Natular® G30 Exposure Assessment, Summary

January 29, 2014

Prepared by: Karen Larson
Vice President, Regulatory Affairs
klarson@clarke.com
(630) 671-3123

Prepared for: Vermont Agency of Agriculture and the Vermont Pesticides Advisory Council

Thank you for you recent inquiry regarding our Natular™ larvicide and its suitability for use in Vermont mosquito abatement programs consistent with Department objectives to deliver optimally effective performance in the most environmentally sensitive manner. I know that consideration of the Council complements the standards and decisions made by the regulatory and registration authorities that have reviewed this product, and I appreciate that the Council is seeking to learn as much as possible about this comparatively new product, its active ingredient, and its proposed use.

I understand the proposed use of Natular G30 in Vermont includes freshwater floodplains with variable flooding and dry cycles offering repeat wet periods sufficient to produce sequential broods of mosquitoes. This area is traditionally treated repeatedly with single-brood formulations, perhaps 4 to 6 times per year, or up to 10 times annually in seasons with heavy precipitation and prolonged flooding. A multi-brood formulation, like Natular G30, with effective program planning can offer operational efficiencies in requiring half as many applications for the same period.

A discussion of estimated aquatic concentration subsequent to application of the extended (slow) release formulation follows. Peak expected environmental concentration (EEC), when applied at the recommended rate, is below the no observed effect concentrations (NOEC) of the most sensitive non-target freshwater invertebrates. The discussion below includes the environmental and physiological inputs into the derivation of the EEC. All of the information sourced for this summary is in the public literature and is consistent with the summaries provided previously to the Council.

<u>Summary of Environmental Fate & Behavior</u>

Spinosad is relatively rapidly dissipated in water. Photolysis is predominant, with hydrolysis and dissipation to sediment also contributing. The degradation in sediment is slower than from the water column but is still regarded as rapid, with 15-20% of the applied dose being found after 35 days. Photolytic degradation is via a fairly complex pathway beginning with an initial rapid loss of parent compound activity and ending in complete breakdown to CO_2. Tables 1 and 2 summarize the physicochemical endpoints and environmental degradation of Spinosad, respectively.

In the shallow and open aquatic environment, as is often applied for mosquito larva population management, photolysis predominates the degradative pathways with a half-life of less than 24 hours.

TABLE 1. Physicochemical endpoints of Spinosad

Property	Spinosyn A	Spinosyn D	Notation
Dissociation constant (pKa)	8.10	7.87	Amino group protonates in neutral and environmentally relevant acidic pH conditions
Water Solubility mg/L (Distilled Water	89.4	0.495	Slightly to moderately soluble
pH 5	290	28.7	Becomes less soluble as pH increases (consistent with protonation of the amino group)
pH 7	235	0.332	
pH 9	16	0.53	
Henry's law Constant ($Pa.m.^3mol^{-1}$)	2.46×10^{-7}	3.01×10^{-5}	Very slightly volatile from water
Partition Coefficient (log Pow)	3.9	4.4	Affinity for the organic phase increases with PH (consistent with protonation of the amino-group)
pH 5	2.8	3.2	
pH 7	4.0	4.5	
pH 9	5.2	5.2	

TABLE 2. Route and rate of degradation

Property	Findings
Aerobic metabolism	Half-lives of 9.4 to 17 days for spinosyn A and 15 days for spinosyn D in a sandy loam soil.
Field Dissipation	Half-lives of spinosyn A determined to be < 1 d
Residues on leaves	Half-lives of 2-3 days
Hydrolysis	Spinosyn A and spinosyn D are hydrolytically stable at pH 5 and 7. At pH 9, the extrapolated half-life calculated by first order kinetics was estimated at 200 and 259 days for spinosyns A and D, respectively
Photolysis	The half-lives of spinosyn A and spinosyn D were determined in a buffer of pH 7 in natural sunlight to be 22.3 and 19.7 *hours*, respectively. In natural pond water, the half-life of the total spinosad component was 4.3 hours. In an aquatic microcosm study, at 2 days spinosyn A accounted for < 15% of that applied (it had more than 85% degraded)

<u>Effects on Aquatic Organisms</u>

A summary of the acute and chronic toxicity endpoints for aquatic organisms is provided in Table 3. Spinosad is slightly or moderately toxic to fish on an acute and chronic basis. All algal species tested showed some sensitivity to spinosad but the difference was marked. Spinosad is slightly to moderately *acutely* toxic to daphnia and other aquatic invertebrates such as oysters and shrimp. However, on a chronic basis spinosad is highly toxic to the freshwater daphnia. In an outdoor aquatic microcosm study after a single treatment of a suspension concentrate agricultural spinosad formulation, daphnia populations were reduced yet recovered to levels similar to the control in the 8 µg /L treatment, but recovery was not observed at the higher treatment levels (17 and 33 µg /L) during the evaluation period *(Duchet et al. 2008. Chemosphere 74:70-77*). In further work with

Daphnia, Duchet et al. conclude that, although effects conclusions based exclusively on laboratory tests are protective, a combination of laboratory and field evaluations are more ecologically relevant and more adequately estimate real effects (*Duchet et al. 2010. Ecotoxicology 19:1224-1237*). The microcosm work of Duchet et al has been referenced in risk assessments by the U.S. EPA as well as its Australian counterpart, the Australian Pesticides and Veterinary Medicine Authority.

Because Natular G30 offers extended activity over a 30-day period, a discussion of toxicity from both acute and chronic exposure follows. Daphnia is the organism most sensitive to chronic exposure. You will note the variability in the available data even for this organism. In risk assessment considering chronic exposure in the outdoor environment, the field microcosm study is most often used as the chronic toxicity endpoint.

TABLE 3. Acute and Chronic Toxicity Endpoints for Aquatic Organisms

Species	Study-Type	Endpoints
Fish	Acute LC50	Carp (*Cyprinus carpio*): 4.99 mg/L Sheepshead minnow (*Cyprinodon variegatus*): 7.87 mg/L Bluegill sunfish (*Lepomis macrochirus*): 5.94 mg/L Trout (*Oncorhynchus mykiss*): 30 mg/L
	Early Life Stage (62 days)	*Oncorhynchus mykiss*: 0.498 mg/L
Daphnia	48 hour static test	EC50 1.48 mg/L
	48 hour semi-static (renewal at 24 hours)	EC50 = 14 mg/L
	21 day chronic flow through	NOEC = 0.62 µg/L
	21 day outdoor microcosm	NOEC < 8 µg /L (recovered populations)
Marine Amphipod	10 day acute test	NOEC > 63 mg/kg dry sediment; > 1.38 mg/L pore water; 0.327 mg/L overlying water
Grass Shrimp	96-hr semi-static (renewal every 24 hours)	NOEC = 9.76 mg/L
Mysid Shrimp	28 day chronic test	NOEC = 84 µg /L
Eastern Oyster	96 hour flow through	EC50 (shell growth) 295 µg /L
Algae	96 hour static	Green algae EC50: > 110 mg/L Freshwater diatom EC50, 0.11 mg/L Marine diatom EC50, 0.23 mg/L Cyanobacteria EC50 8.1 mg/L
Duckweed	14 day acute	EC50 based on growth rate: 12.7 mg/L

Except where noted, these endpoints identify the threshold at which toxic effect is observed in static (or semi-static) laboratory conditions. They are useful in assessing and comparing organism sensitivity. These measures, however, do not indicate the likelihood or magnitude of effect from environmental exposure. More information about the magnitude and the duration of exposure, each independent of the hazard potential described here, is necessary to bridge these hazard values

to a conclusion on risk in the environment. An assessment of exposure from the proposed use of Natular G30 in Vermont follows.

Peak Expected Environmental Concentration (EEC)

In application of single-brood formulations like Natular 2EC emulsifiable concentrate and Natular G granules, spinosad is applied directly to the aquatic environment and the peak aquatic concentration may be simply calculated from the application rate and water depth.

For Natular G30, a sustained release formulation, the steady state concentration is calculated. Based on first order kinetics, the steady state concentration may be calculated based on the peak amount of chemical according to A = K1/K2, where K1 is the rate of introduction and K2 is the rate of degradation (ln2/t ½). Since the photolytic pathway predominates, consideration will be given to degradation using the photolytic half-life in water of 1 day. The value of K2, then, is 0.6931 g spinosad day $^{-1}$ (ln2/1).

For Natular G30, 56.7 g (0.125 lb) spinosad are applied in the recommended 5 pound per acre application rate. However, because slow-release formulation encases the spinosad active constituent within a protective matrix of plaster, the entire insecticide load in the layered granule is not released at once. Spinosad is released in metered doses over the intended control period as the plaster layers on the granule surface react with water to form gypsum and release the spinosad to the water. Repeated measures of product performance in the laboratory and in varying field conditions demonstrate rapid decline in bioactivity beginning day 30-32 post-application. As mosquito larvae mortality is typically observed 48-72 hour after exposure to a lethal concentration, it is evident that at the end of the control period spinosad is not available for release to the water column and aquatic concentrations rapidly fall below a lethal dose. The magnitude of the decline in effectiveness supports the rapid decline in available spinosad in the aquatic phase due to photolytic and other degradative pathways. Of the 56.7 g applied in the granule formulation, in conservative estimates of constant rate of release, 1/30th may be expected to be released daily, or 1.89 g spinosad-day^{-1}. This is the value assumed for K1.

At a steady state, the amount of spinosad in a one-acre body of water will be 2.73 g (1.89/0.6931).

To obtain the Peak EEC, the depth of the treated acre of water must be considered, acknowledging that the depth of the typical treated water body is not expected to remain completely static. The water levels fluctuate seasonally and during the control season, subsiding through dry-down and expanding with rainfall or other inflow. Using scenarios of 10, 15, 18, and 30 cm deep water bodies, the EEC is 0.0067, 0.0045, 0.0037, and 0.0022 mg/L. This represents water of variable depth from 4 inches to 1 foot. At 2-ft depths, EEC is 0.0011 mg/L (Table 4). Shallower waters are not likely to be treated with an extended release control product unless they are expected to be flooded again – and thus increasing in depth – during the control period. Deeper waters are likely to harbor significant predator species or water current/flow, and are likewise not anticipated to be treated.

As one acre is 4046.825 square meters, Peak EEC expressed in mg/L is

$$[A] / [4046.825m2 * d(m)]$$

TABLE 4. Spinosad Peak Expected Environmental Concentrations (Peak EEC) in varying water depth subsequent to application of Natular G30 at 5 lb/acre

Natular G30 (lb/acre)	Spinosad (g/acre)	K1 (g a.i./day)	Half-life (d)	K2 (g a.i./day)	A (g a.i.)	Depth (m)	Peak EEC mg/L
5.0	56.7	1.89	1	0.6931	2.73	0.1	0.0067
						0.15	0.0045
						0.18	0.0037
						0.3	0.0022
						0.45	0.0015
						0.6	0.0011

Given the low application rate and the dominance of the initial photolytic processes, the use of first order kinetics is not likely to under predict the EEC even though degradation may slow with time. In turbid situations, degradation by photolysis may be slightly slower, but is likely to be balanced by spinosad's proclivity for organic sorbtion. Exceptionally turbid environments are likewise not anticipated to support significant non-target populations.

Risk Assessment

Once obtained, EEC is compared to the toxicity thresholds for sensitive indicator organisms to assess risk to these organisms from acute or chronic exposure to the concentrations.

Normally, environmental risk from ACUTE exposure is assessed by calculating the Environmental Risk Quotient [RQ = EEC/LC50 or EC50]. The following guidelines are generally considered appropriate for the assessment of acute risk:

RQ > 0.5; risk is unacceptable

0.1 ≤ RQ ≤ 0.5; risk may be able to be mitigated by some form of risk management, such as label restraints

RQ < 0.1; risk is considered acceptable (minimal, not likely to impact populations)

Table 5 summarizes the acute and chronic RQ values. The Acute exposure is < 0.1 for applications of Natular G30 at 5 lb per acre, indicating that this acute exposure to non-target aquatic life is not of significant concern.

For CHRONIC exposure, risk is considered to be acceptable in environmental risk assessment if the EEC estimated for prolonged exposure is less than the chronic no-observed effect concentration (NOEC) for the species most sensitive to chronic exposure (EEC < NOEC), and is expressed as the quotient RQ = EEC/NOEC.

RQ values less than or equal to 1 are considered to represent acceptable risk from prolonged exposure. RQ values greater than 1 may be mitigated by risk management tools, including use restrictions and rate restraints. As described in Table 5, the chronic exposure RQ values are less than 1 for all species except the daphnid, and when considering the outdoor microcosm threshold

for this organism, the chronic exposure EEC is less than that concentration which allowed for population recovery (0.008 mg/L).

TABLE 5. Acute and Chronic RQ values to aquatic biota after proposed application of Natular G30 (5 lb/acre) in varying water depths (10 cm to 30 cm).

	Depth	**0.1 m**	**0.15 m**	**0.18 m**	**0.3 m**
	EEC Spinosad mg/L	**0.0067**	**0.0045**	**0.0037**	**0.0022**
Most Sensitive Species	***Toxicity***	***RQ Value***			
	Acute				
Fish (carp)	96 h LC50 4.99 mg/L	< 0.01	< 0.01	< 0.01	< 0.01
Grass shrimp	96 h EC50 > 9.76 mg/L	< 0.001	< 0.001	< 0.001	< 0.001
Oyster	96 h EC50 0.295 mg/L	0.02	0.015	0.012	< 0.01
Duckweed	14 d EC50 12.7 mg/L	< 0.001	< 0.001	< 0.001	< 0.001
	Chronic				
Daphnia	21d NOEC 0.00062 mg/L (1) Outdoor microcosm NOEC < 0.008 mg/L(2)	10.8 0.84	7.2 0.56	6.0 0.46	3.5 0.27
Fish (trout)	NOEC 0.498 mg/L	0.01	< 0.01	< 0.01	< 0.01
Mysid shrimp	28 d NOEC 0.084 mg/L	0.08	0.05	0.04	0.03
Freshwater diatom	NOEC 0.049 mg/L	0.13	0.09	0.07	0.04
Duckweed	NOEC 1.9 mg/L	< 0.01	< 0.01	< 0.01	< 0.01

(1) Laboratory static flow-through protocol assessment of chronic toxicity
(2) Field mesocosm assessment of chronic exposure toxicity

The risk model described above, despite some general assumptions of suitability of the surrogate test organism as environmentally relevant, considers physiochemical properties and principal degradative pathways to refine estimates of risk, yet is still conservative. In this model constant water depth is assumed, without movement, dilution or rainfall, and without sorption to sediment or other environmental degradative pathways. In this assessment, exposure concentrations are suggestive of daphnid population recovery and survival during the period of mosquito control, as well as that of other freshwater organisms represented by the daphnid surrogate.

Sediment Dwelling Organisms. As Natular is added directly to the aquatic environment and spinosad has an affinity for organic matter, consideration is given here to sediment dwelling organisms. The adsorption behavior of spinosad is partially irreversible and spinosad is not expected to be bound to suspended matter when introduced to the sediment/water system. Accordingly, the concentration in sediment may be calculated from the equilibrium partitioning method (EPHC 2009, http://www.ephc.gov.au/taxonomy/term/75). As a worst case, the long term peak concentration is calculated. Based on the steady state concentration in overlying water of 2.73 g/L, the concentration in sediment may be calculated as follows.

Partitioning in the sediment is described by the equation:

Kp = Koc x OC (organic carbon), where OC (default) = 0.05 for sediment

There is a large spread in the Koc values for spinosyn. A value of 50,000 is estimated as representative worst case for both spinosyns A and D, representing spinosad. This results in Kp = 2500 [50,000 x 0.05].

For the whole sediment compartment, including pore water, assumed to comprise 80% water and 20% solids, the concentration is calculated as follows:

$$K_{sediment\text{-}water} = 0.8 + (0.2 \times Kp/1000) \times \text{Bulk density of solid}$$

This results in 0.8 + (0.5 x 2400 kg/m^3 (default value)) = 1201

Finally, the EEC in sediment can be calculated based on the $K_{sediment\text{-}water}$ partition coefficient and the bulk density of the solid.

$$EEC_{sediment} = K_{sediment\text{-}water} / (BD_{sediment} \times 100 \times EEC_{water})$$

(the default value for $BD_{sediment}$ is 1280 kg/m^3)

Acute toxicity of spinosad in whole sediment to the marine amphipod (*Leptochirus plumulosus*) was studied in 2009 under static conditions for ten days (unpublished report by Dow AgroSciences LLC). Test levels were spiked dry sediment of 6.5, 13, 25, 50, and 100 mg spinosad/kg dry sediment. No sub-lethal effects were observed and the survival at all test levels, including the control, was between 95-98%. The 10 day LC50 of spinosad to the marine amphipod is ≥ 63.1 mg/kg dry sediment. Based on the mean measured total radioactive residue (TRR) in pore water the NOEC is ≥ 1.33 mg/L. This disparity between the sediment and aquatic exposures illustrates that spinosad is not particularly toxic once bound to the sediment portion. Neither acute nor chronic exposure exceeds risk thresholds (RQ values).

TABLE 6. Acute and Chronic RQ Values to sediment dwelling biota after proposed application of Natular G30 (5 lb/acre) in varying water depths (10 cm to 60 cm), *L. plumulosus*.

Depth (m)	EEC water mg/L	EEC sediment mg/kg	Toxicity	RQ Value
0.10	0.0067	0.632		*Acute*
0.15	0.0045	0.421	LC50 ≥ 63.1 mg/kg	< 0.01
0.18	0.0037	0.351		
0.30	0.0022	0.211		*Chronic*
0.45	0.0015	0.141	NOEC Pore water ≥ 1.33 mg/L	< 0.5
0.60	0.0011	0.103		

Use of surrogate organisms. Risk assessment by the U.S. EPA and other federal and international agencies for the registration of pesticide products attempts to harmonize on commonly evaluated, data-robust, and known sensitive 'indicator' organisms as representative of organisms of similar life-cycle, habitat, and habit. The toxicology endpoints and characteristics of spinosad presented here, as well as additional information on the risk assessment process, can be found in EPA's public dockets for spinosad (http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1).