Role of biodegradability tests in efforts for minimising the environmental risk of pesticides application

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Abstract

In EU countries the classification of pesticides is based on their toxicity, bioaccumulation and duration in water, soils and sediments. This paper reviews some information on our own works (particularly on ready biodegradability tests in aquatic systems) as well as on other tests preferred by OECD. The compatibility of OECD, ISO and DIN methods has been given too. The applicability of test methods has been described as well as the principle of the tests. The own results of OECD 301 F ready biodegradability test of some pesticides from the most important pesticides group have been described. The test pesticides contained such active ingredients like atrazine, simazine, carbendazim, alpha-cypermethrin, prometryn and propachlor. The determined biodegradability, using described step-by-step 301 F OECD manometric respirometry test revealed that most of tested pesticides couldn't be classified as ''readily biodegradable''.

Introduction

After plenty of time and never-estimated costs, the high sustainable economic growth and the high standard of living have been achieved in some countries. But this activity has created some unwanted effects too. One of these effects is the hazard posed to the environment by human chemical products as well as their associated wastes.

Pesticides in the environment can arise from application, which can be proper or improper due to overdosing, or application at the wrong time. But there are also other sources, which should be prevented: accidents during production, transport, storage, washing of equipment etc. The problem of pesticides in soil and groundwater has been exhaustively presented during the 5th Forum (1, 2). The risk, which is caused to the environment by production and application of chemicals - particularly pesticides -, should be considered as a function of the exposure of the environment, the toxicity of the chemicals and the time of exposure. Pesticides due to their destination are designated to kill agrofags and thus they can't be completely neutral for the environment. But their undesirable side effects should be identified far before commercial practical application. The proper assessment of the environmental hazard of a pesticide of interest needs some information about the course of interactions in the environment leading to degradation of the chemical or its accumulation.

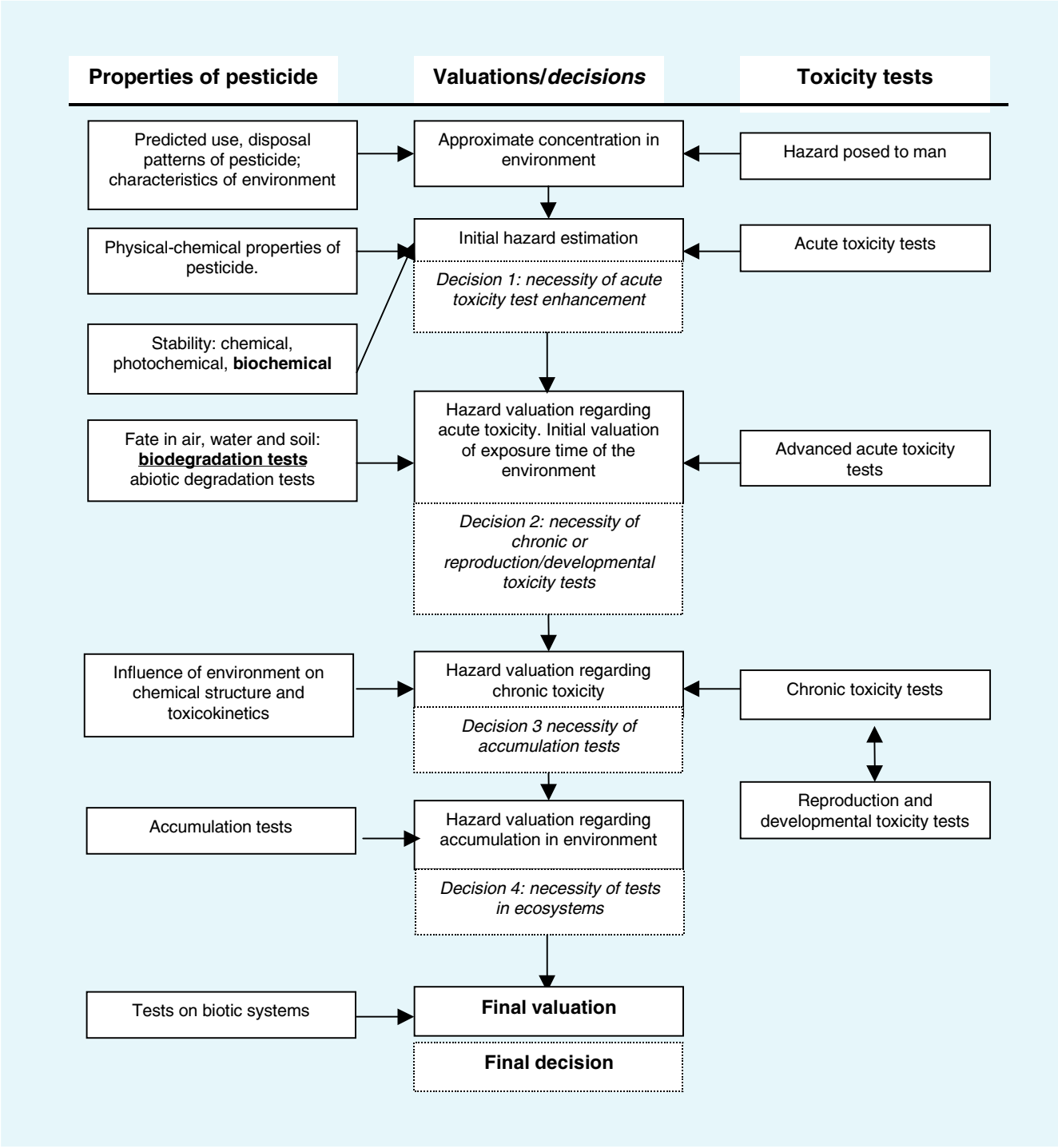

Figure 1. Strategy for evaluation of environmental hazard of a pesticide

Pesticide fate in the environment - biodegradation

The pesticide life cycle in the environment is rather multistage. The microbial degradation of many pesticides has been revealed for several years, in the environment as well as using activated sludge or other aerobic and anaerobic processes. (3,4). The biodegradation and hydrolysis are the most effective loss mechanisms of organic substances in the environment.

Biodegradation of a substance commonly means the molecular degradation of it due to complex action of microorganisms. The complex structure can be broken down into fundamental compounds. The total decomposition of organic materials is only possible via biodegradation leading to mineralisation:

$$\text{organic materials} + O_2 \xrightarrow{\text{microorganisms}} CO_2 + \text{water} + \text{salts} + \text{biomass}$$

Because of greatly integrated and complex routes of a pesticide and its derivatives in the environment and of many interactions, a strategy of testing should cover many areas of interest as described in (5) and shown in Figure 1. The areas are as follows: physicochemical properties of the pesticide, effects on biotic systems, degradation and accumulation abilities and short and long term health effects.

Biodegradation tests

General

The Organisation for Economic Cooperation and Development (OECD) created the most commonly used standard methods for biodegradation testing (6). The assessment of the environmental fate of a novel pesticide is now required for its registration. The crucial feature (regarding environment) of human products, particularly pesticides, is the capacity for biodegradation. The biodegradability tests focus on the measurement, at standard conditions, of the degradation rate of the compound in environment.

Biodegradability tests in water

This test system enables us to make preliminary screening using simple tests of ultimate biodegradation of pesticides. These tests are organised into logical series of steps of increasing complexity and cost:

Ready biodegradability

Tests provide us with the reliability regarding that a pesticide presenting positive result in this type of test can be rapidly biodegraded in the environment.

Inherent biodegradability

Tests of prolonged exposure of the test compound to microorganisms. The compound, which gave a positive result because of more favourable conditions of this test, may not be assumed to be rapid and reliable biodegraded in the environment.

Simulation tests

Present the biodegradation rate under some environmental conditions: a) biological treatment (aerobic); b) biological treatment (anaerobic); c) river; d) estuary; f) sea and g) soil. The sequence of testing for biodegradability in aquatic environment is shown in Figure 2.

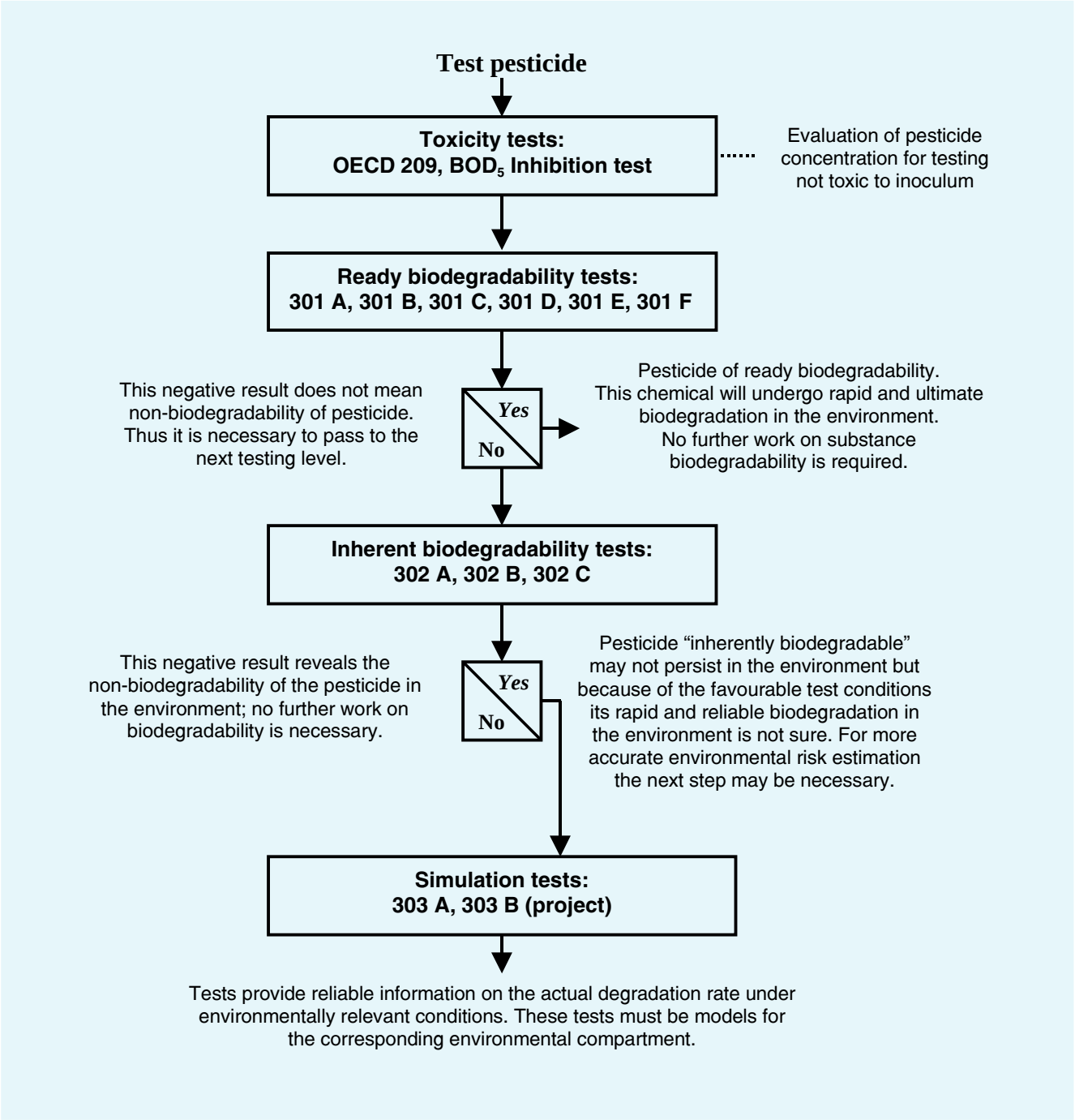

Figure 2. Sequence of testing for biodegradability in aquatic environment

General principle of ready biodegradability tests

A solution, or suspension, of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The amount of DOC in the test solution due to the inoculum should be kept as low as possible compared with the amount of organic carbon of the test substance. Allowance is made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test substance, although the endogenous activity of cells in the presence of a chemical will not exactly match that in the endogenous control. A reference compound (sodium acetate or sodium benzoate) is run in parallel to check the operation of the procedures.

Table 1. Applicability of biodegradability tests in an aerobic aqueous medium: dependence on the test compound properties and compatibility of European tests.

	Test			Analytical method	Suitability for compounds which are:		
	OECD	ISO	DIN/EN		poorly soluble	Volatile	absorbing
Ready biodegradability							
DOC die-away- [IPO]	301 A	7827	7827	Dissolved organic carbon	-	-	+/-
CO_2 evolution	301 A	9439	9439	Respirometry: CO_2 evolution	+	-	+
CO_2 evolution (sealed flasks)		14593		Respirometry: CO_2 evolution	+	+	+
MITI (I)	301 C			Respirometry: oxygen consumption	+	+/-	+
Closed bottle	301 D	10707	10707	Respirometry: dissolved oxygen	+/-	+	+
Modified Screening [IPO]	301 E			Dissolved organic carbon	-	-	+/-
Manometric respirometry [IPO]	301 F	9408	9408	Oxygen consumption	+	+/-	+
Two-phase closed bottle		10708		Respirometry: dissolved oxygen	+	+	+
Inherent biodegradability							
Zahn-Wellens [IPO]	302 B	9888	9888	Dissolved organic carbon or chemical oxygen demand	-	-	+/-
Modified SCAS	302 A	9887	9887	Dissolved organic carbon	-	-	-
Simulation tests							
Activated sludge [IPO]	303 A	11733	11733	Dissolved organic carbon	-	-	+/-

- Uselessness + usefulness +/- at some conditions

[IPO] test used at Institute of Industrial Organic Chemistry

These OECD test guidelines defined limits for the requirements of a test substance for ready biodegradability. In the case of DOC measurement the "pass level" for this are 70% removal and 60% of ThOD or $ThCO_2$ for respirometric methods. For the specific analysis of the test chemical 80% removal must be achieved. The abovementioned removal values are expected to be reached within the 10-day window within the 28-day period of the test. This window begins (Initiation of biodegradation) when 10% of DOC, ThOD or $ThCO_2$ is obtained and must end before day 28 of the test.

An applicability of biodegradability tests in an aerobic aqueous medium and the dependence on the test compound properties and compatibility of European tests are shown in Table 1 (7, 8).

The test report is expected to be included as described in (6). The 28-day biodegradability test, the biodegradability graph and the principle of determination of: time window, duration of the lag phase and of degradation phase is presented in Figure 3.

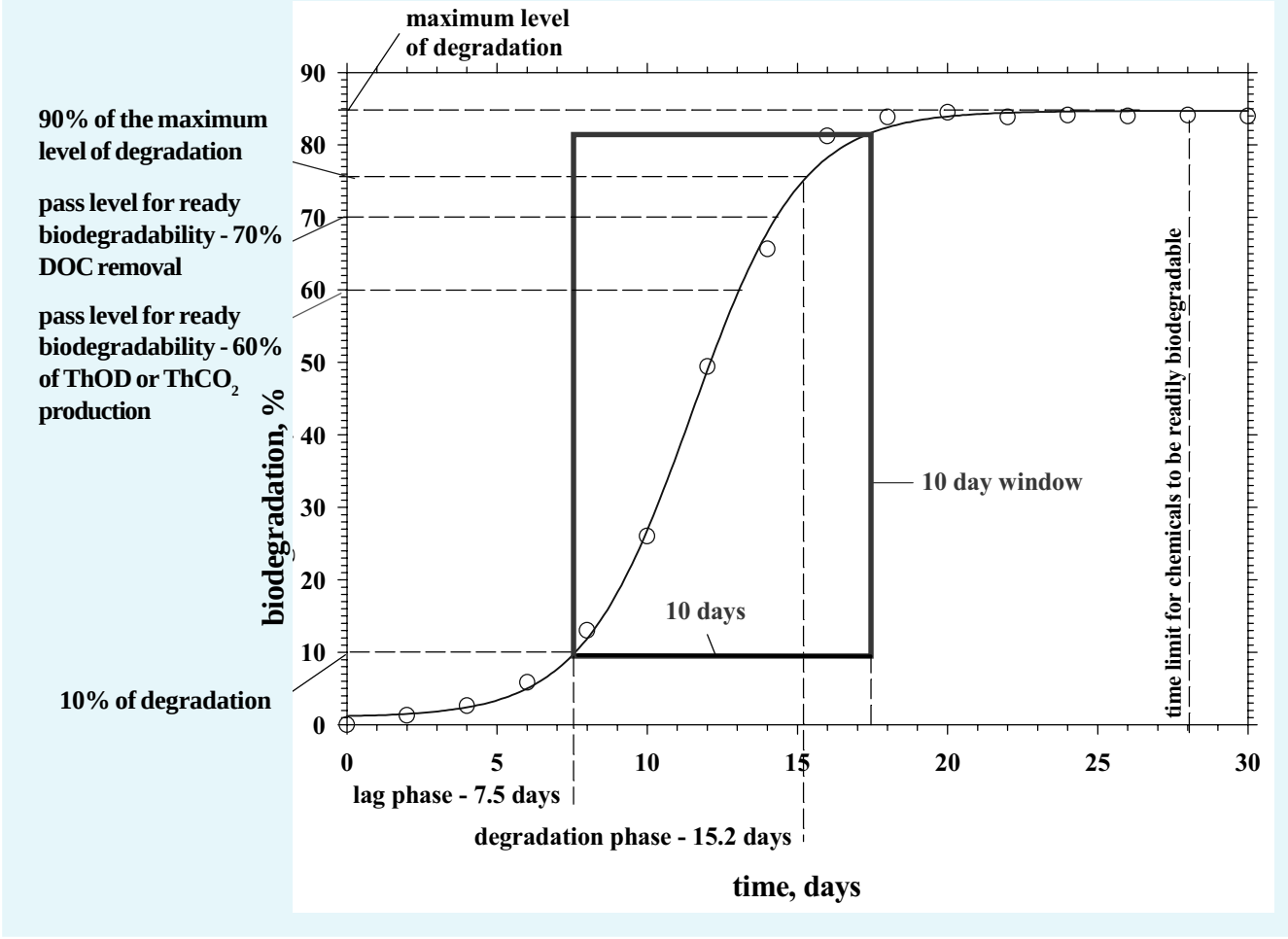

Figure 3. 28-day biodegradability test, the biodegradability graph. The principle of determination of: time window, duration of the lag phase and of degradation phase.

Example of test - 301 F OECD manometric respirometry test

The 301 F OECD manometric respirometry test has been used for determination of ready biodegradability of some pesticides from the most important pesticides group. The tested pesticides contained such active ingredients like atrazine, simazine, carbendazim, alpha-cypermethrin, prometryn and propachlor. The course of this test is shown in Figure 4.

The consumption of oxygen was determined by measuring the change of pressure in a flask. This value was stored in an intelligent head and each time on request it was able to transmit this information via infrared waves into a pilot device. Evolved carbon dioxide was absorbed in a solution of potassium hydroxide. Biodegradation of organic in water usually undergoes in two phases (9). First the carbon breakdown undergoes and next nitrification occurs. This later one consumes additional oxygen amount. Two groups of nitrifying bacteria catalyse the following synthesis:

$$NH_4^+ \xrightarrow{+1.5\ O_2} NO_2^- \xrightarrow{+1.5\ O_2} NO_3^-$$

This has shown conversion results in oxygen uptake of 4.57 mg/l per mg of NH_4^+ which is a significant contribution to the whole oxygen uptake. Of course that phenomenon falsifies the BOD values. To eliminate the influence of nitrification, some nitrification inhibitor N-allylthiourea (ATH) was added. The BOD value was calculated as follows:

$$BOD = \frac{\text{mg } O_2 \text{ uptake by test pesticide - mg } O_2 \text{ uptake by blank}}{\text{mg test pesticide in vessel}}$$

and hence biodegradation could be described as:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2 \text{ / mg test pesticide)}}{\text{ThOD (mg } O_2 \text{ / mg test pesticide)}} \text{ x100}$$

or using measured initial COD value:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2 \text{ / mg test pesticide)}}{\text{COD (mg } O_2 \text{ / mg test pesticide)}} \text{ x100}$$

It is often found that COD is usually not as high as the ThOD and some chemicals are very poorly oxidised in the COD test. This can result in too high biodegradation values. The results of the 301 F OECD ready biodegradability test (10, 11, 12) are given in Figure 5. As it can be seen all these test pesticides except carboxin are not readily biodegradable.

Carboxin can be expected to pass a level of ready biodegradability. On the basis of a literature it is well known that the majority of test compounds of all kinds do not fit into ready biodegradable category. But it should be borne in mind that the ready biodegradability tests are not definitive ones.

Conclusion

- The biodegradability of some pesticides, containing various active ingredients like atrazine, simazine, carbendazim, alpha-cypermethrin, prometryn and propachlor from the most important pesticides group using OECD 301 F ready biodegradability test has been measured.
- It was found that the determined biodegradability of most tested pesticides, using described step-by-step 301 F OECD manometric respirometry test couldn't be classified as ''ready biodegradability''.
- However the tests we have performed are just a preliminary screening or a start of the studies about the environmental fate of the pesticides.
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- We are aware of much more test work that must be done using inherent biodegradability tests and may be simulation tests finally.
- International cooperation is highly recommended in this field of global environmental investigations. UNIDO seems to be the very place to make all international participants meet together (13).
- The efforts should be expanded towards the invention and application of environmentally friendly pesticides; this means among others properties with more biodegradability.
- Everyone, including the companies should be interested in environmental fate of the pesticides and actual degradation rate of their products in the environmental compartment.

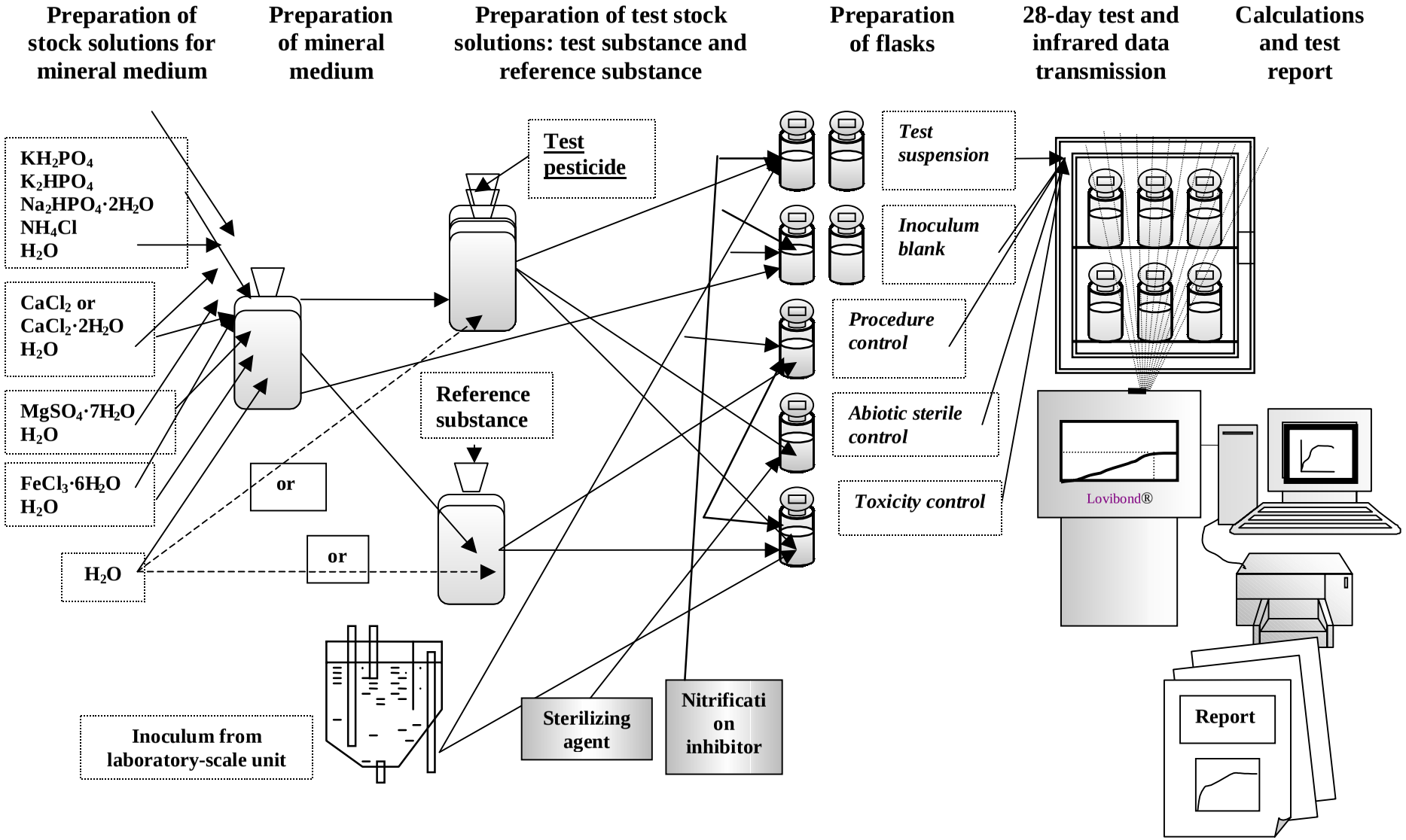

Figure 4. Course of 301 F OECD manometric respirometry test.

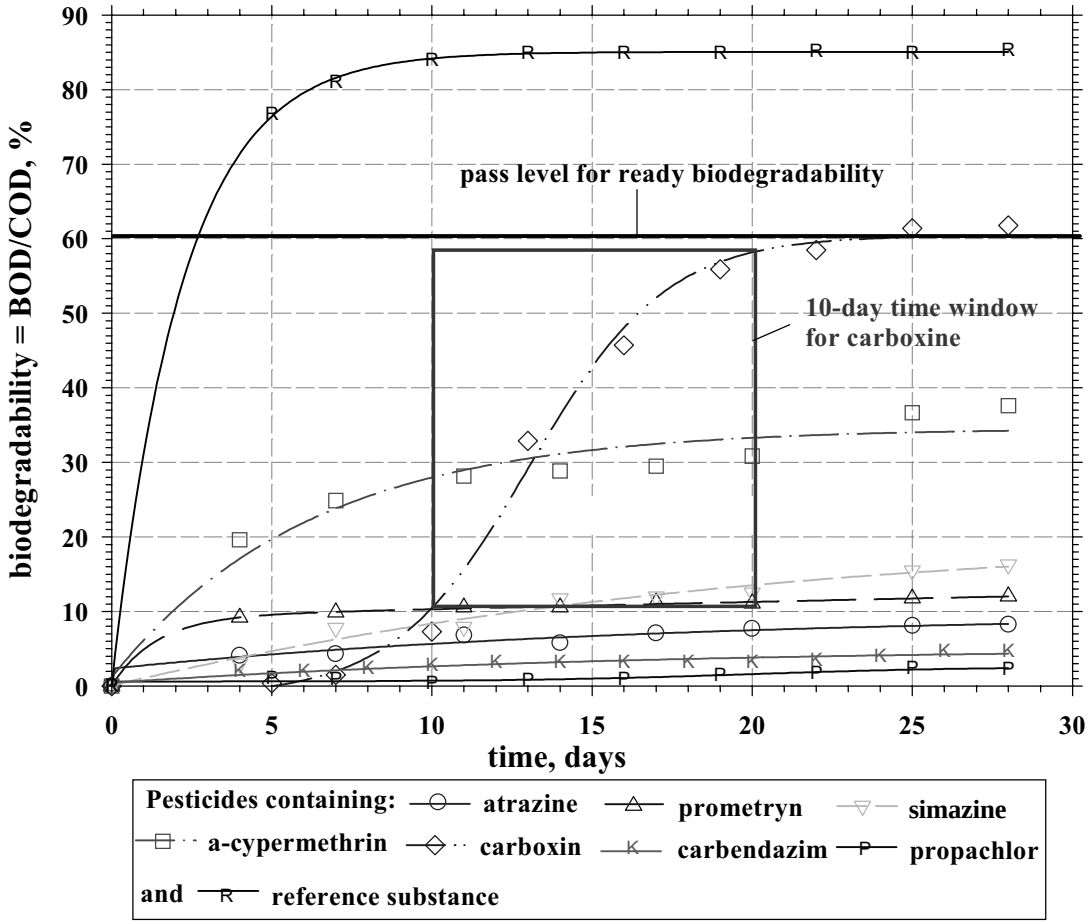

Figure 5. 301 F OECD biodegradability test for seven pesticides of various active ingredients.

Used abbreviations and definitions in accordance with OECD Guidelines

BOD: Biochemical oxygen demand (mg) is the amount of oxygen consumed by microorganisms when metabolising a test compound. Also expressed as mg oxygen uptake per mg test compound.

COD: Chemical oxygen demand (mg) is the amount of oxygen consumed during oxidation of a test compound with hot, acidic dichromate. It provides a measure of the amount of oxidisable matter present; also expressed as mg oxygen consumed per mg test compound.

DOC: Dissolved organic carbon is the organic carbon presents in solution or that which passes through a 0.45 micrometer filter or remains in the supernatant after centrifuging at approx. 4,000 g (about 40,000 m sec^{-2}) for 15 min.

ThOD: Theoretical oxygen demand (mg) is the total amount of oxygen required to oxidise a chemical completely. It is calculated from the molecular formula and is also expressed, as mg oxygen required per mg test compounds.

$ThCO_2$: Theoretical carbon dioxide (mg) is the quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test compound when fully mineralised. Also expressed as mg carbon dioxide evolved per mg test compound.

Primary biodegradation: The alternation in the chemical structure of a substance, brought about by biological action, resulting in the loss of a specific property of that substance.

Ultimate biodegradation (aerobic): The level of degradation achieved when the test compound is totally utilised by microorganisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

Readily biodegradable: An arbitrary classification of chemicals, which have passed certain, specified screening tests for ultimate biodegradability. These tests are so stringent that it is assumed that such compounds will rapidly and completely biodegrade in aquatic environments under aerobic conditions.

Inherently biodegradable: A classification of chemicals for which there is unequivocal evidence of biodegradation (primary or ultimate) in any test of biodegradability.

Lag phase: The period from inoculation in a die-away test until the degradation percentage has increased to about 10%. The lag time is often variable and poorly reproducible.

Degradation phase: The time from the end of the lag period to the time when 90% of the maximum level of degradation has been reached.

10-day window: The 10 days immediately following the attainment of 10% biodegradation.

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