Development of Toxicity Test Protocols for Corals

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The risk assessment which is generally performed for any registration of chemical products does not cover corals up to now. First research on the effects of UV-filters started. However, for the generation of reliable and comparable toxicity endpoints it is essential to develop a standard toxicity test protocol. For risk assessment larvae or nubbins (branch tips consisting of several polyps) can be used. Beside acute tests also long-term tests using growth parameters can be performed under lab conditions. First studies showed that in case of extended test duration to 1-2 months, some coral species could be suitable to fulfil validity criteria according to OECD 239 to generate robust and reproducible data. Endpoints could be assessed on growth rate and yield based on fresh weight, polyp number or total length of corals.

First data show that tests with focus on coral growth parameters can be established and can assist in risk assessment of chemicals on corals.

In case effects on corals could be determined in acute tests a further testing strategy is needed to address higher TIER testing.

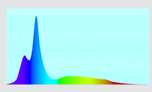
We present data of an acute testing with focus on toxicity on the zooxanthellae (symbiotic algae). First results indicate that toxicity seems to be higher on algae living in symbiosis with corals than on solitary living algae or macrophytes.

We established a testing strategy in order to cover the increased sensitivity of corals compared to standard species.

Beside the data of an acute short-term testing, we discuss data of a higher tier long term testing and finally end with a proposed microcosm study design.

For risk assessment based on long term testing, growth rates and yield (e.g. length and biomass), necrosis (loss of tissue) and coral bleaching could be assessed. These growth parameters which are based on metric data will assist in refining risk assessments. Toxic endpoints that are based on visual observations such as necrosis, bleaching, polyp contraction, change in color, loss of tissue and mucous formation can also be determined. In a microcosm study endpoints can be combined to refine risk assessment.

Material & Methods

Temperature:	25°C±1°C	Phosphate:	0 - 0.05 mg/L	Carbonate hardness (°dkH):	6.7 ± 0.3	Test Strategy :	
pH-value:	8.2±0.2	Nitrate:	2 - 5 mg/L	Oxygen content:	8.02-8.48 mg/L	TIER 1 acute testing over 48 hours	
Calcium:	420-430 mg/L	Light spectrum:		Test Design:	based on OECD Guideline 202, 235, 239, 221	TIER 2 long term testing over 1-2 months	
Magnesium:	1230-1290 mg/L					TIER 3 microcosm study 3-4 months	
Salinity:	34.8-35.2 g/L						

Results

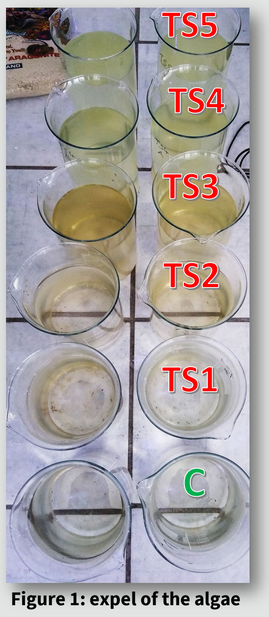


Figure 1: expel of the algae

Table 1: Effects on corals treated with 3,5-Dichlorophenol (3,5-DCP) over 48 hours (daily reneval) and a 42 days recovery phase

3,5-DCP		Effects on *Seriatopoa caliendrum*				
Treatment	Conc. [mg/L]	4h (toxicity phase)	24h (toxicity phase)	48h (toxicity phase)	14 days (revovery phase)	42 days (recovery phase)
control	0.00				good growth, dark green colour	good growth, dark green colour
TS1	0.625		water pale green	half of the corals look bright	less colourful, pale, no growth but polyps well formed and look vital	polyps recovered and started growth, but significantly recuced growth compared to the control
TS2	1.25		water pale green and yellow	loss of green colour but polyps look vital	less colourful, pale, no growth but polyps well formed and look vital	polyps recovered and started growth, but significantly recuced growth compared to the control
TS3	2.50		two corals of 6 replicates show in-turned polyps, water green/yellow	all polyps in-turned	less colourful, pale, no growth but polyps well formed and look vital, 10 % of the corals died off	complete necrosis, 100 % lethal
TS4	5.00	slightly in-turned polyps	all polyps deformed and in-turned, water slightly cloudy with green/yellow colour	polyps black, necrosis, water cloudy, tissue losses	complete necrosis, 100 % lethal	complete necrosis, 100 % lethal
TS5	10.0	half in-turned polyps	all polyps deformed and in-turned, brown polyps, water cloudy with green/yellow colour	polyps black, necrosis, water cloudy, tissue losses	complete necrosis, 100 % lethal	complete necrosis, 100 % lethal

Figure 2: coral bleaching

Table 1: % Effects on *Seriatopora caliendrum* treated with 3,5-DCP

3,5-DCP		Effects		
Treatment	Conc. [mg/L]	4h	24h	48h
control	0.00	0%	0%	0%
TS1	0.625	0%	0%	50%
TS2	1.25	0%	0%	100%
TS3	2.50	0%	33%	100%
TS4	5.00	100%	100%	100%
TS5	10.0	100%	100%	100%

Figure 3: untreated control

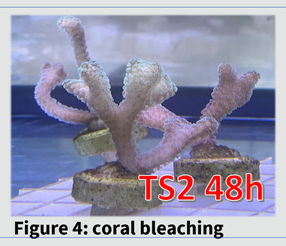


Figure 4: coral bleaching

Figure 5: in-turned polyps

Figure 6: coral bleaching

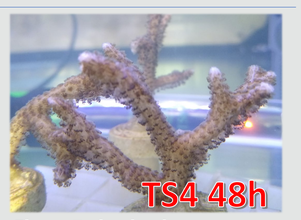
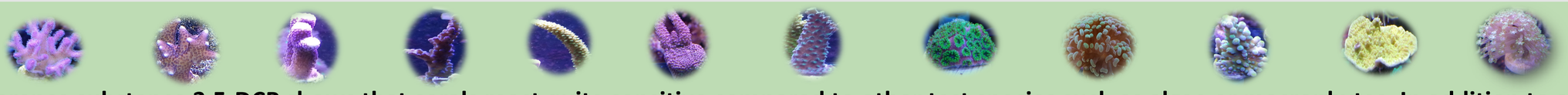


Figure 7: dead polyps

Discussion



A first test with the reference substance 3,5-DCP shows that corals react quite sensitive compared to other test species such as algae or macrophytes. In addition to acute effects, long-term effects could also be observed. It became clear that corals are indicator species, especially corals living in symbiosis with algae. It was shown that the algae were released, but the polyps still looked healthy at lower concentrations in acute tests over 48 hours. However, no growth of the coral was observed within 14 days after treatment. Therefore, it may be appropriate for coral species with small polyps (SPS) to test for a longer period of time based on OECD 239 to obtain robust and reproducible data for growth parameters. Selected endpoints could be growth rates and yields based on fresh weight, polyp count, or total coral length. For species with large polyps (LPS), the test could also be adapted to OECD 221. In this case, the polyps could be counted. When doubling of the number of polyps is reached, the endpoints can be calculated based on polyp number and fresh weight. It should be mentioned that acute testing with corals can indicate potential risk, but further testing might be needed to refine the risk assessment. To determine risk for different coral species and combine acute and chronic endpoints, microcosm studies could be a useful tool to fill data gaps.

Proposal for Testing Strategy	
TIER Approach	Parameter and Guidelines (only describes general test design for choosing number of replicates, statistic; other parts have to be adopted to the test species e.g. water parameters, environmental conditions)
TIER 1 Acute Testing (Risk Assessment Factor 100)	Necrosis, bleaching, polyp contraction, change in colour, loss of tissue, mucous formation; Following as far as possible for the test species to OECD 202 or OECD 235; Immobilisation corresponds to polyp contraction
TIER 2 Long Term Testing (Risk Assessment Factor 10)	Growth rates and yields (e.g. length and biomass or number of polyps) + visual observation (polyp contraction, change in colour, loss of tissue and mucous formation); Following as far as possible for the test species to OECD 221 and OECD 239
TIER 3 Multi-species Testing, Microcosm Studies (Risk Assessment Factor 2-5)	Combined species sensitivity distribution testing in microcosm studies, including fresh weight, number of polyps, or optionally length of corals at test start and test end; Endpoints based on yields, growth rates and mortality; Further assessment of visual parameters like polyp contraction, change in colour and mucous formation

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