁻³	10	4.71 × 10 ⁻⁵
4-Hydroxytamoxifen	3	387.5	100	2.58 × 10 ⁻⁴	100	2.58 × 10 ⁻⁴	10	2.58 × 10 ⁻⁵
4- <i>tert</i> -Octylphenol	3	206.3	1000	4.85 × 10 ⁻³	100	4.85 × 10 ⁻⁴	10	4.85 × 10 ⁻⁵
5α-Dihydrotestosterone	3	290.4	1000	3.44 × 10 ⁻³	100	3.44 × 10 ⁻⁴	10	3.44 × 10 ⁻⁵
Actinomycin D	3	1255.4	1000	7.97 × 10 ⁻⁴	100	7.97 × 10 ⁻⁵	100	7.97 × 10 ⁻⁵
Bisphenol A	3	228.3	1000	4.38 × 10 ⁻³	100	4.38 × 10 ⁻⁴	100	4.38 × 10 ⁻⁴
Bisphenol B	3	242.3	1000	4.13 × 10 ⁻³	100	4.13 × 10 ⁻⁴	100	4.13 × 10 ⁻⁴
Clomiphene citrate	3	598.1	100	1.67 × 10 ⁻⁴	100	1.67 × 10 ⁻⁴	10	1.67 × 10 ⁻⁵
Coumestrol	3	268.2	1000	3.73 × 10 ⁻³	100	3.73 × 10 ⁻⁴	10	3.73 × 10 ⁻⁵
Daidzein	3	254.2	1000	3.93 × 10 ⁻³	100	3.93 × 10 ⁻⁴	10	3.93 × 10 ⁻⁵
Dexamethasone	3	392.5	100	2.55 × 10 ⁻⁴	100	2.55 × 10 ⁻⁴	100	2.55 × 10 ⁻⁴
Di - <i>n</i> -butyl phthalate	3	278.3	1000	3.59 × 10 ⁻³	1000	3.59 × 10 ⁻³	10	3.59 × 10 ⁻⁵
Dicofol	3	370.5	10	2.70 × 10 ⁻⁵	1000	2.70 × 10 ⁻³	10	2.70 × 10 ⁻⁵
Diethylhexyl phthalate	3	330.2	100	3.03 × 10 ⁻⁴	1000	3.03 × 10 ⁻³	10	3.03 × 10 ⁻⁵
Diethylstilbestrol	3	268.4	100	3.73 × 10 ⁻⁴	100	3.73 × 10 ⁻⁴	10	3.73 × 10 ⁻⁵
Estrone	3	270.4	100	3.70 × 10 ⁻⁴	10	3.70 × 10 ⁻⁵	10	3.70 × 10 ⁻⁵
Ethyl paraben	3	166.2	1000	6.02 × 10 ⁻³	1000	6.02 × 10 ⁻³	1000	6.02 × 10 ⁻³
Fluoranthene	3	202.3	1000	4.94 × 10 ⁻³	100	4.94 × 10 ⁻⁴	10	4.94 × 10 ⁻⁵
Hydroxyflutamide	3	292.2	1000	3.42 × 10 ⁻³	1000	3.42 × 10 ⁻³	100	3.42 × 10 ⁻⁴
Kaempferol	3	286.2	100	3.49 × 10 ⁻⁴	100	3.49 × 10 ⁻⁴	10	3.49 × 10 ⁻⁵
Kepona	3	490.6	1000	2.04 × 10 ⁻³	1000	2.04 × 10 ⁻³	10	2.04 × 10 ⁻⁵

214 **Table 4-12 Antagonist Range Finder Starting Concentrations (continued)**

Chemical Name	Study Phase	FW	XDS Max Concentration Tested		ECVAM Max Concentration Tested		Hiyoshi Max Concentration Tested	
			µg/mL	M	µg/mL	M	µg/mL	M
<i>meso</i> -Hexestrol	3	270.4	100	3.70×10^{-4}	100	3.70×10^{-4}	10	3.70×10^{-5}
Methyl testosterone	3	302.5	1000	3.31×10^{-3}	1000	3.31×10^{-3}	100	3.31×10^{-4}
Morin	3	302.2	1000	3.31×10^{-3}	100	3.31×10^{-4}	100	3.31×10^{-4}
Norethynodrel	3	298.4	1000	3.35×10^{-3}	1000	3.35×10^{-3}	10	3.35×10^{-5}
<i>p,p'</i> -DDE	3	318.0	1000	3.14×10^{-3}	100	3.14×10^{-4}	10	3.14×10^{-5}
<i>p,p'</i> -Methoxychlor	3	345.7	10	2.89×10^{-5}	1000	2.89×10^{-3}	10	2.89×10^{-5}
Phenobarbital	3	232.2	1000	4.31×10^{-3}	1000	4.31×10^{-3}	NT	NT
Phenolphthalin	3	320.3	1000	3.12×10^{-3}	1000	3.12×10^{-3}	1000	3.12×10^{-3}
Propylthiouracil	3	170.2	1000	5.87×10^{-3}	1000	5.87×10^{-3}	100	5.87×10^{-4}
Raloxifene HCl	3	510.1	10	1.96×10^{-5}	100	1.96×10^{-4}	10	1.96×10^{-5}
Sodium azide	3	65.0	100	1.54×10^{-3}	100	1.54×10^{-3}	100	1.54×10^{-3}
Testosterone	3	288.4	1000	3.47×10^{-3}	1000	3.47×10^{-3}	100	3.47×10^{-4}
Vinclozolin	3	286.1	1000	3.50×10^{-3}	100	3.50×10^{-4}	10	3.50×10^{-5}
17β-Trenbolone	4	270.4	1000	3.70×10^{-3}	NT	NT	NT	NT
19-Nortestosterone	4	274.4	1000	3.64×10^{-3}	NT	NT	NT	NT
4-OH Androstenedione	4	302.4	100	3.31×10^{-4}	NT	NT	NT	NT
Ammonium perchlorate	4	117.5	1000	8.51×10^{-3}	NT	NT	NT	NT
Apomorphine	4	267.3	1000	3.74×10^{-3}	NT	NT	NT	NT
Bicalutamide	4	430.4	1000	2.32×10^{-3}	NT	NT	NT	NT
Chrysin	4	254.2	1000	3.93×10^{-3}	NT	NT	NT	NT
Cycloheximide	4	281.4	1000	3.55×10^{-3}	NT	NT	NT	NT
Cyproterone acetate	4	416.9	1000	2.40×10^{-3}	NT	NT	NT	NT
Fenarimol	4	331.2	1000	3.02×10^{-3}	NT	NT	NT	NT
Finasteride	4	372.5	100	2.68×10^{-4}	NT	NT	NT	NT
Fluoxymestrone	4	336.4	100	2.97×10^{-4}	NT	NT	NT	NT
Flutamide	4	276.2	1000	3.62×10^{-3}	NT	NT	NT	NT
Haloperidol	4	375.9	100	2.66×10^{-4}	NT	NT	NT	NT
Ketoconazole	4	531.4	100	1.88×10^{-4}	NT	NT	NT	NT
L-Thyroxine	4	776.9	100	1.29×10^{-4}	NT	NT	NT	NT
Linuron	4	249.1	1000	4.01×10^{-3}	NT	NT	NT	NT
Medroxyprogesterone acetate	4	386.5	10	2.59×10^{-5}	NT	NT	NT	NT
Mifepristone	4	429.6	1000	2.33×10^{-3}	NT	NT	NT	NT
Nilutamide	4	317.2	1000	3.15×10^{-3}	NT	NT	NT	NT
Oxazepam	4	286.7	1000	3.49×10^{-3}	NT	NT	NT	NT
Pimozide	4	461.6	100	2.17×10^{-4}	NT	NT	NT	NT
Procymidone	4	284.1	100	3.52×10^{-4}	NT	NT	NT	NT
Reserpine	4	608.7	100	1.64×10^{-4}	NT	NT	NT	NT
Spirolactone	4	416.6	1000	2.40×10^{-3}	NT	NT	NT	NT

Abbreviations: ECVAM = European Centre for the Validation of Alternative Methods; FW = formula weight; Hiyoshi = Hiyoshi Corporation; M = molar; Max = maximum; NT = not tested; XDS = Xenobiotic Detection Systems, Inc.

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218 4.4 Test Results for Coded Test Substances

219 4.4.1 Cell Viability Assessment

220 An assessment of cell viability was made in order to determine if reduction of ER TA activity is the result
221 of cell loss. The visual observation method described in **Section 2.4.3** was used to assess cell viability in
222 all wells of the test plates. Cell viability results from range finder testing were used to establish starting
223 concentrations for comprehensive testing (see **Section 2.4.2**) and to identify cytotoxic concentrations in
224 comprehensive testing, which was of particular importance in antagonist testing since it is critical for
225 distinguishing whether reduction of ER TA activity is caused by cell loss or ER antagonism. The lowest
226 concentrations that produced cell viability scores of 2 or greater for each substance evaluated in agonist
227 and antagonist range finder and comprehensive testing are provided in **Annex G3**. Results were evaluated
228 to determine if differences in cell viability were responsible for ER TA activity discordances among the
229 laboratories. Ten substances were identified that were discordant for ER TA agonist activity (atrazine,
230 corticosterone, flavone, vinclozolin, 2-sec-butylphenol, 4-androstenedione, clomiphene citrate, dicofol,
231 fluoranthene, and resveratrol see **Table 4-11**) but evaluation of range finder and comprehensive testing
232 results indicated that the discordance was not due to differences in cell viability. Three substances were
233 identified that were positive for ER TA antagonist activity at one laboratory but negative at the other two
234 laboratories (17- α estradiol was positive at XDS but negative at ECVAM and Hiyoshi, and clomiphene
235 citrate and diethylstilbestrol were positive at Hiyoshi and negative at XDS and ECVAM, see **Table 4-12**).
236 However, all cells for these substances were viable below the 1.0×10^{-5} M limit concentration for
237 determining ER TA antagonist activity, indicating that the discordance was not due to differences in cell
238 viability.

239 4.4.2 BG1Luc ER TA Agonist and Antagonist Data

240 Test substances were evaluated in a phased approach as follows:

- 241 • Phase 2a - four coded agonist and four coded antagonist substances were tested independently
242 at least three times at each laboratory.
- 243 • Phase 2b – eight coded agonist and eight coded antagonist substances were tested
244 independently at least three times at each laboratory
- 245 • Phase 3 - up to 41 coded agonist and 41 coded antagonist substances were tested at least once
246 at each laboratory.
- 247 • Phase 4 - the lead laboratory (XDS) tested 25 coded substances (once each) to further
248 characterize the ICCVAM Reference Substances for several substances for which no *in vitro*
249 ER TA data were previously available.

250 The results from Phases 2 and 3 are provided in **Table 4-11** (agonist) and **Table 4-12** (antagonist). **Table**
251 **4-13** provides the Phase 4 data generated by the lead laboratory.

252 **Table 4-13 Agonist Summary Data for Phases 2a, 2b, and 3**

Chemical	Phase	Laboratory	EC ₅₀ (M)	SD	CV (%)	# Plates for EC ₅₀ / # Plates Tested ^a	Classification ^b
Bisphenol A	2a	XDS	3.86×10^{-7}	3.27×10^{-8}	8	3/8	P (3/3)
		ECVAM	8.18×10^{-7}	2.53×10^{-8}	3	3/16	P (3/3)
		Hiyoshi	3.95×10^{-7}	1.86×10^{-8}	5	3/4	P (3/3)
Bisphenol B	2a	XDS	1.60×10^{-7}	2.56×10^{-8}	16	3/7	P (3/3)
		ECVAM	1.74×10^{-7}	5.25×10^{-8}	30	3/14	P (3/3)
		Hiyoshi	2.52×10^{-7}	7.44×10^{-9}	3	3/4	P (3/3)
Corticosterone	2a	XDS	-	-	-	0/8	N (3/3)
		ECVAM	NC	-	-	0/16	P (3/3)
		Hiyoshi	-	-	-	0/4	N (4/4)
Diethylstilbestrol	2a	XDS	4.87×10^{-11}	1.98×10^{-11}	41	3/9	P (3/3)
		ECVAM	3.60×10^{-11}	2.55×10^{-11}	71	2/14	P (3/3)
		Hiyoshi	2.07×10^{-11}	7.97×10^{-12}	39	4/4	P (4/4)
Atrazine	2b	XDS	-	-	-	4/6	N (4/4)
		ECVAM	7.43×10^{-5}	1.25×10^{-4}	168	3/11	P (3/3)
		Hiyoshi	-	-	-	0/4	N (3/3)
Butylbenzyl phthalate	2b	XDS	1.18×10^{-6}	3.57×10^{-7}	30	3/3	P (3/3)
		ECVAM	2.17×10^{-6}	9.92×10^{-7}	46	3/3	P (3/3)
		Hiyoshi	2.92×10^{-6}	3.69×10^{-7}	13	2/3	P (3/3)
<i>o,p'</i> -DDT	2b	XDS	6.12×10^{-8}	1.87×10^{-8}	30	3/3	P (3/3)
		ECVAM	4.22×10^{-7}	6.20×10^{-8}	15	3/5	P (3/3)
		Hiyoshi	6.98×10^{-7}	9.19×10^{-8}	13	3/3	P (3/3)
17- α Ethinyl estradiol	2b	XDS	7.60×10^{-12}	2.32×10^{-12}	31	4/7	P (4/4)
		ECVAM	5.85×10^{-12}	1.44×10^{-12}	25	3/3	P (3/3)
		Hiyoshi	8.38×10^{-12}	1.99×10^{-12}	24	3/4	P (3/3)
Flavone	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	7.05×10^{-6}	8.82×10^{-7}	13	3/5	P (3/3)
		Hiyoshi	NC	-	-	0/4	P (3/3)
Genistein	2b	XDS	2.09×10^{-8}	6.01×10^{-9}	29	3/3	P (3/3)
		ECVAM	3.00×10^{-7}	3.24×10^{-8}	11	3/5	P (3/3)
		Hiyoshi	4.39×10^{-7}	1.76×10^{-7}	40	4/5	P (4/4)
<i>p-n</i> -nonylphenol	2b	XDS	1.78×10^{-6}	6.95×10^{-8}	4	3/6	P (3/3)

Chemical	Phase	Laboratory	EC ₅₀ (M)	SD	CV (%)	# Plates for EC ₅₀ / # Plates Tested ^a	Classification ^b
		ECVAM	2.50×10^{-6}	1.06×10^{-6}	43	3/5	P (3/3)
		Hiyoshi	5.83×10^{-6}	2.89×10^{-7}	5	2/4	P (3/3)
Vinclozolin	2b	XDS	-	-	-	0/6	N (4/4)
		ECVAM	4.45×10^{-6}	3.57×10^{-6}	80	3/8	P (6/6)
		Hiyoshi	-	-	-	0/5	N (4/4)
Actinomycin D	3	XDS	-	-	-	0/3	I (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
4-Androstenedione	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	NC	-	-	0/1	P (1/1)
Apigenin	3	XDS	2.74×10^{-6}	-	-	1/1	P (1/1)
		ECVAM	1.63×10^{-6}	1.09×10^{-6}	67	3/4	P (3/3)
		Hiyoshi	1.62×10^{-6}	-	-	1/1	P (1/1)
Clomiphene citrate	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	4.38×10^{-8}	-	-	1/1	P (1/1)
Coumestrol	3	XDS	2.40×10^{-12}	-	-	1/3	P (1/1)
		ECVAM	2.58×10^{-7}	-	-	1/4	P (1/1)
		Hiyoshi	5.00×10^{-9}	-	-	1/1	P (1/1)
4-Cumylphenol	3	XDS	2.62×10^{-7}	-	-	1/1	P (1/1)
		ECVAM	3.03×10^{-7}	-	-	1/1	P (1/1)
		Hiyoshi	3.95×10^{-7}	-	-	1/1	P (1/1)
Daidzein	3	XDS	6.84×10^{-7}	-	-	1/1	P (1/1)
		ECVAM	1.19×10^{-6}	-	-	1/1	P (1/1)
		Hiyoshi	7.39×10^{-7}	-	-	1/1	P (1/1)
Dibenzo[<i>a,h</i>] anthracene	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/2	N (2/2)
Di - <i>n</i> -butyl phthalate	3	XDS	NC	-	-	0/1	P (1/1)
		ECVAM	1.91×10^{-7}	-	-	1/1	P (1/1)
		Hiyoshi	7.98×10^{-6}	6.60×10^{-7}	8	2/2	P (2/2)
<i>p,p'</i> -DDE	3	XDS	-	-	-	0/4	I (2/2)
		ECVAM	-	-	-	0/1	I (1/1)
		Hiyoshi	-	-	-	0/4	N (4/4)
Diethylhexyl phthalate	3	XDS	NC	-	-	0/1	P (1/1)
		ECVAM	-	-	-	0/1	I (1/1)
		Hiyoshi	-	-	-	0/1	I (1/1)
Dexamethasone	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	9.63×10^{-6}	-	-	1/1	P (1/1)

Chemical	Phase	Laboratory	EC ₅₀ (M)	SD	CV (%)	# Plates for EC ₅₀ / # Plates Tested ^a	Classification ^b
		Hiyoshi	-	-	-	0/1	N (1/1)
5 α -Dihydrotestosterone	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	I (1/1)
		Hiyoshi	8.97×10^{-8}	2.56×10^{-8}	29	2/2	P (2/2)
Dicofol	3	XDS	2.22×10^{-6}	-	-	1/1	P (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	NC	-	-	0/1	P (1/1)
17- α Estradiol	3	XDS	4.85×10^{-12}	-	-	1/2	P (1/1)
		ECVAM	2.46×10^{-9}	3.53×10^{-9}	143	3/4	P (3/3)
		Hiyoshi	3.32×10^{-10}	-	-	1/1	P (1/1)
17- β Estradiol	3	XDS	1.34×10^{-11}	-	-	1/2	P (1/1)
		ECVAM	NC	-	-	0/2	P (1/1)
		Hiyoshi	3.37×10^{-12}	-	-	1/1	P (1/1)
Ethyl paraben	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	3.19×10^{-5}	-	-	1/2	P (1/1)
		Hiyoshi	2.12×10^{-5}	1.96×10^{-6}	9	2/2	P (1/1)
Estrone	3	XDS	3.52×10^{-10}	-	-	1/1	P (1/1)
		ECVAM	2.36×10^{-10}	-	-	1/2	P (1/1)
		Hiyoshi	1.82×10^{-10}	-	-	1/2	P (1/1)
Fluoranthene	3	XDS	2.03×10^{-5}	-	-	1/1	P (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	9.30×10^{-6}	-	-	1/1	P (1/1)
<i>meso</i> -Hexestrol	3	XDS	2.36×10^{-11}	-	-	1/2	P (1/1)
		ECVAM	1.16×10^{-11}	-	-	1/4	P (1/1)
		Hiyoshi	1.53×10^{-11}	3.77×10^{-12}	25	2/2	P (2/2)
Hydroxyflutamide	3	XDS	-	-	-	0/6	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Kepone	3	XDS	9.19×10^{-7}	-	-	1/2	P (1/1)
		ECVAM	1.23×10^{-7}	-	-	1/1	P (1/1)
		Hiyoshi	4.32×10^{-7}	-	-	1/1	P (1/1)
Kaempferol	3	XDS	7.65×10^{-6}	-	-	1/2	P (1/1)
		ECVAM	NC	-	-	0/1	P (1/1)
		Hiyoshi	3.35×10^{-7}	-	-	1/1	P (1/1)
<i>p,p'</i> -Methoxychlor	3	XDS	2.88×10^{-6}	-	-	1/4	P (2/2)
		ECVAM	1.22×10^{-6}	-	-	1/1	P (1/1)
		Hiyoshi	1.80×10^{-6}	1.09×10^{-6}	61	2/2	P (2/2)
Morin	3	XDS	2.62×10^{-5}	-	-	1/2	P (1/1)
		ECVAM	2.68×10^{-5}	-	-	1/1	P (1/1)

Chemical	Phase	Laboratory	EC ₅₀ (M)	SD	CV (%)	# Plates for EC ₅₀ / # Plates Tested ^a	Classification ^b
		Hiyoshi	4.80×10^{-5}	-	-	1/1	P (1/1)
Methyl testosterone	3	XDS	5.22×10^{-7}	4.50×10^{-7}	86	3/6	P (3/3)
		ECVAM	1.25×10^{-5}	-	-	1/1	P (1/1)
		Hiyoshi	2.36×10^{-6}	-	-	1/2	P (2/2)
Norethynodrel	3	XDS	1.39×10^{-9}	7.25×10^{-10}	52	2/4	P (2/2)
		ECVAM	3.65×10^{-10}	-	-	1/2	P (1/1)
		Hiyoshi	6.03×10^{-10}	-	-	1/2	P (2/2)
4-tert-Octylphenol	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	5.38×10^{-8}	-	-	1/1	P (1/1)
		Hiyoshi	1.01×10^{-8}	-	-	1/3	P (3/3)
4-Hydroxy-tamoxifen	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/3	N (3/3)
Phenobarbital	3	XDS	-	-	-	0/4	N (2/2)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	NT	NT	NT	0/0	NT
Phenolphthalein	3	XDS	2.40×10^{-5}	-	-	1/2	P (1/1)
		ECVAM	9.99×10^{-5}	-	-	1/1	P (1/1)
		Hiyoshi	8.33×10^{-5}	1.24×10^{-5}	15	2/2	P (2/2)
Progesterone	3	XDS	5.06×10^{-6}	-	-	1/4	P (2/2)
		ECVAM	1.27×10^{-6}	-	-	1/1	P (1/1)
		Hiyoshi	1.18×10^{-6}	5.08×10^{-7}	43	1/2	P (2/2)
Propylthiouracil	3	XDS	-	-	-	0/3	N (2/2)
		ECVAM	-	-	-	0/3	I (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Raloxifene HCl	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/2	N (2/2)
Resveratrol	3	XDS	3.97×10^{-6}	-	-	1/2	P (1/1)
		ECVAM	-	-	-	0/1	I (1/1)
		Hiyoshi	-	-	-	0/3	N (3/3)
Sodium azide	3	XDS	-	-	-	0/4	N (3/3)
		ECVAM	-	-	-	0/3	I (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
2-sec-Butylphenol	3	XDS	1.18×10^{-9}	-	-	1/1	P (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	2.95×10^{-5}	8.24×10^{-6}	28	2/2	P (2/2)
Tamoxifen	3	XDS	-	-	-	0/2	I (1/1)
		ECVAM	-	-	-	0/1	I (1/1)

Chemical	Phase	Laboratory	EC ₅₀ (M)	SD	CV (%)	# Plates for EC ₅₀ / # Plates Tested ^a	Classification ^b
		Hiyoshi	6.73×10^{-8}	-	-	1/2	P (2/2)
2,4,5-Trichloro-phenoxyacetic acid	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/2	N (2/2)
Testosterone	3	XDS	4.88×10^{-7}	5.77×10^{-7}	118	3/4	P (3/3)
		ECVAM	NC	-	-	0/1	P (1/1)
		Hiyoshi	9.95×10^{-5}	-	-	1/2	P (2/2)
12 - O - Tetradecanoylphorbol-13-acetate	3	XDS	-	-	-	0/5	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)

Abbreviations: # = number; CV = coefficient of variation; ECVAM = European Centre for the Validation of Alternative Methods; EC₅₀ = the half maximal effective concentration; Hiyoshi = Hiyoshi Corporation; I = inadequate (positive or negative classification could not be determined because of poor quality data); M = molar; N = negative; NC = not calculated; NT = not tested; P = positive; SD = standard deviation; XDS = Xenobiotic Detection Systems, Inc.

^aNumber of acceptable plates used to determine the EC₅₀ value vs. the total number of plates tested (includes all acceptable and non-acceptable plates)

^bNumber in parentheses represents test results (P, N, or I) over the total number of acceptable trials.

Table 4-14 Antagonist Summary Data for Phases 2a, 2b, and 3

Chemical	Phase	Laboratory	IC ₅₀ (M)	SD	CV (%)	# Plates for IC ₅₀ / # Plates Tested ^a	Classification ^b
Dibenzo[<i>a,h</i>] anthracene	2a	XDS	NC	-	-	0/6	P (3/3)
		ECVAM	NC	-	-	0/4	P (3/3)
		Hiyoshi	NC	-	-	0/3	P (3/3)
Progesterone	2a	XDS	-	-	-	0/6	N (3/3)
		ECVAM	-	-	-	0/4	N (3/3)
		Hiyoshi	-	-	-	0/3	N (3/3)
<i>p-n</i> -nonylphenol	2a	XDS	-	-	-	0/6	N (3/3)
		ECVAM	-	-	-	0/4	N (2/3)
		Hiyoshi	-	-	-	0/3	N (3/3)
Tamoxifen	2a	XDS	8.28×10^{-7}	2.36×10^{-7}	29	4/8	P (4/4)
		ECVAM	4.31×10^{-7}	2.69×10^{-7}	6	3/10	P (3/3)
		Hiyoshi	1.19×10^{-6}	3.67×10^{-6}	31	3/3	P (3/3)
Apigenin	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	-	-	-	0/5	N (3/3)
		Hiyoshi	-	-	-	0/4	N (4/4)
Atrazine	2b	XDS	-	-	-	0/5	N (4/4)
		ECVAM	-	-	-	0/5	N (3/3)
		Hiyoshi	-	-	-	0/3	N (3/3)
Butylbenzyl phthalate	2b	XDS	-	-	-	0/3	N (3/3)

Chemical	Phase	Laboratory	IC ₅₀ (M)	SD	CV (%)	# Plates for IC ₅₀ / # Plates Tested ^a	Classification ^b
		ECVAM	-	-	-	0/4	N (3/3)
		Hiyoshi	-	-	-	0/4	N (4/4)
Corticosterone	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	-	-	-	0/4	N (3/3)
		Hiyoshi	-	-	-	0/3	N (3/3)
o,p'-DDT	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	-	-	-	0/4	N (3/3)
		Hiyoshi	-	-	-	0/4	N (4/4)
Flavone	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	-	-	-	0/5	N (3/3)
		Hiyoshi	-	-	-	0/4	N (4/4)
Genistein	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	-	-	-	0/4	N (3/3)
		Hiyoshi	-	-	-	0/3	N (3/3)
Resveratrol	2b	XDS	-	-	-	0/3	N (3/3)
		ECVAM	-	-	-	0/5	N (3/3)
		Hiyoshi	-	-	-	0/3	N (3/3)
Actinomycin D	3	XDS	2.67×10^{-7}	-	-	1/6	P (1/1)
		ECVAM	1.98×10^{-8}	-	-	1/3	P (1/1)
		Hiyoshi	NC	-	-	0/1	P (1/1)
Bisphenol A	3	XDS	-	-	-	0/5	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Bisphenol B	3	XDS	-	-	-	0/1	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Diethylstilbestrol	3	XDS	-	-	-	0/2	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	1.70×10^{-5}	-	-	1/1	P (1/1)
17- α Ethinyl estradiol	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
4-Androstenedione	3	XDS	-	-	-	0/2	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Clomiphene citrate	3	XDS	-	-	-	0/2	I (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	NC	-	-	0/1	P (1/1)
Coumestrol	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/3	N (0/2)

Chemical	Phase	Laboratory	IC ₅₀ (M)	SD	CV (%)	# Plates for IC ₅₀ / # Plates Tested ^a	Classification ^b
		Hiyoshi	-	-	-	0/1	N (1/1)
4-Cumylphenol	3	XDS	-	-	-	0/2	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Daidzein	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Di - n -butyl phthalate	3	XDS	-	-	-	0/5	N (2/2)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
<i>p,p'</i> -DDE	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Diethylhexyl phthalate	3	XDS	-	-	-	0/3	N (1/1)
		ECVAM	-	-	-	0/3	N (2/2)
		Hiyoshi	-	-	-	0/2	N (1/1)
Dexamethasone	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
5 α -Dihydrotestosterone	3	XDS	-	-	-	0/6	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Dicofol	3	XDS	-	-	-	0/2	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/2	N (1/1)
17- α Estradiol	3	XDS	4.26 $\times 10^{-6}$	-	-	1/2	P (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	I (1/1)
17- β Estradiol	3	XDS	-	-	-	0/4	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Ethyl paraben	3	XDS	-	-	-	0/2	N (1/1)
		ECVAM	-	-	-	0/3	N (2/2)
		Hiyoshi	-	-	-	0/2	N (1/1)
Estrone	3	XDS	-	-	-	0/2	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Fluoranthene	3	XDS	-	-	-	0/6	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/2	N (1/1)

Chemical	Phase	Laboratory	IC ₅₀ (M)	SD	CV (%)	# Plates for IC ₅₀ / # Plates Tested ^a	Classification ^b
meso-Hexestrol	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	I (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Hydroxyflutamide	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/3	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Kepone	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/2	N (1/1)
Kaempferol	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
<i>p,p'</i> -Methoxychlor	3	XDS	-	-	-	0/5	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Morin	3	XDS	-	-	-	0/3	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/2	N (1/1)
Methyl testosterone	3	XDS	-	-	-	0/6	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Norethynodrel	3	XDS	-	-	-	0/3	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
4- <i>tert</i> -Octylphenol	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	-	-	-	0/2	N (1/1)
4-Hydroxy-tamoxifen	3	XDS	4.13×10^{-7}	5.77×10^{-7}	140	2/3	P (3/3)
		ECVAM	-	-	-	0/2	I (1/1)
		Hiyoshi	3.87×10^{-9}	-	-	1/1	P (1/1)
Phenobarbital	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/2	N (1/1)
		Hiyoshi	NT	NT	NT	0/0	NT
Phenolphthalein	3	XDS	-	-	-	0/6	N (1/1)
		ECVAM	-	-	-	0/3	N (2/2)
		Hiyoshi	-	-	-	0/1	N (1/1)
Propylthiouracil	3	XDS	-	-	-	0/5	N (1/1)
		ECVAM	-	-	-	0/3	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Raloxifene HCl	3	XDS	2.16×10^{-9}	-	-	1/1	P (1/1)

Chemical	Phase	Laboratory	IC ₅₀ (M)	SD	CV (%)	# Plates for IC ₅₀ / # Plates Tested ^a	Classification ^b
		ECVAM	5.41×10^{-10}	-	-	1/1	P (1/1)
		Hiyoshi	8.84×10^{-10}	-	-	1/1	P (1/1)
Sodium azide	3	XDS	-	-	-	0/4	N (1/1)
		ECVAM	-	-	-	0/4	N (1/1)
		Hiyoshi	-	-	-	0/2	N (1/1)
2-sec-Butylphenol	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
2,4,5-Trichloro-phenoxyacetic acid	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/3	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Testosterone	3	XDS	-	-	-	0/6	N (1/1)
		ECVAM	-	-	-	0/4	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
12 - O - Tetradecanoylphorbol-13-acetate	3	XDS	-	-	-	0/4	N (2/2)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)
Vinclozolin	3	XDS	-	-	-	0/1	N (1/1)
		ECVAM	-	-	-	0/1	N (1/1)
		Hiyoshi	-	-	-	0/1	N (1/1)

Abbreviations: # = number; CV = coefficient of variation; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation; I = inadequate (positive or negative classification could not be determined because of poor quality data); IC₅₀ = the half maximal inhibitory concentration; M = molar; N = negative; NC = not calculated; NT = not tested; P = positive; SD = standard deviation; XDS = Xenobiotic Detection Systems, Inc.

^aNumber of acceptable plates used to determine the EC₅₀ value vs. the total number of plates tested (includes all acceptable and non-acceptable plates).

^bNumber in parentheses represents test results (P, N, or I) over the total number of acceptable trials.

Table 4-15 Phase 4 Results from XDS

Chemical	AGONIST			ANTAGONIST		
	EC ₅₀ ^a (M)	Classification	# Plates Tested	IC ₅₀ ^c (M)	Classification	# Plates Tested
17β-Trenbolone	9.58×10^{-8}	P (1/1)	2	-	N (2/2)	4
19-Nortestosterone	1.80×10^{-6}	P (1/1)	1	-	N (1/1)	1
4-hydroxyandrostenedione	3.91×10^{-5}	P (1/1)	2	-	N (1/1)	1
Ammonium perchlorate	-	N (1/1)	3	-	N (1/1)	1
Apomorphine	-	N (2/2)	3	NC	P (1/1)	1
Bicalutamide	-	N (1/1)	2	-	N (1/1)	1
Chrysin	3.20×10^{-6}	P (2/2)	3	-	N (1/1)	1
Cycloheximide	-	I (2/2)	1	9.67×10^{-7}	P (1/1)	1
Cyproterone acetate	-	N (1/2)	4	-	N (1/1)	1

Fenarimol	4.59×10^{-6}	P (2/2)	6	-	N (1/1)	4
Finasteride	-	N (1/1)	3	-	N (1/1)	2
Fluoxymestrone	2.22×10^{-5}	P (2/2)	4	-	N (1/1)	1
Flutamide	-	I (1/1)	3	-	N (1/1)	1
Haloperidol	-	N (1/1)	3	-	N (1/1)	1
Ketoconazole	-	N (1/1)	3	1.23×10^{-6}	P (1/1)	3
L-Thyroxine	-	N (2/2)	4	-	N (1/1)	1
Linuron	-	N (2/2)	5	-	N (1/1)	1
Medroxyprogesterone acetate	-	N (2/2)	5	NC	P (1/1)	1
Mifepristone	-	N (2/2)	2	-	N (1/1)	1
Nilutamide	NC	P (1/1)	2	-	N (2/2)	4
Oxazepam	-	I (1/1)	3	-	N (1/1)	1
Pimozide	-	N (1/1)	1	-	N (1/1)	1
Procymidone	-	I (1/1)	3	-	N (1/1)	3
Reserpine	-	N (2/2)	5	-	I (1/1)	1
Spirolactone	-	N (1/1)	2	-	N (1/1)	1

271 Abbreviations: # = number; EC₅₀ = the half maximal effective concentration; I = inadequate (positive or negative classification could not be
 272 determined because of poor quality data); IC₅₀ = the half maximal inhibitory concentration; M = molar; N = negative; NC = not calculated; NT
 273 = not tested; P = positive.

274 ^aEC₅₀ values are from one test except 4-OH androstenedione (mean value from two tests [SD = 3.91×10^{-5} ; coefficient of variation = 52%]).

275 ^bNumber in parentheses represents test results (P, N, or I) over the total number of acceptable trials

276 ^cIC₅₀ values are from one test.