EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON HEALTH AND ENVIRONMENTAL RISKS

SCHER

Opinion on

"Compatibility of the ISO standard 10708 (biodegradability test method) with the ultimate biodegradability requirements imposed through Annex III of Regulation 648/2004 of Parliament and of the Council"

Adopted by the SCHER
during the 3rd plenary of 28 January 2005

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1. BACKGROUND

Annex III of Regulation 648/2004 lists a set of OECD / ISO test methods aiming at providing International standardised methodologies to quantify the ultimate biodegradability of surfactants in aerobic conditions.

The international ISO 10708 standard, not included in Annex III of Regulation 648/2004, aims to determine the ultimate aerobic biodegradability of organic compounds in an aqueous medium. This test method is apparently similar to the "closed bottle test" (OECD 301 D).

The Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) already examined a request addressing a similar issue (ultimate test methods on biodegradability currently listed in Annex III, Regulation 648/2004) and adopted an opinion on "a proposed ready biodegradability approach to update detergent legislation" at its 12th plenary meeting of 25 November 1999.

According to Industry, ISO standard 10708 method provides an equivalent level on reliability compared to the other OECD test methods addressing the ultimately biodegradability features of surfactants (Annex III of Regulation 648/2004).

2. TERMS OF REFERENCE

The Scientific Committee on Health and Environmental Risks (SCHER) is requested to address this issue and give an opinion whether

1) the methodology and

2) the pass criterion

of ISO 10708 provide an equivalent level of reliability and stringency to the international test methods set out in Annex III of the Regulation 648/2004.

3. OPINION

3.1. Methodology

The biodegradability test method ISO 10708 was developed to have a relatively inexpensive and simple method for the ultimate aerobic degradability of poorly soluble chemicals. It was called BODIS (BOD of insoluble chemicals) or two-phase Closed bottle test, the latter because BOD bottles and an oxygen electrode are used like in the "Closed-Bottle-Test (OECD 301 D)". One third of the bottle (headspace) acts as an oxygen reservoir. Consequently, higher test substance concentrations than in the "Closed-Bottle-Test (OECD 301 D)" can be used, as the oxygen concentration in the (saturated) medium is no longer the limiting factor for degradation. Concentrations of up to 100 mg COD/l can be weighed directly into the test flasks. The method was included in an OECD ring-test (OECD, 1989) which will be discussed under section 3.2.

The procedure and technical requirements of the ISO 10708 test are relatively simple.

Considering the test conditions, the method is congruent with most of the biodegradability methods included in Annex III of Regulation 648/2004 in terms of the test medium, inoculum type and concentration, test duration, etc.(Table 1).

Table 1 - Comparison of the ISO 10708 test conditions and those included in Annex III of the Regulation 648/2004

Test method	ISO 14593	OECD 301B	OECD 301D	OECD 301F	OECD 301C	ISO 10708
Principle	CO_2 release	CO_2 release	BOD	BOD	BOD	BOD
Concentration test subst.	2-40 mg DOC/L	10-20 mg DOC/L	2-10 mg/L	50-100 mg COD/L	100 mg/L	100mg COD/L
Inoculum	act. Sludge 4 mg SS/L	act. sludge ≤ 30 mg SS/L	secondary effluent ≤5 mg/L	act. sludge ≤ 30 mg SS/L	act. Sludge ≤ 30 mg SS/L	act. sludge ≤ 30 mg SS/L

Considering the test design, it is equivalent to the ISO 14593, test which is the reference method in the Annex III of the Regulation 648/2004 except for the detection parameter. Eisentraeger et al. (202) presents a combined biodegradation test system where both parameters are measured, the test results for 15 samples (poorly soluble lubricants, ester oil or mineral oil) after 28 days of incubation demonstrated that results obtained for both parameters are in a comparable range.

Overall, the ISO 10708 test is comparable in terms of the methodology to the tests described in Annex III of the Regulation 648/2004; combining in its methodological design elements of the reference method ISO 14593 and of other methods described in OECD 301. The reasons for not including this test in the OECD guideline 301 does not seem to be related to scientific issues but are rather of a managerial nature. In this context OECD (1989) states "*The Set I Two Phase Closed-Bottle test does not appear to offer any significant advantages over the existing respirometric tests, being labour and space intensive*".

3.2. Pass criterion

There are three main sources of information allowing a comparison of the biodegradation results obtained with the ISO 10708 to those acquired by other ready biodegradation methods.

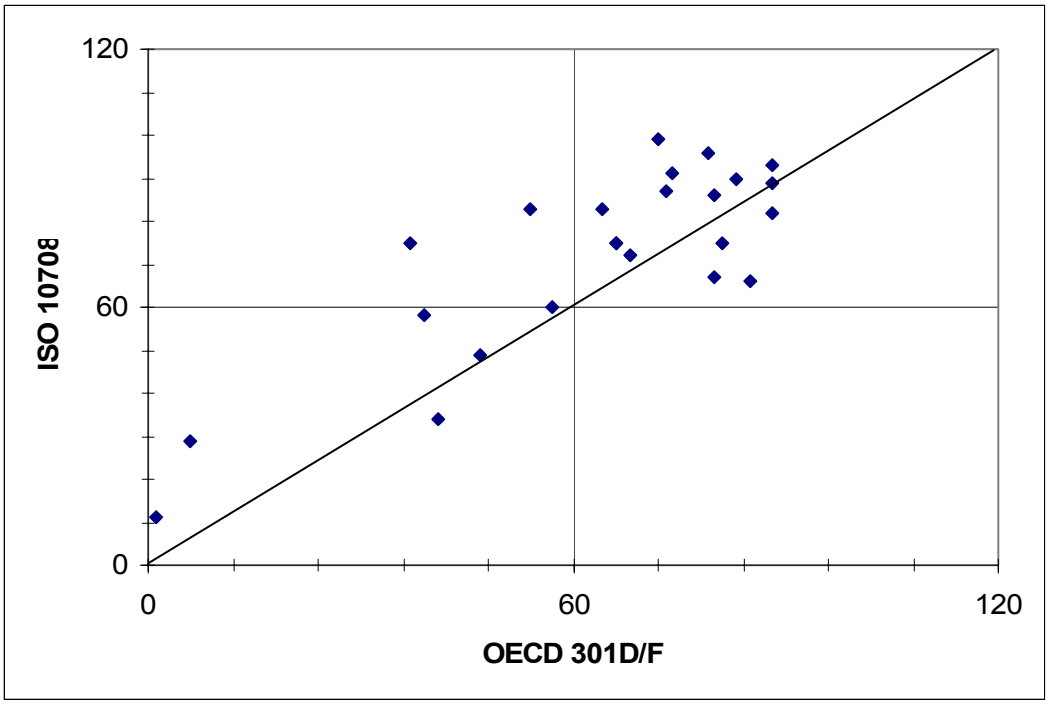
Ritcherich *et al.* (1998) compared biodegradation data from the "Two- phase closed bottle test" (BODIS test) and the OECD 301D (Closed Bottle test). 54 poorly soluble compounds were compared. For 46 % of the substances the ISO 10708 biodegradation was significantly higher (i.e., data differed ≥10% relatively to each other) than that observed in the Closed Bottle test; 7% of the substances degraded better in the Closed Bottle test and 44 % gave comparable results (difference less than 10%). Taking the 60 % BOD/COD pass criterion, for 83% of the substances the same conclusion on ready

biodegradability was obtained, whereas for the remaining 17 % only the ISO 10708 test indicated readily biodegradability.

The CESIO (European Committee of Organic Surfactants and their Intermediates) has provided biodegradation test (OECD 301 D/F tests and ISO-10708) results for a set of anionic, cationic and non-ionic surfactants, allowing a comparative evaluation. The information was supplied by CESIO (Appendix 2) in tabular form without additional information and, therefore, the SCHER cannot confirm the quality and validity of the data.

Figure 1 presents a comparison of both methods. In general a relatively good linear correlation between both methods is observed. On average, the degradation value obtained in the BODIS test tend to be higher than in the OECD methods (as suggested by the slope of the line); for three out of 24 surfactants the differences in biodegradability were larger than 20%.

Figure 1 - Comparison of ISO 10708 and OECD 301D/F using degradation data on surfactants provided by CESIO (2004).



Using the biodegradability pass criterion of the 60% BOD/COD, comparison of the biodegradability test data of 24 surfactants shows that 21 compounds (88%) reached the same evaluation (14 above and 7 below the pass level) whereas 3 compounds (12%) only passed the ISO-10708 test.

A calibration exercise of methods for determining ready biodegradability was performed by the OECD in 1988 and a final report on the evaluation of these results was issued in 1989. This report compared the biodegradability data obtained for 4 organic compounds applying different OECD ready biodegradability tests (301B, 301C, 301 D and 301F) and the ISO 10708 test.

The variability inter- and intra-assays observed in the inter-calibration exercise was high, as expected for these biological assays. The results obtained in the OECD

intercalibration exercise must be considered for putting into context the comparison presented in Figure 1. Similar levels of discrepancy were observed among other tests included in Annex III of the Regulation 648/2004 and even among laboratories conducting the same tests (OECD, 1989).

From the evaluation of the above mentioned studies and considering the observed variability, the biodegradation results obtained with the ISO 10708 are consistent (i.e., results in a similar final evaluation) with those obtained with the OECD ready biodegradability methods.

4. CONCLUSION

Referring to the terms of reference, the SCHER concludes that:

the ISO 10708 test is comparable in terms of the methodology to the tests described in Annex III of the Regulation 648/2004, and

the ISO 10708 provides an equivalent level of reliability and stringency to the international test methods set out in Annex III of the Regulation 648/2004.

5. LIST OF ABBREVIATIONS

BOD	Biological Oxygen Demand
CBT	Closed Bottle Test (OECD 301 D)
COD	Chemical Oxygen Demand
DOC	Dissolved Organic Carbon

6. REFERENCES

CESIO (2004) Appendix 2: Comparison of Biodegradability Test Data. CESIO Oct 2004

Eisenträger A., Schmidt M., Murrenhoff H., Dott W. and Hahn S. (2002) Biodegradability testing of synthetic ester lubricants – Effects of additives and usage, Chemosphere 48, 89-96

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7. ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

Prof. M. T. Garcia Ramon (external expert) Dr. S. Hahn (external expert), Prof. C. Janssen, Prof. J. Tarazona (rapporteur).