OECD 試験法に係る最近の動向について

国立環境研究所　環境リスク・健康領域

山本 裕史

化審法セミナー@Web
（令和4年2月15日）

National Institute for Environmental Studies, Japan

簡単な自己紹介

National Institute for Environmental Studies, Japan

- 専門は生態毒性学、環境化学、環境工学
- 現職は国立環境研究所、環境リスク･健康領域副領域長･生態毒性研究室長（http://www.nies.go.jp/index-j.html）
- 東京大学大学院新領域創成科学研究科自然環境学専攻客員教授
- 環境省の化審法、農取法、内分泌かく乱、医薬品による環境汚染、土壌汚染、海洋プラスチックごみ等の約30の委員を担当

長引くCOVID-19の影響

National Institute for Environmental Studies, Japan

- OECDの試験法関係の会議も軒並みWeb (Zoom等）での開催
- 欧州での10-17時程度の開催が多く、米国では早朝、日本では深夜のことが多い

OECDテストガイドライン作業グループ

WNT: Working Group of National Co-ordinators of the TGs programme

- 化学品の試験のためのOECDテストガイドライン（テストガイドラインの開発ならびに試験方法の検証の円滑化及び調和化を含む）
- 試験の諸問題に関するガイダンス文書
- 特定の有害性領域の先端科学に関する詳細レビュー文書

に関する作業の指揮・監督をおこなう。

WNT-33は2021年4月20～23日に開催

OECD WNT33の主要議題(1)

National Institute for Environmental Studies, Japan

Item 4: 新規試験法や試験法改訂の提案書（SPSF）の承認

各国のSPSFの採否を審議

日本から提案した、OECD TG201（藻類生長阻害試験法）の改訂プロジェクト案が承認された

OECD WNT33の主要議題(2)

National Institute for Environmental Studies, Japan

Item 7: フランスからの提案のミツバチの帰巣行動に関する試験法

ガイダンス文書として承認された。

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)7

Unclassified

English - Or. English
16 July 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

GUIDANCE DOCUMENT ON HONEY BEE (APIS MELLIFERA L.) HOMING FLIGHT TEST, USING SINGLE ORAL EXPOSURE TO SUBLETHAL DOSES OF TEST CHEMICAL

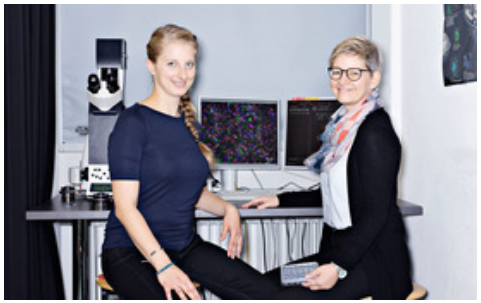
Series on Testing and Assessment,
No. 332

削除
（ミツバチの主な帰巣行動の実例）

OECD WNT33の主要議題(3)

National Institute for Environmental Studies, Japan

Item 8: ノルウェー・スイスからの提案のニジマスのエラ細胞株RTgill-W1を用いた急性毒性試験



https://www.eawag.ch/en/news-agenda/news-portal/news-detail/eawag-test-with-fish-cells-replaces-animal-experiments/

https://www.eawag.ch/en/news-agenda/news-portal/news-detail/eawag-test-with-fish-cells-replaces-animal-experiments/

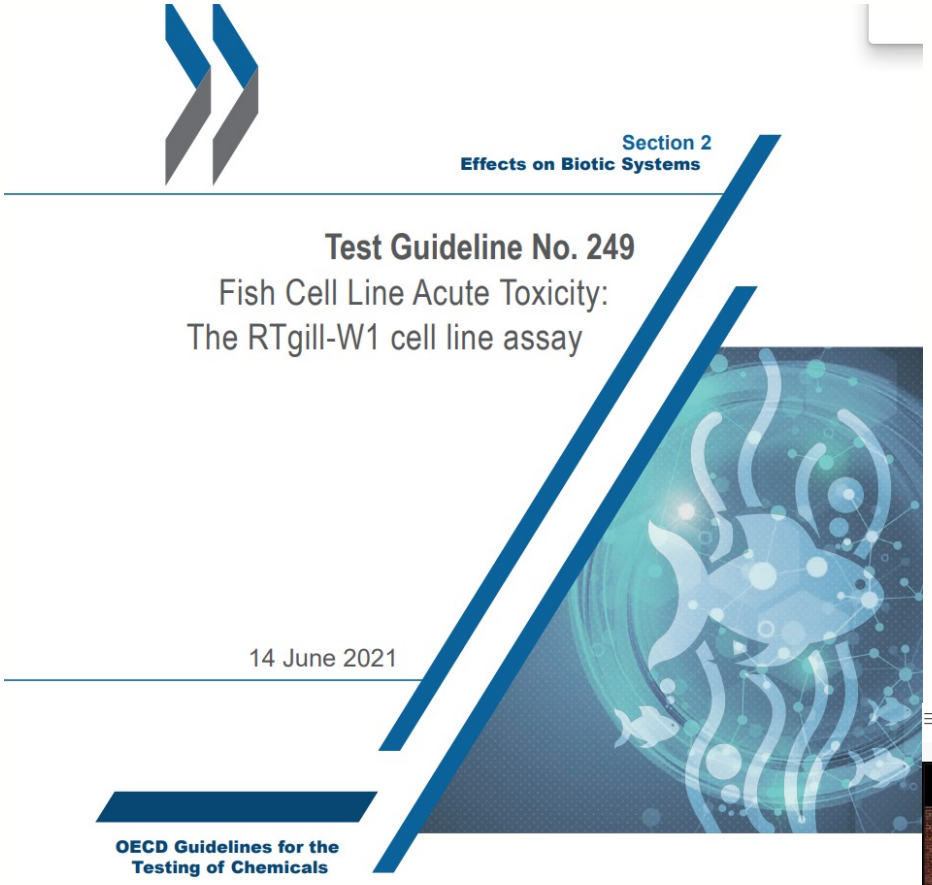
削除
（試験系模式図）

スイス連邦水科学技術研究所（Eawag)のKristin Schirmer教授らが開発した手法

OECD WNT33の主要議題(4)

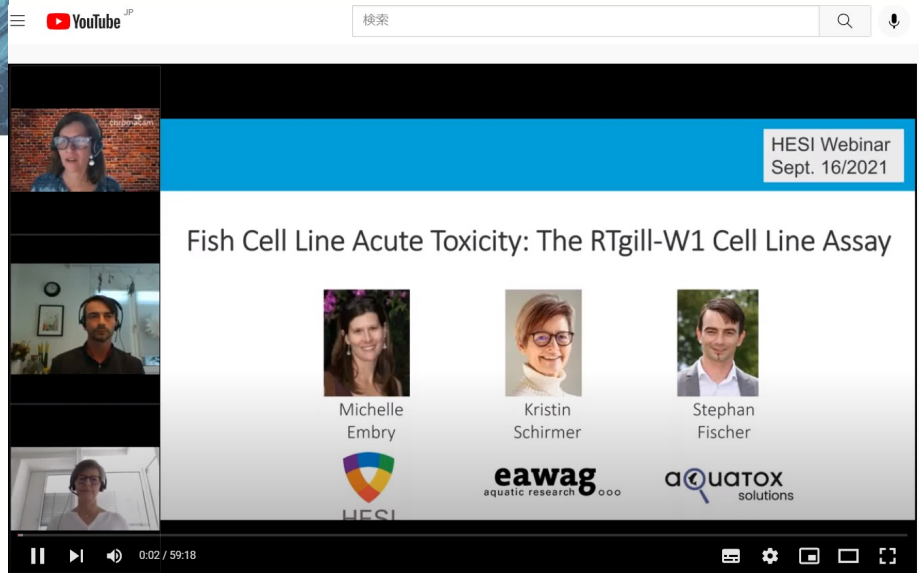
National Institute for Environmental Studies, Japan

OECD TG249として承認



エラ細胞株の利用では、全身毒性物質、伝達化学物質などの評価は不可能であることに注意が必要

９月にはWebinarが開催



YouTubeに動画は公開
https://www.youtube.com/watch?v=DteBMHHNEYI

OECD WNT33の主要議題(5)

National Institute for Environmental Studies, Japan

２日目 Item 9: フランスからの提案のアロマターゼ・GFP遺伝子導入ゼブラフィッシュの胚を用いたエストロゲン様用物質の検出試験法(EASZY)

削除
（試験系模式図）

OECD WNT33の主要議題(6)

OECD TG250として承認

内分泌かく乱化学物質の検出試験法として、利用される
→ほかにも、遺伝子導入メダカを用いたRADAR（抗アンドロゲン作用）、REACTIV（エストロゲン・アンドロゲン作用）も提案中

WNT傘下の組織

- VMG-eco（生態毒性試験バリデーション管理グループ）において、200シリーズ関連の専門家会合を開催し、バリデーションを実施

- ほかにVMG-NA（非動物試験バリデーション管理グループ）など

16th VMG-ecoは2021年10月20～21日にWebで開催、
日本からは内分泌かく乱関係の試験法のバリデーション結果ほか、①鳥類卵内投与試験の進捗報告、②**TG203改訂に伴うメダカの診断症状**、③**ヨコエビ試験法提案**、 ④**TG201の改訂に向けた提案**等の情報提供を発表

VMG-ecoの概要(1)

Item 1のアジェンダ案の承認、
Item 2の事務局からの連絡事項の後、
Item 3ではTG210のSolvent Controlプロジェクト（ICAPO)の紹介

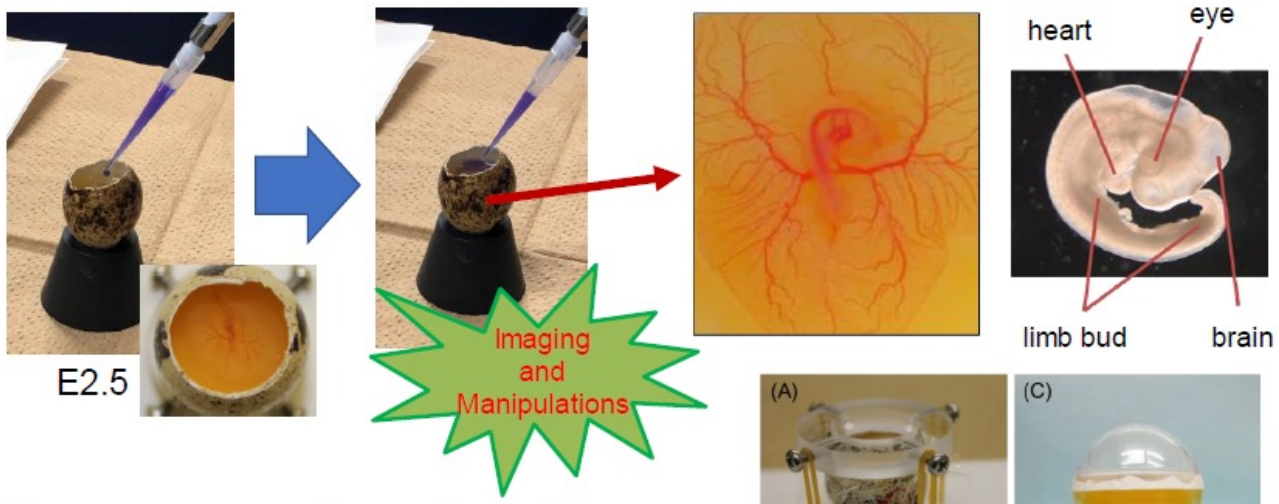
日本からも環境省のメダカ4データを提供

VMG-ecoの概要(2)

National Institute for Environmental Studies, Japan

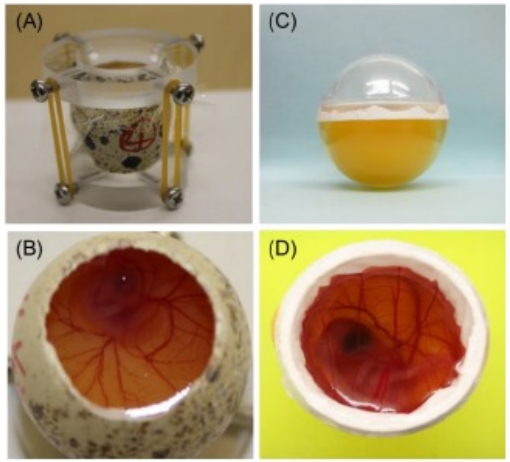
Item 4の各国の活動（この先TGに提案されそうな内容）の紹介部分で、日本の活動として、鳥類卵内試験の進捗状況を国立環境研究所から紹介

ex ovo assay (Japanese Quail)



The avian surrogate eggshell culture method can be applied as a new method of administration due to more flexibility in setting the dose of test substances.

We are also developing a method for culturing quail embryos in artificial vessel.



Kawashima et al. (2016) Frontiers Neurosci 10 (296)
Kawashima et al. (2005) Zoo Biol 24: 519-529

5

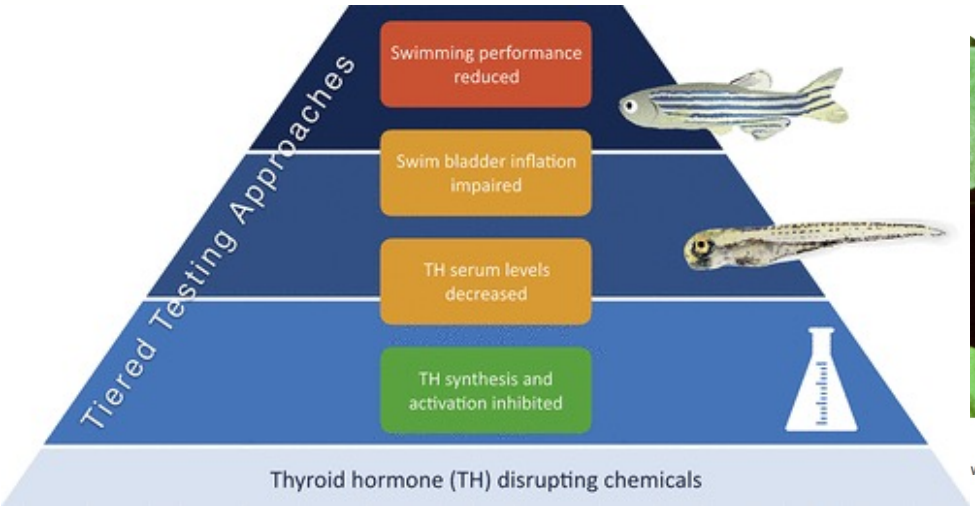
VMG-ecoの概要(3)

National Institute for Environmental Studies, Japan

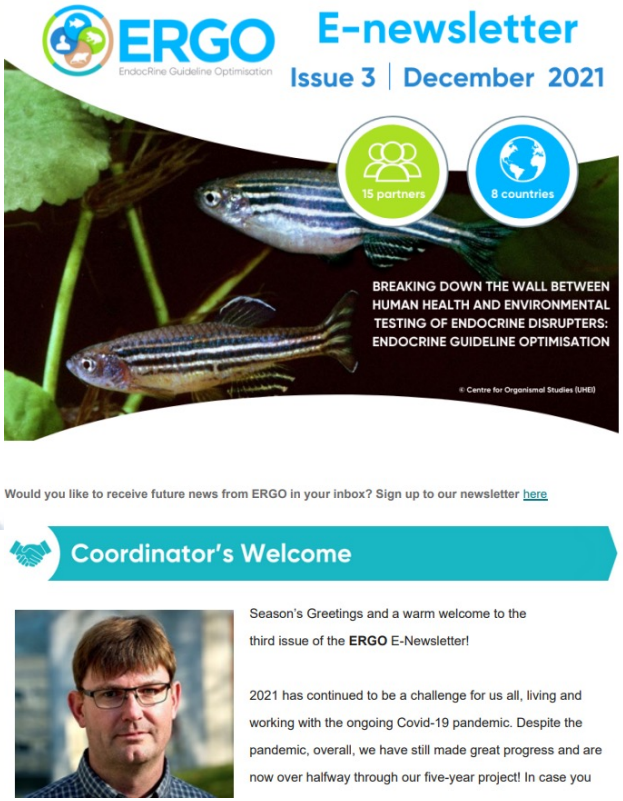
Item 5: 魚（主にゼブラフィッシュのTG234）をつかった甲状腺ホルモン作用物質の検出に関するプロジェクト

デンマークなどからの発表

浮袋の発達や眼球など、ゼブラフィッシュの表現型と甲状腺ホルモンとの間の関係を解明し、既存のTG234の改良により甲状腺ホルモンかく乱物質を検出するエンドポイントを確立したり、AOPを確立したりする欧州のプロジェクトERGO



Knappen et al. (2020) Environ Sci Technol, 54, 8491-8499.



https://ergo-project.eu/

VMG-ecoの概要(4)

National Institute for Environmental Studies, Japan

Item 6: HYBIT（ヨコエビを用いたBCF)のプロジェクト紹介

フランス・ドイツからの提案で、欧州CEFIC/LRIなどのFundingで進む

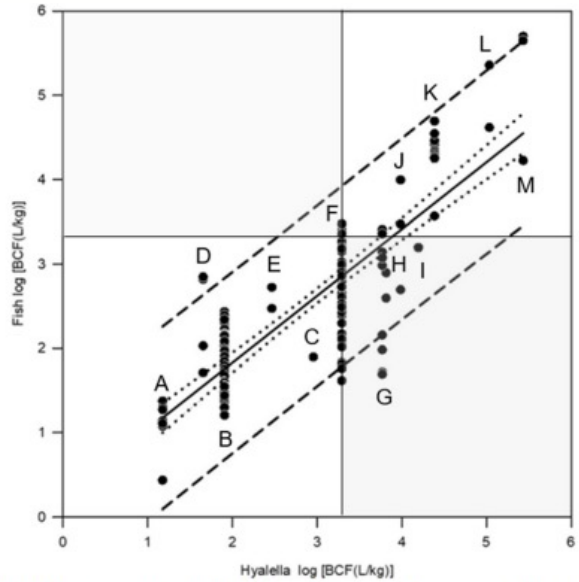


Fig. 4 Experimental fish BCFs from different studies versus individual experimental kinetic BCFs estimated for male *Hyalella azteca* for thirteen chemicals with different log K_{ow}. All *Hyalella* BCF values are normalized to 5% lipid content except for ^{14}C-pyrene (G). The thin black lines mark the regulatory threshold of log BCF 3.3 (BCF 2000). Data points in the hatched area would relate to substances which highly accumulate in fish (log BCF ≥ 3.3) but not in *H. azteca* and vice versa representing type II and I error, respectively. Correlation: black regression line [fish log BCF = 0.251 + (0.792 × *Hyalella* log BCF)]; $R^2 = 0.687$) with 95% confidence interval (dotted lines) and prediction interval (short dash). Standard error of the estimate ($s_{y\,x}$) of the regression line = 1.1248. A, ^{14}C-simazine; B, diazinon; C, ^{14}C-low hydrophobic compound; D, 1,2,3-trichlorobenzene; E, 2,4,5-trichlorophenol; F, chlorpyrifos; G, ^{14}C-pyrene; H, benzo(a)pyrene; I, methoxychlor; J, o-terphenyl; K, hexachlorobenzene; L, PCB77; M, PCB 153. References for fish BCF estimates are presented in Table S2.1. For detailed results of regression analysis see Electronic Supplementary Material, Part 3. A comparison of kinetic BCFs estimated for male *H. azteca* and fish BCF estimates for single species is presented in Figs. 5a–c

Schlechtriem et al. (2019) Environ Sci Pollut Res, 26, 1628-1641

VMG-ecoの概要(5)

National Institute for Environmental Studies, Japan

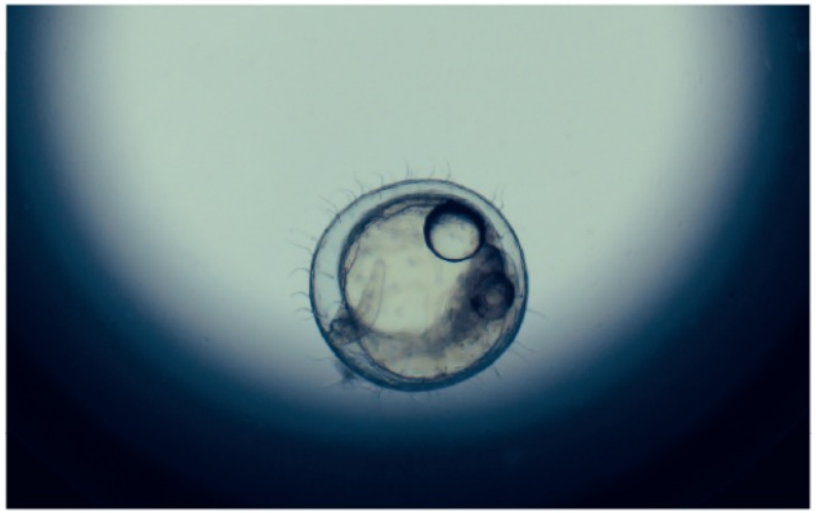
Item 7: ChoriogeninHを導入したメダカの胚を用いた試験(REACTIV)

フランス・Watchfrog社の提案

国立環境研究所でValidation試験を実施

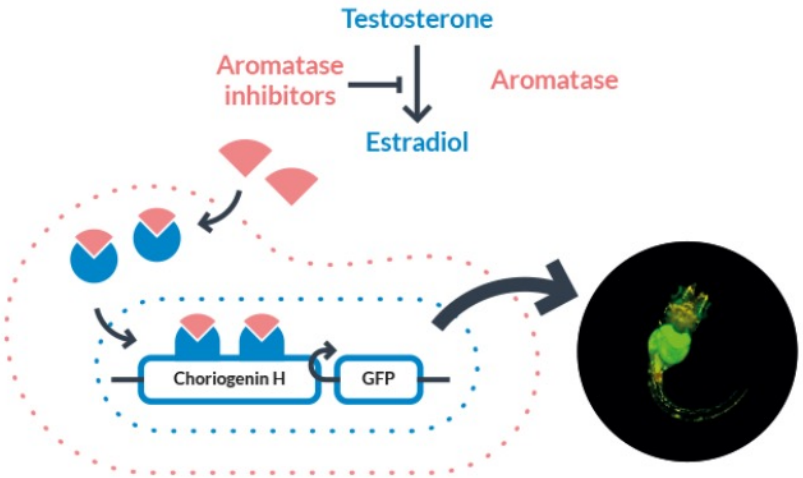
Key event

OECD physiological criteria: Egg production in fish, sex determination in fish



Modes of actions

Provide additional information to authorities to assess MOA



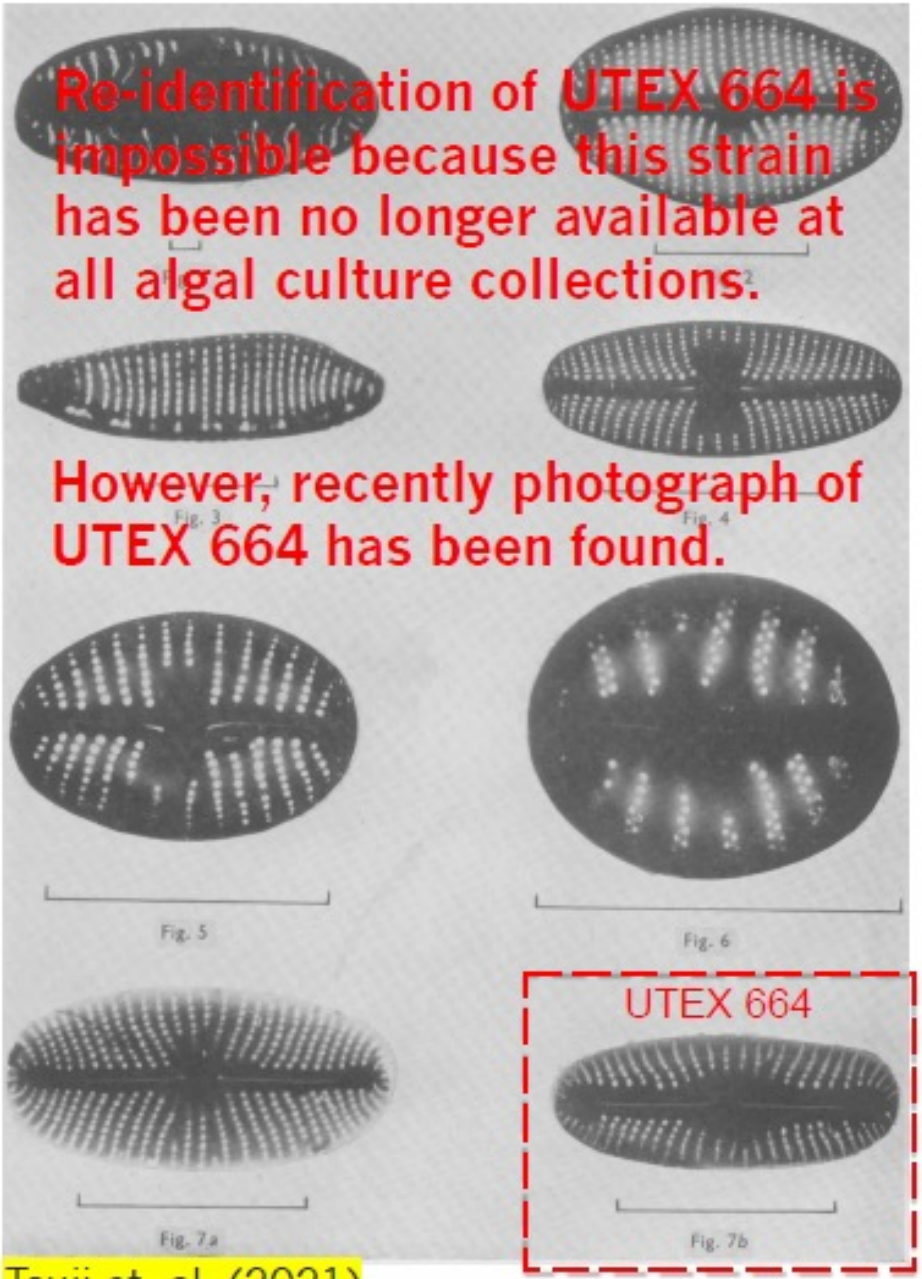
Watchfrog社HPより
(https://www.watchfrog.fr/en/test_oestrogenique.php)

VMG-ecoの概要(6)

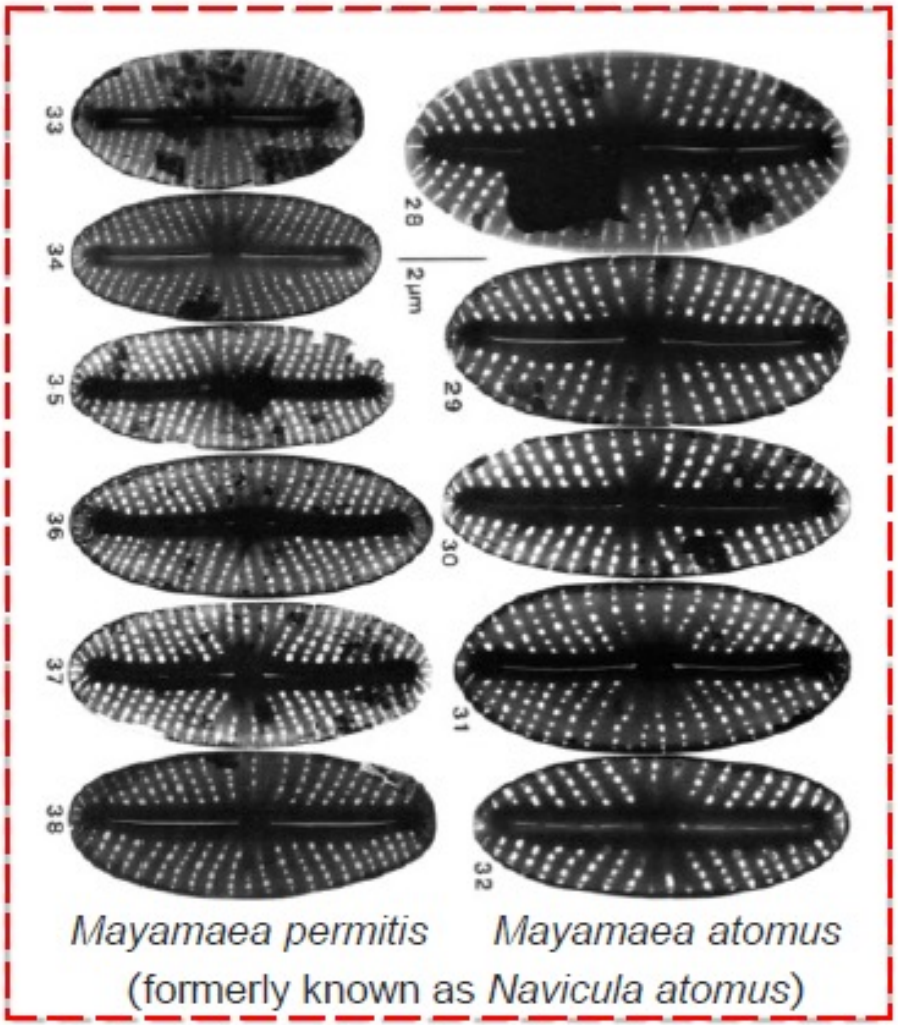
Item8: WNT(2021年4月）で採択された藻類TG201改訂プロジェクトについて、国立環境研究所から紹介

Navicula pelliculosa (UTEX 664)

National Institute for Environmental Studies, Japan



Tsuji et. al. (2021)
Bull. Nati. Mus. Vol. 47, No. 4 in press.



- Currently, the use of *Mayamaea* sp. is most appropriate for UTEX 664 (Tsuji et. al. 2021).
- Alternative strain for UTEX 664 should be chosen from the strains of *Mayamaea* sp..

Navicula pelliculosa (UTEX 664)

National Institute for Environmental Studies, Japan

Growth rates on three candidate strains

		Specific growth within 72 h	Mean CV for section-by-section growth rates	CV of the average specific growth rates
Criterion		>16 time	<35%	<7%
***Mayamaea permitis* (NIES-2724)**	OECD medium	125±12.5	13.7±8.15	1.63±0.97
***Mayamaea pseudoterrestris* (NIES-4280)**	OECD medium Modified OECD*	12.2±1.71 25.2±3.22	11.6±0.68 10.2±0.74	0.99±0.54 1.23±0.41
Mayamaea terrestris (NIES-4281)	OECD medium Modified OECD*	7.32±2.21 11.3±8.21	22.7±1.68 22.7±1.68	3.86±1.46 3.86±1.46

*Fe-EDTA was replaced with ferric citrate at the modified OECD medium.

n=3, mean±SD

Comparison of EC50 values on two typical toxicants among three candidate strains

	3,5-DCP $EC50_{72h}$	Cr(VI) $EC50_{72h}$	Reference
***Mayamaea permitis* (NIES-2724)**	1.2±0.24	0.12±0.07	In this study
***Mayamaea pseudoterrestris* (NIES-4280)**	1.40±0.24	0.24±0.10	In this study
	1.0	0.51	Okamoto et al. (2021)
Navicula seminulum	1.80±0.20	0.12-1.22	Academy of Natural Science (1960)

n=3, mean±SD

- We propose *M. permitis* (NIES-2724) and *M. Pseudoterrestris* (NIES-4280) as alternative strain of UTEX 664.
- Interlaboratory validation work for the candidate strain is necessary.

Summary

National Institute for Environmental Studies, Japan

Proposal for revision of scientific name of recommended algal strains

OECD/OCDE

ANNEX 2

STRAINS SHOWN TO BE SUITABLE FOR THE TEST

Green algae

- ~~*Pseudokirchneriella subcapitata*, (formerly known as *Selenastrum capricornutum*) , ATCC 22662,~~ CCAP 278/4, 61.81 SAG
- *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) 86.81 SAG, NIES-4282

Raphidocelis subcapitata, (formerly known as *Selenastrum capricornutum* = *Pseudokirchneriella subcapitata*), ATCC 22662, CCAP 278/4, 61.81 SAG, NIES-35

Diatoms

- ~~*Navicula pelliculosa*, UTEX 664~~

Mayamaea sp, (formerly misidentified as *Navicula* (= *Fistulifera*) *pelliculosa*), UTEX 664
Mayamaea permitis or *Mayamaea pseudoterrestris*, NIES-2724 or NIES-2480

Cyanobacteria

- ~~*Anabaena flos-aquae*, UTEX 1444, ATCC 29413, CCAP 1403/13A~~
- *Synechococcus leopoliensis*, UTEX 625, CCAP 1405/1, NIES-3277

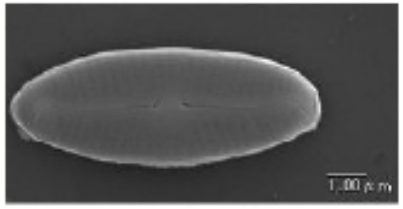
Anabaena valiabilis, (formerly misidentified as *Anabaena flos-aquae*) UTEX 1444, ATCC 29413, CCAP 1403/13A, NIES-2095

Summary

National Institute for Environmental Studies, Japan

Interlaboratory validation work to add a new diatom strain

Test method

Test strain	 *Mayamaea permitis* (NIES-2724) *Mayamaea Pseudoterrestris* (NIES-2480)
Initial concentration	5 x 10^3 cells/mL
endpoint	Growth inhibition
measurements	• Cell count using electronic particle counter, microscope, or a flow cytometer. • Fluorescence or optical density
Test condition	axenic temperature：23±2℃ Light intensity：60-120 $\mu E^{-2}S^{-2}$ cycle：continuous light Agitation: about 150 / min
medium	OECD+Si meduim
volume	100 mL
Test duration	72 hours
Validity criteria	> 16 times < 35% < 7.0%
Test chemicals	• 3,5-DCP • Potassium dichromate (VI)

Key milestone

1. The SPSF was approved at WNT in April 2021.
2. Inter-laboratory validation is conducted in 2021-2022.
3. A final report of the inter-laboratory and draft of revised test guideline including the update of the scientific name will be prepared in 2022-2023.

Testing laboratories

1. National Institute for Environmental Studies (NIES), Japan
2. Other testing laboratory from Japan
3. Kindly offered from National Institute for Industrial Environment and Risks (INERIS), France
4. Scymaris, UK is also interested in the interlaboratory validation.

VMG-ecoの概要(7)

Item9: 日本からのミジンコ幼若ホルモンJHのスクリーニング試験のTG提案の進捗報告

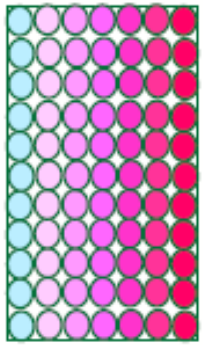
主にノンケミカルストレスの結果を発表

Develop Juvenile Hormone Activity Screening Assay (JHASA)

National Institute for Environmental Studies, Japan

5

OECD TG211



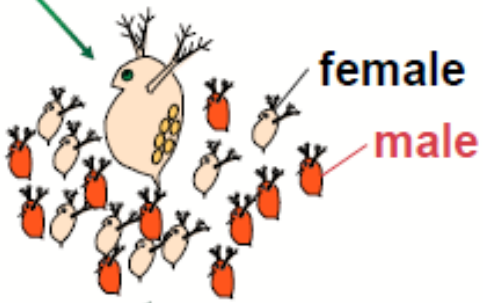
offspring

Control +
6 concentrations

100-200/female=1,000-2,000 neonates/treatment should be checked!

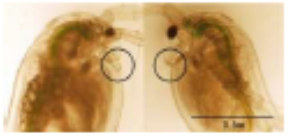
21 days

1st week | 1st brood | 2nd brood | 3rd brood | 4th brood | 5th brood | 6th brood



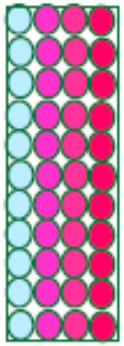
Annex 7

Identify sex of all neonates



male female

JHASA



10-17d-old adults with embryos in their brood chambers

Control
+3 concentrations
(Maximum: ½ of 48h-EC50)

5-7 days

1st brood | 2nd brood | 3rd brood

Identify sex of the neonates of 2nd brood after exposure

20/female=200 neonates/treatment

Effect of non-chemical stressors in JHASA

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11

Temperature

- Culturing and JHASA at 20°C, 28°C, 30°C: High temperature
- Culturing (30°C)→JHASA (20°C) : Higher to test temperature
- Culturing (10°C)→ JHASA (20°C) : Lower to test temperature

Hardness

High hardness: 250, 500, 1000 mg$CaCO_3$/L
(x1, x2, x4 hardness of M4 medium)

High Density

35, 70, 105 daphnids/L (x1, x2, x3 of normal density)
(same food amount per daphnids)

High Density + Low Nutritional status

35, 70, 105 daphnids /L (x1, x2, x3 of normal density)
(food amount per daphnids: x1, x1/2, x1/3)

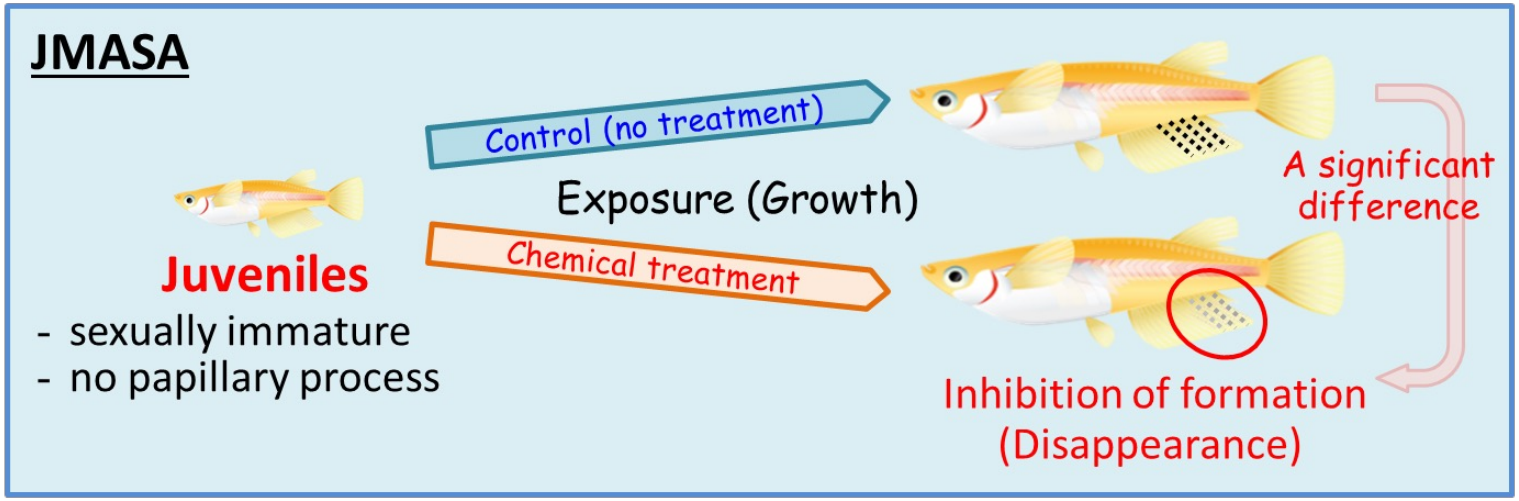
No conditions induced male offspring production!

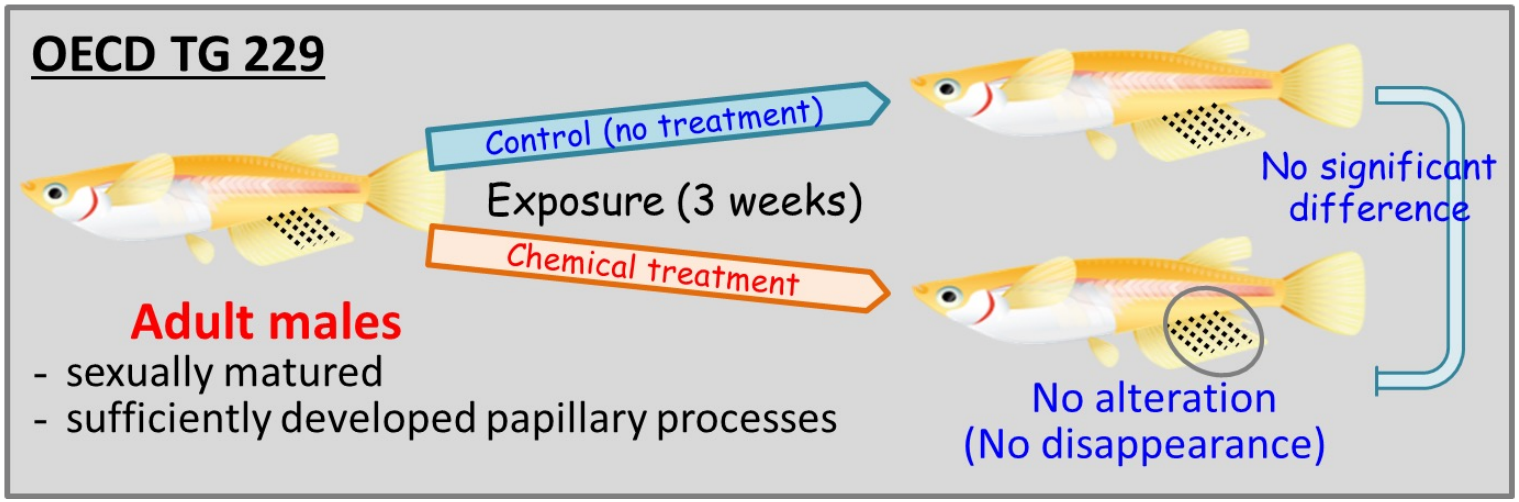
VMG-ecoの概要(8)

National Institute for Environmental Studies, Japan

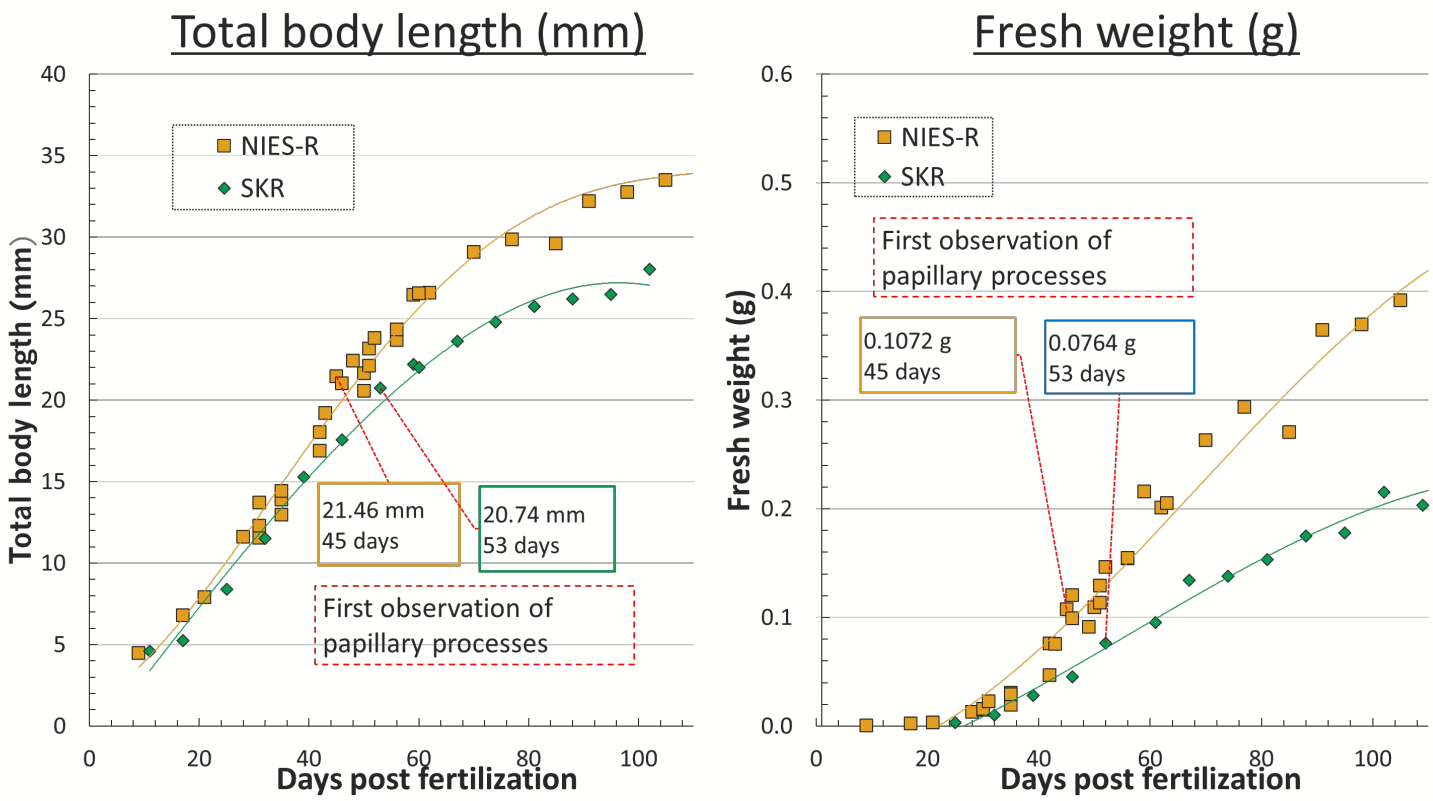
Item 10: ２日目の最初は国立環境研究所から、幼若メダカ抗アンドロゲン検出試験（JMASA)の進捗報告

- Juvenile Medaka Anti-androgen Screening Assay (JMASA)



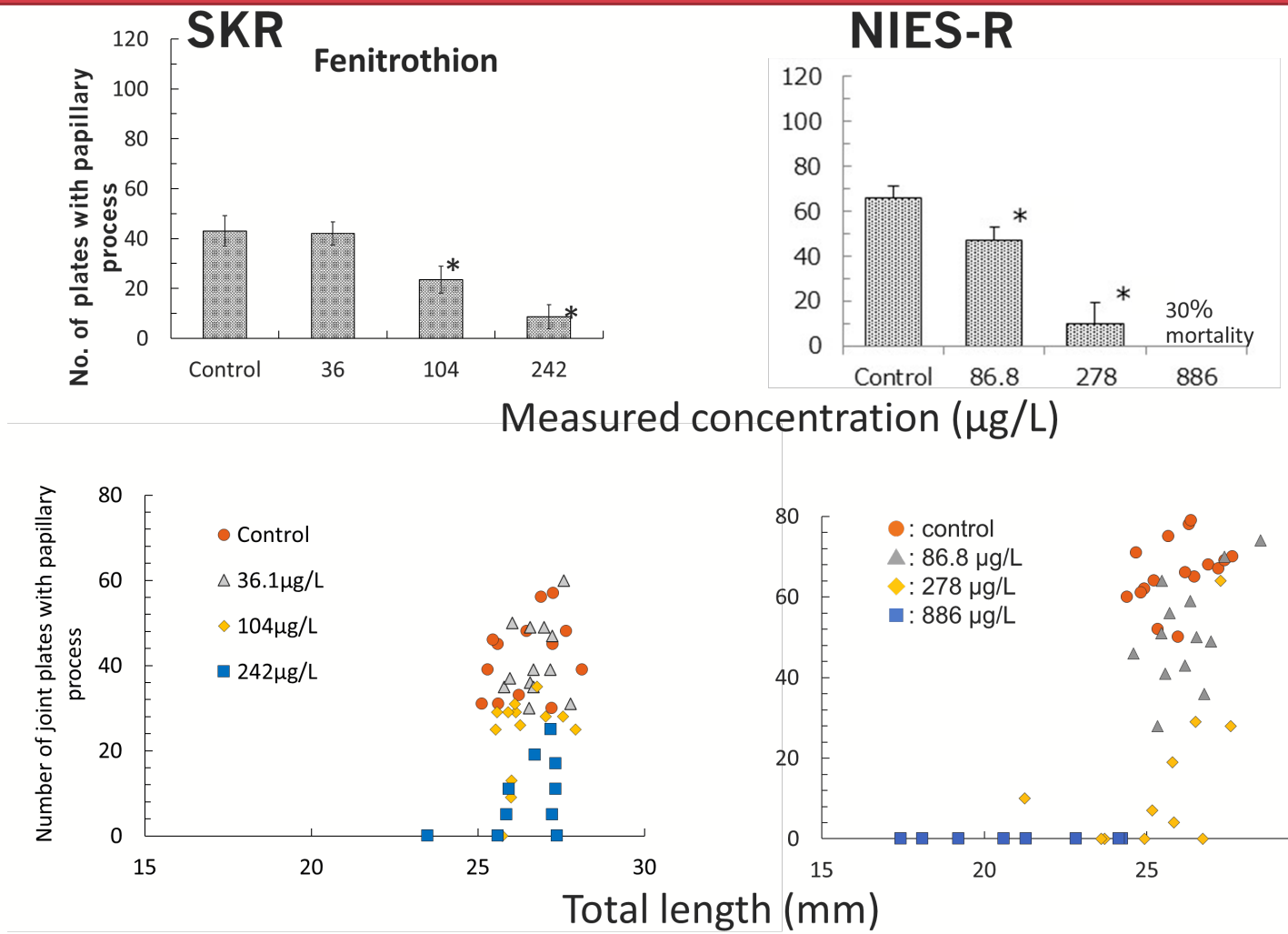


First observation day of papillary process after fertilization on two different strains of *O. latipes*, NIES-R and SKR



- First observation day of papillary process at SKR was eight days behind that of NIES-R

Results of validation studies (Fenitrothion)



VMG-ecoの概要(9)

National Institute for Environmental Studies, Japan

Item 11: REACTIVと同じWatchFrog社によるRADARアッセイの検証結果

イトヨのオス特有のスピギンタンパクの遺伝子を導入したメダカ胚の試験

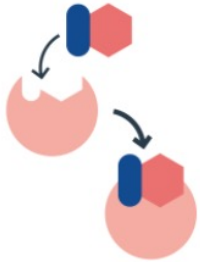
（抗）男性ホルモン作用物質の検出に利用

検証レポートやTGに関する専門家コメントへの対応案に関して議論

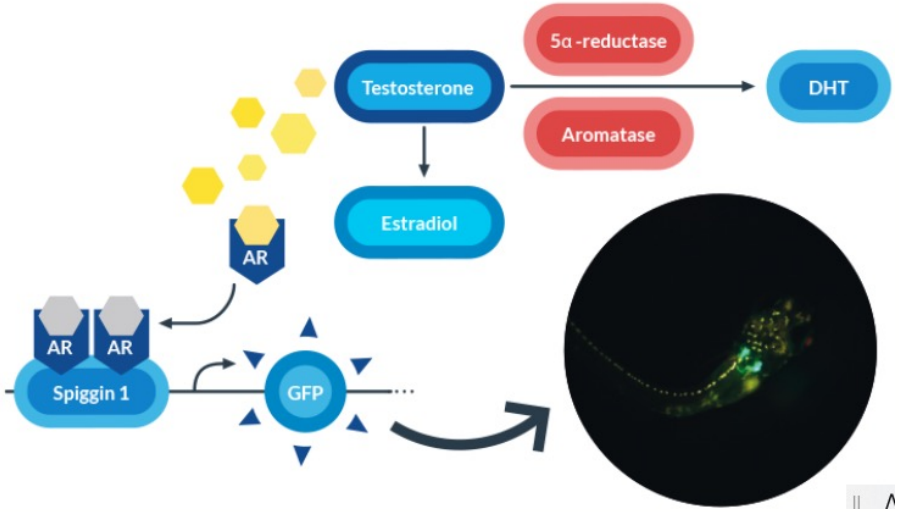
Key event

OECD physiological criterion: Spiggin production in the Three Spined Stickleback

Modes of actions



Provide additional information to authorities to assess MOA



Watchfrog社HPより
(https://www.watchfrog.fr/en/test_androgenique.php)

VMG-ecoの概要(10)

Item12: MEOGRT試験のゼブラフィッシュ版、ZEOGRT（ゼブラフィッシュ拡張一世代繁殖毒性試験）

性比の偏りが課題であるほか、検証実施機関を募集

VMG-ecoの概要(11)

National Institute for Environmental Studies, Japan

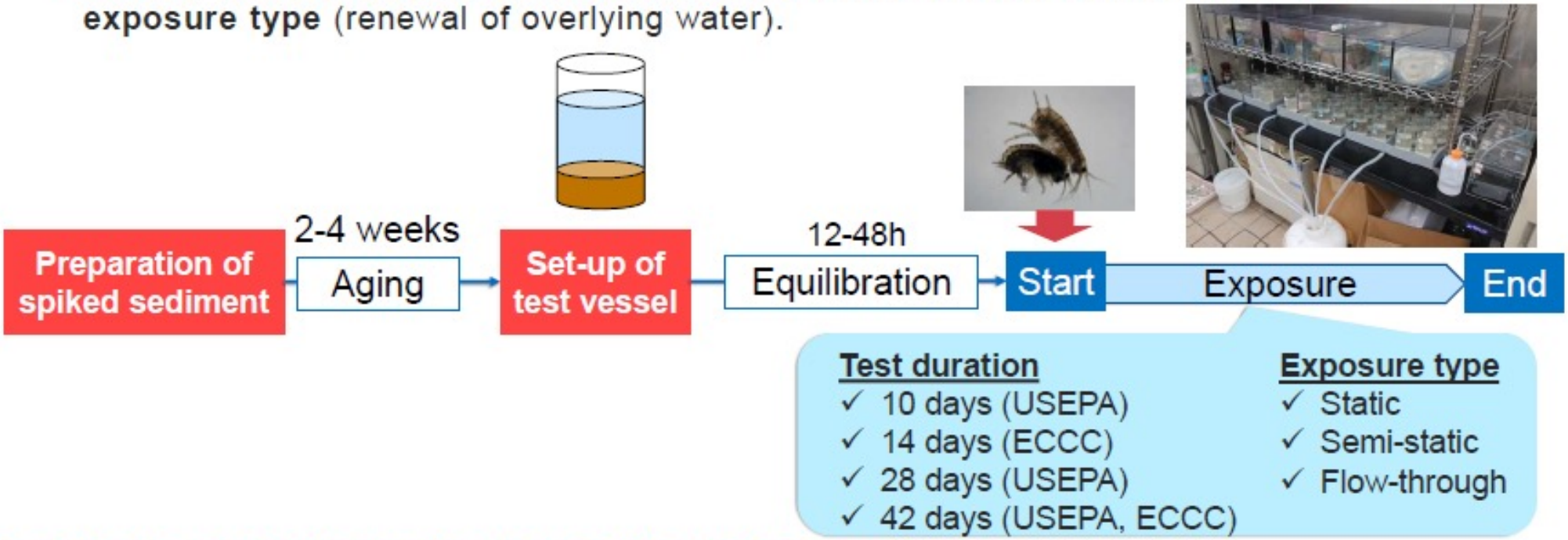
Item 13: 新規プロジェクト提案について

1つ目として、国立環境研究所から、ヨコエビを用いた新規の底質試験法提案について説明

Harmonization of Test Protocols

Existing protocols have different test conditions

- USEPA/ASTM, Environment and Climate Change Canada/ISO, OSPAR, French Standard
- Several test conditions are different among test protocols, such as **test duration** and **exposure type** (renewal of overlying water).



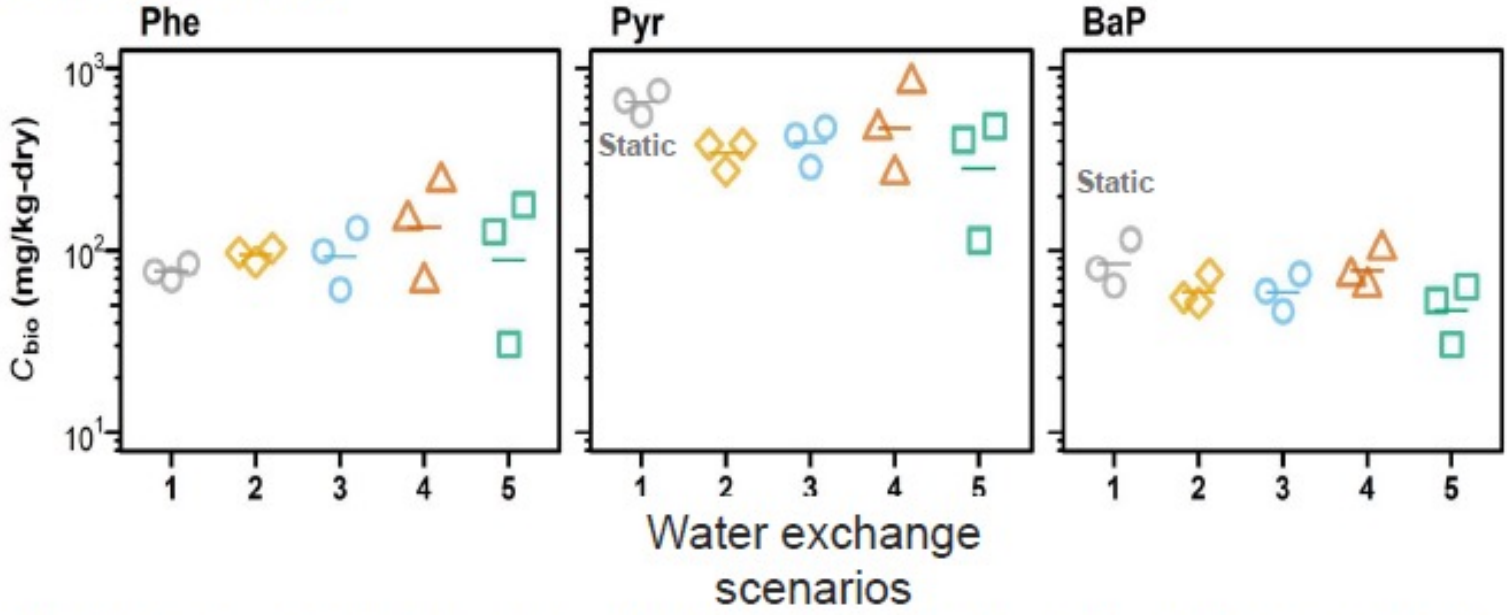
Conduct a questionnaire to testing laboratories

- **France**, INERIS (French National Institute for Industrial Environment and Risks)
- **Switzerland**, Swiss Centre for Applied Ecotoxicology
- **Canada**, ECCC (Environment and Climate Change Canada, 2 researchers)
- **Germany**, Fraunhofer Institute for Molecular Biology and Applied Ecology
- **UK**, CEFAS (Center for Environment, Fisheries and Aquaculture Science)
  Scymaris Ltd.
  UKCEH (UK Centre for Ecology and Hydrology)
- **US**, USEPA (US Environmental Protect Agency)

Results: Amphipod survival & body concentration

- ✓ Amphipod survival was > 70% in all the tested scenarios.
- ✓ Dry weight of surviving amphipods was not different among scenarios.
- ✓ Also, there were no statistically significant differences in body concentrations among scenarios. The static condition caused the highest body concentrations of Pyr and BaP, though.

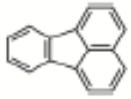
This suggests that pore water concentration, not overlying water concentration, was the major contributor to the transfer of the tested chemicals to amphipods and the resulting toxicity.



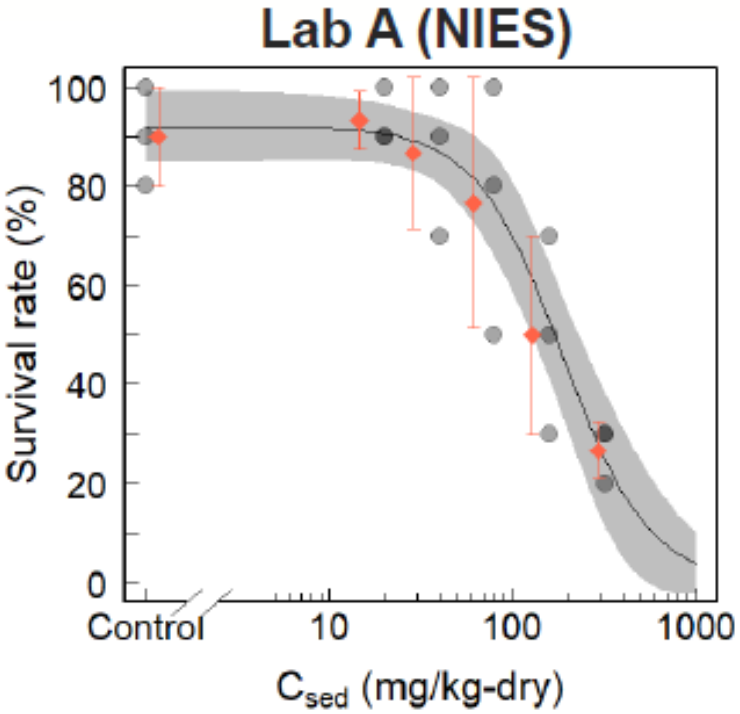
- ✓ The effect of exposure types is limited. In particular, the difference between semi-static and flow-through is minor.
- ✓ Rather, we should focus on other factors (e.g., sediment types).

Preliminary ring-test

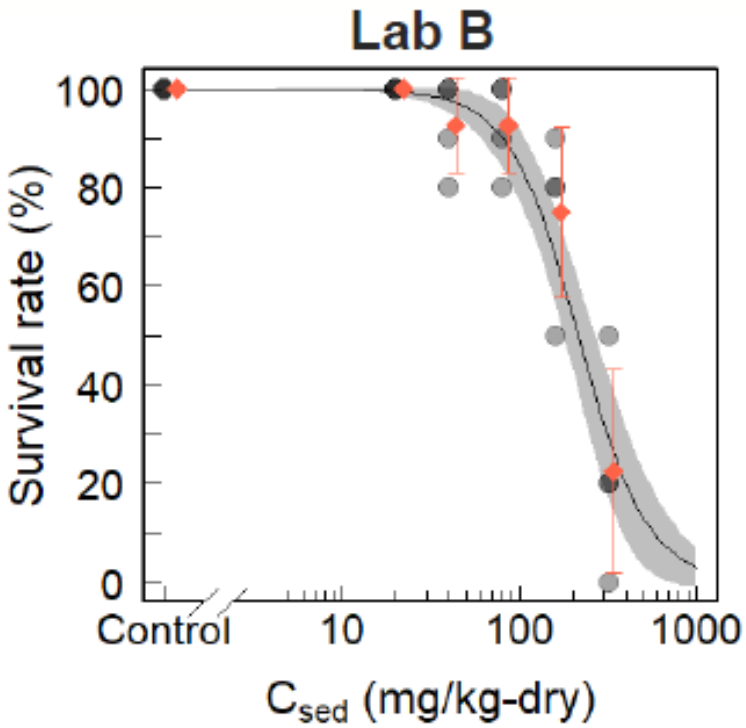
As a preliminary study for future ring-test, sediment toxicity tests with *H. azteca* were performed by two laboratories in Japan. Fluoranthene was used as a model toxicant.



Both tests were done under the same condition (e.g., flow-through, artificial sediment).



LC50: 187 mg/kg
(95%CI 130–244 mg/kg)



LC50: 215 mg/kg
(95%CI 172–258 mg/kg)

Highly reproducible!

Schedule

Apr 2019: Call for co-lead or participant countries (WNT)

→ France (INERIS)

Oct 2021: Propose SPSF (VMG-eco) ←Now!

2021年11月にSPSFを提出

Nov 2021: Submit SPSF draft

Apr 2022: Approval of SPSF draft

Call for participants of validation study (ring-test) (WNT)

2022–2023: Ring test

Oct 2023: Present draft TG and ring test's result (VMG-eco)

Apr 2024: Approval? (WNT)

We welcome your feedback about the SPSF draft!

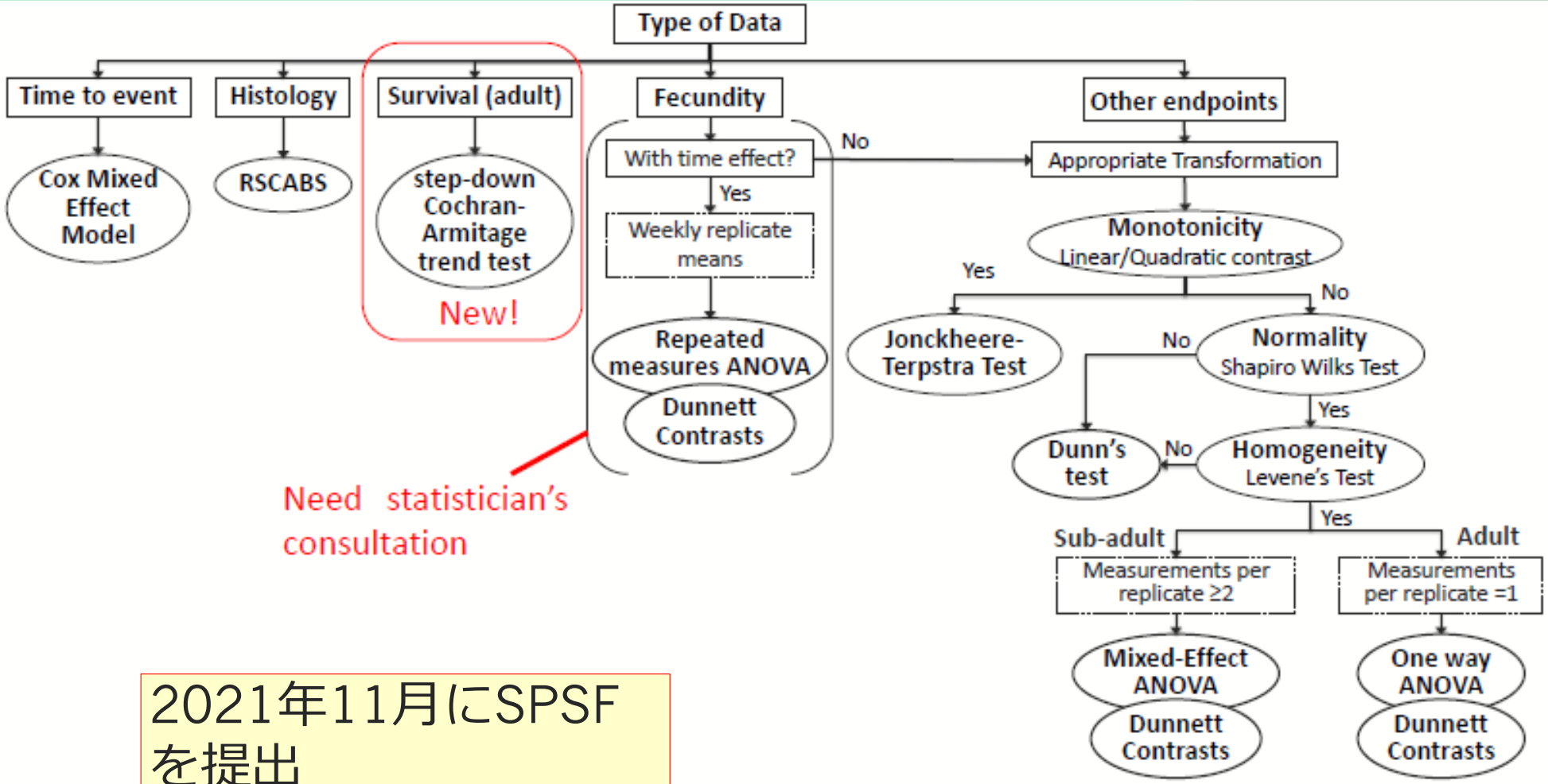
VMG-ecoの概要(12)

Item 13: 新規プロジェクト提案について

2つ目として、国立環境研究所から、MEOGRTの統計部分などの改訂案について説明

（USEPAのガイドラインとOECD TG240との整合性を取るための提案）

Revised flow chart (draft)



2021年11月にSPSFを提出

VMG-ecoの概要(13)

Item 13: 新規プロジェクト提案について

3つ目として、ドイツから、ユスリカ毒性試験の改訂案についての検討

試験濃度測定の義務化、マスバランスや統計などの改訂案

Item 14: OECDのTGを最新化するための見直しに関する意見聴取

OECD WNT34が4月開催予定

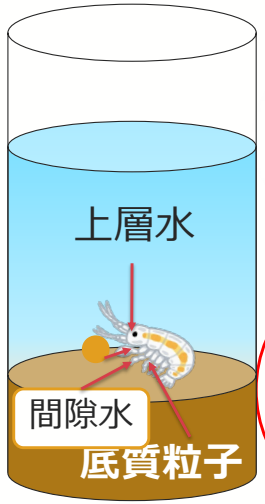
National Institute for Environmental Studies, Japan

ヨコエビ試験法、MEOGRT改訂の２件のSPSFの承認に努める

底生生物に対する曝露経路と生物利用性を考慮した包括的な底質リスク評価手法の構築

National Institute for Environmental Studies, Japan

環境省環境研究総合推進費(H31-R3)



底質毒性試験

水曝露と底質-水曝露

サブテーマ１

底生生物を用いた底質-水曝露による有害化学物質の毒性評価

東京大学・国立環境研究所

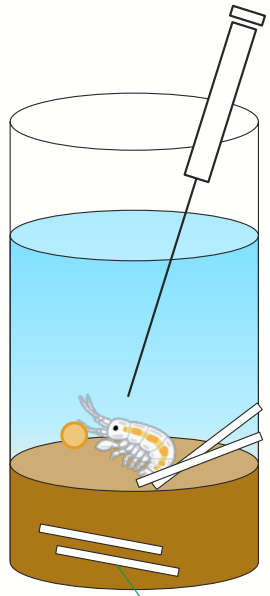
底質毒性試験中の分配・挙動

サブテーマ２

底質中有害化学物質の分配挙動と生物利用性の評価

国立環境研究所

化学分析



パッシブサンプリング手法

$$C_{free} = \frac{C_{PDMS}}{K_{PDMS/w}}$$

K_{pw}：シリコン/水分配係数

生物利用性と毒性

サブテーマ３

平衡分配法の最適化と包括的な底質リスク評価手法の構築

国立環境研究所

試験対象化学物質

平衡分配法

$PNEC_{底質} = \underline{Kd} \cdot PNEC_{水}$

底質/水分配係数

水生生物の予測毒性値

暫定リスク評価

平衡分配式の補正

PNECおよびPECの換算

評価体系

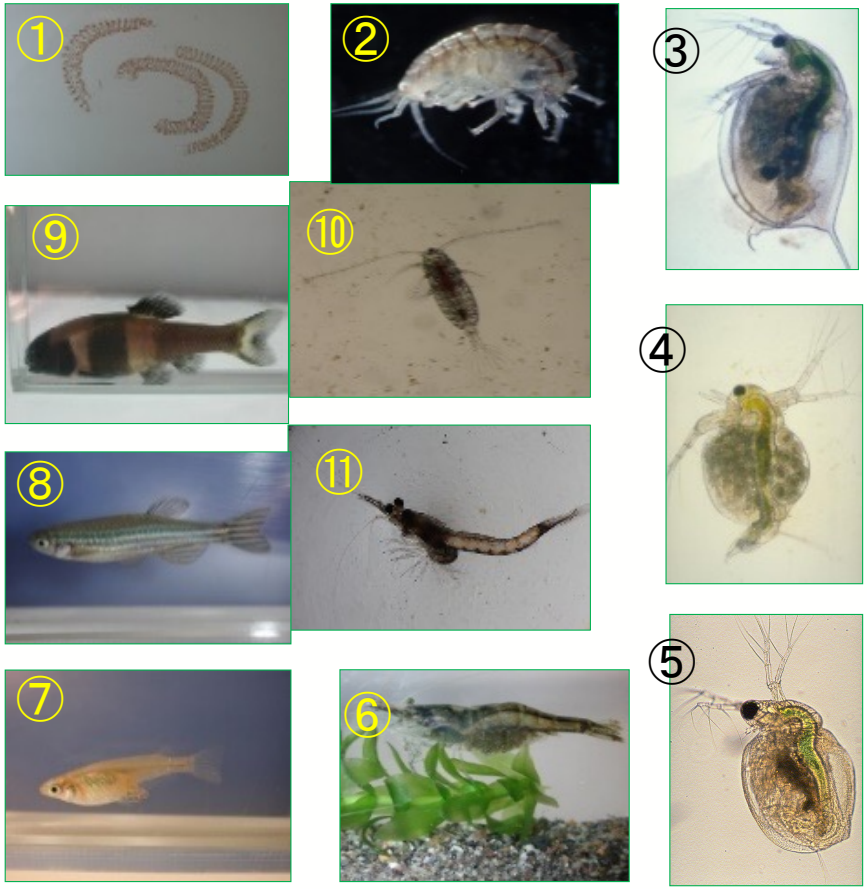
水生生物分譲業務について

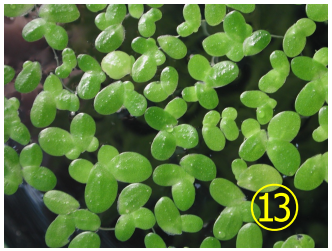
National Institute for Environmental Studies, Japan

化審法対象の試験生物種の販売

実験水生生物の有償分譲の対象種・系統

①	セスジユスリカ	*Chironomus yoshimatsui*
②	ヨコエビ	*Hyalella azteca*
③	オオミジンコ	*Daphnia magna*
④	タマミジンコ	*Moina macrocopa*
⑤	ニセネコゼミジンコ	*Ceriodaphnia dubia*
⑥	ヌカエビ	*Paratya compressa improvisa*
⑦	ヒメダカ	*Orizias latipes*
⑧	ゼブラ	*Danio rerio*
⑨	ファッドヘッドミノー	*Pimephales promelas*
⑩	アカルチア	*Acartia tonsa*
⑪	アミ	*Americamysis bahia*
⑫	ミジンコ6種	*Daphnia pulex* 他 5種
⑬	コウキクサ	*Lemna minor*





https://www.nies.go.jp/kenkyu/yusyo/suisei/index.html