



**Committee of Experts on the Transport of Dangerous Goods
and on the Globally Harmonized System of Classification
and Labelling of Chemicals****Sub-Committee of Experts on the Transport of Dangerous Goods****Forty-ninth session**

Geneva, 27 June – 6 July 2016

Item 3 of the provisional agenda

Listing, classification and packing**Additional entry for SP 308 of Fish Meal (Fish Scrap),
Stabilised (UN 2216): Class 9****Transmitted by the International Fishmeal and Fish Oil Organization
(IFFO)¹****Introduction**

1. Stabilising fishmeal by addition of the anti-oxidant, ethoxyquin (EQ) has been done for many years, and IFFO estimates that approximately 66% of globally traded fishmeal is stabilised with ethoxyquin. The addition levels of ethoxyquin were determined more than 40 years ago, and may be at levels well above any requirement for stabilisation. Unnecessarily high levels of ethoxyquin are undesirable and may lead to high residue levels in the animal which has been fed feed which incorporates the treated fishmeal.
2. Increased negative publicity in the European Union, and difficulties with high levels of ethoxyquin found in shrimp originating from Asian countries exporting to Japan, has drawn attention to the use of ethoxyquin. In addition, the potential carry-over of fat soluble ethoxyquin into omega-3 oils produced from by-products of farmed fish may be a cause for concern.
3. Ethoxyquin is in the process of being reauthorized as a feed additive according to the requirements of European Parliament and Council Regulation (EC) No 1831/2003, that sets out new rules for the authorisation, supervision and labelling of feed additives.

¹ In accordance with the programme of work of the Sub-Committee for 2015–2016 approved by the Committee at its seventh session (see ST/SG/AC.10/C.3/92, paragraph 95 and ST/SG/AC.10/42, para. 15).

4. The European Food Safety Authority (EFSA) published their Opinion on ethoxyquin in November 2015 which states that the safety regarding the use of ethoxyquin remains inconclusive in terms of the risk associated with ethoxyquin to consumers, fed livestock, and the environment. EFSA identified a number of knowledge gaps where data is required to make an overall assessment of the safety of ethoxyquin. The European Commission is currently in the process of drafting a regulation based on the EFSA Opinion that may partially suspend or even ban the use of ethoxyquin within EU-28.

5. In addition, reduced levels of ethoxyquin in compound feed, taking into account the level of ethoxyquin in feed ingredients such as fishmeal, may be introduced. If that is the case, it is likely that maximum permitted levels in farmed fish will also be set, requiring that the fishmeal, aquafeed and fish farming industries all work towards maintaining as low a level of ethoxyquin in the end product as possible. It is critical to look at optimisation of the levels of ethoxyquin for the stabilisation of fishmeal as well as alternative antioxidants to ethoxyquin.

6. As previously notified to the Sub-Committee at the meeting held in December 2015, IFFO commenced with a fishmeal stability trial on 9 July 2015 that will continue for 12 months. The aim of this trial is to compare the stability of fishmeal treated with lower inclusion rates of ethoxyquin, as well as other antioxidants, butylated hydroxytoluene (BHT) and a natural tocopherol/rosemary extract blend. Four out of five sampling intervals have been performed (Day 0, Week 2, Month 3, Month 6) with Month 12 to be performed in July 2016. The reactive anchovy fishmeal for the trial was provided by a producer from within the IFFO membership, and the trials were conducted under standard conditions, but also comparing storage volume through a comparison of 50kg and regular 1 tonne bags. The concentrations and analyses are detailed in the treatment plan in Table 1 along with the content of the active component in the treatments calculated from the percentage in the dosing solution.

7. The specifications of the antioxidants are as follows:

- Ethoxyquin (EQ): Minimum 95% solution
- Naturox Premium liquid: 23.2% tocopherols and <1% rosemary extract (containing ≈ 5% carnosic acid).
- Rendox T: 20% BHT solution

Table 1: Antioxidant treatments, content of the active component and analyses plan

Treatments	Antioxidant content (active component) (ppm)	Sampling intervals				
		Day		Month		
		0	14	2	6	12
EQ: 300 ppm	285	AO x 5 PV, AV, FFA, PUFA	AO, PV, AV, FFA.	AO, PV, AV, FFA	AO, PV, AV, FFA. Self heating test (50 kg bags)	AO, PV, AV, FFA, PUFA Self- heating test (all sizes)
EQ: 600 ppm	570					
BHT solution: 2,000 ppm	400					
BHT solution: 4,000 ppm	800					
Tocopherol/ rosemary extract blend: 2,000 ppm	460 ppm tocopherols + < 20 ppm rosemary extract (containing 1ppm carnosic acid)					
Tocopherol/ rosemary extract blend: 4,000 ppm	920 ppm tocopherols + < 40 ppm rosemary extract (containing 2 ppm carnosic acid)					

Where AO = antioxidant; PV = Peroxide value; AV = Anisidine value; FFA = Free Fatty Acid value; PUFA = polyunsaturated fatty acids

8. All the fishmeal treatments are stored in two sizes: 50 kg bags as well as 1,000 ton bags. The two sizes will show whether there is a difference in the deterioration rate of fishmeal in different storage volumes.

9. The 1 ton bags were only analysed intermittently due to cost restraints. However, the corresponding available results will be compared to the results of the 50kg trials and will be assessed for differences in deterioration rate within the two different storage volumes. Table 2. Presents the results at the 6-month sample point.

Table 2: Results of the treatments after the 6 month's storage period

Treatments		Sampling intervals			
		Day 0	Month 6		
		Antioxidant level (ppm)	Antioxidant level (ppm)	Self heating test	Oxygen Bomb induction period (hrs)
EQ: 300 ppm	50 kg bag	318	259	Neg (pass)	9.5
	1 ton bag	297			9.5
EQ: 600 ppm	50 kg bag	28	<15		1.2
	1 ton bag	48			2.7
BHT solution: 2,000 ppm	50 kg bag	438	368	Neg (pass)	2.4
	1 ton bag	438			2.6
BHT solution: 4,000 ppm	50 kg bag	858	751		2.8
	1 ton bag	866			2.9
Tocopherol/rosemery extract blend: 2,000 ppm	50 kg bag	385	243	Neg (pass)	2.5
	1 ton bag	400			3.8
Tocopherol/rosemery extract blend: 4,000 ppm	50 kg bag	628	488		3.4
	1 ton bag	752			3.5

10. Four of the five planned samples have been performed. The last sample point is at 12 months and will conclude the trial in July 2016.

11. Unfortunately, there was a problem with the dosing of the 600 ppm ethoxyquin treatment and the fishmeal was dosed with ≤ 50 ppm ethoxyquin, but this material remains in the trial for comparison.

12. The results show that the antioxidant levels after 6 months of storage have decreased to levels that are still more than sufficient to provide continued protection to the fishmeal. The percentage remaining antioxidants for each treatment along with the corresponding percentage reduction is shown in Table 3.

Table 3: Percentage residual antioxidant and reduction after 6 month's storage

Antioxidant treatment	Residual antioxidant content (%)	Decrease in antioxidant content (%)
Ethoxyquin: 300 ppm	81.4	18.6
Ethoxyquin: 600 ppm	< 53.6	< 46.4
BHT: 2,000 ppm	84.0	16.0
BHT: 4,000 ppm	87.5	12.5
Natural blend: 2,000 ppm	63.1	36.9
Natural blend: 4,000 ppm	77.7	22.3

13. The self-heating test performed on the lowest antioxidant concentration in the 50kg (i.e. 300 ppm EQ, 2,000 ppm BHT and 2,000 ppm Natural blend) bags were all negative which indicated that none of the treatments had self-heating properties at 6 months. The tests were performed on the low levels only as the high levels would pass as well if the low levels have passed. The low ethoxyquin dosage of 300 ppm passed which indicates that lower dosage levels of ethoxyquin would effectively stabilise fishmeal. The current ethoxyquin application levels prescribed in the IMDG Code Special Provision 945 is 400 to 1000 mg/kg (ppm) ethoxyquin. The self-heating test of the final analyses interval at 12 months will be performed on all the samples.

14. The Oxygen Bomb Test is used to predict stability and evaluate antioxidant systems in fats and finished products. The oxygen uptake of the sample is measured in a closed system. The rate at which oxygen is consumed indicates the oxidative stability of the tested product and measures the stability of the complete product without prior extraction of the fat. A short induction period (in hours) indicate quicker uptake of oxygen and a less stable product whereas a longer uptake period for oxygen indicates a more stable product. The Oxygen Bomb test has shown to correlate well with shelf life and the Schaal Oven accelerated test².

15. Ethoxyquin (even at a low dosage level of 300 ppm) has shown to be the most effective antioxidant with the longest induction period of 9.5 hrs. [Here we can clearly see the lack of antioxidant in the 600 ppm ethoxyquin treatment although the one ton 600 ppm sample had a similar induction time (2.7 hrs) to the 2,000 ppm and 4,000 ppm BHT treated samples (2.4; 2.6; and 2.8; 2.9 hrs respectively). The difference between the two 600 ppm ethoxyquin treatments (1.2 hrs and 2.7 hrs) could be due to uneven distribution of the antioxidant in the fishmeal.] Surprisingly, the natural antioxidant blend seemed to have performed slightly better than BHT.

16. The results indicate that 300 ppm ethoxyquin will effectively stabilise fishmeal, at least over a 6-month period, with the 12-month sample point yet to come. The alternative antioxidants tested could also be used to stabilise fishmeal because of the high levels of remaining antioxidant and in addition none of the alternative antioxidants BHT (2,000 ppm and 4,000 ppm) as well as the natural blend (2,000 ppm and 4,000 ppm) show self-heating properties. BHT is currently written into the IMDG code at levels of 400 and 1000 mg/kg (ppm) liquid BHT or between 1000 and 4000 mg/kg (ppm) BHT in powder form (however, the concentration of the active ingredient is not clear in that entry which can result in uncertainty of the actual active BHT dosage requirement).

17. Once the final results have been obtained in July 2016 a further data point should confirm the effectiveness of the lower level of ethoxyquin (300 ppm), as well as the use of the alternative antioxidants. Depending on the availability of funds, the hope is that the trial will be extended even further to include sampling at 18-month and 24-month intervals.

18. Ethoxyquin has been shown to be the most efficacious of the available synthetic antioxidants^{3,4,5}. The high efficacy of ethoxyquin is not only because of its chemical nature

² Methods to Assess Quality and Stability of Oils and fat-containing Foods, (1995). Eds: Warner, K and Michael Eskin, N.A., AOCS Press, Champaign, Illinois, pp 183-184

³ Aquaculture development and coordination programme, (1980). Fish feed technology, FAO Fisheries and Aquaculture department. Downloaded on 23 March 2016 from <http://www.fao.org/docrep/x5738e/x5738e0b.htm>

⁴ Blaszczyk, A., Augustyniak, A. and Skolimowski, J. Ethoxyquin: An antioxidant used in animal feed., International Journal of Food Science, Volume 2013 (2013), Article ID 585931, 12 pages <http://dx.doi.org/10.1155/2013/585931>

but also due to the fact that its oxidation products also possess strong antioxidant properties^{6,7}. Two of its oxidation products, ethoxyquin-dimer and a quinolone have shown to have efficacy values of 69% and 80% of the value of ethoxyquin respectively^{5,8}. The efficacies of the oxidation products and BHT in fishmeal relative to ethoxyquin can be seen in Table 4. BHT has roughly 2/3 the activity of ethoxyquin in fishmeal and 3/4 in fish oil. The efficacies of the same antioxidant in fishmeal and fish oil are different and therefore antioxidants must be evaluated in the specific matrix that it will be used in.

Table 4: Efficacy values of the oxidation products of ethoxyquin and BHT relative to ethoxyquin⁵

Antioxidant	Efficacy value (%)	
	Fishmeal	Fish oil
Ethoxyquin	100	100
EQ-Dimer	69	35
Quinolone	80	74
BHT	67	77

19. The breakdown rate of the dimer was considerably slower than that of ethoxyquin, which has the beneficial effect that even once the ethoxyquin levels has dropped significantly there are still significant levels of ethoxyquin dimer remaining to act as antioxidant and protect the fishmeal.

20. In a previously performed long term fishmeal stability study where reactive fishmeal dosed at 400-1000 mg/kg ethoxyquin was stored in approximately 5kg polypropene buckets at 25°C for more than a year, the ethoxyquin content as well as the oxidation products, EQ-dimer and quinolone were determined⁹. The ethoxyquin content and its oxidation products as well as the calculated total ethoxyquin equivalents (based on the efficacy value compared to ethoxyquin) on the final days of the trial are shown in table 5.

⁵ Lundebye, A.-K., Hovea, H., Mage, A., Bohne, V.J.B. and Hamre, K., (2010). Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. *Food Additives and Contaminants*, Vol. 27, No. 12, 1652–1657

⁶ De Koning, A.J., (2002). The antioxidant ethoxyquin and its analogues: A Review. *International Journal of Food Properties*, Vol 5, Issue 2, pp 451 - 461

⁷ Thorrison, S., (1987). Antioxidant properties of ethoxyquin and some of its oxidation products. PhD Thesis, Faculty of Science, University of St Andrews, United Kingdom

⁸ De Koning, A.J., (1996). Determination of the antioxidant efficacies in fish meal of two oxidation products of ethoxyquin. *International Fishmeal and Fish oil manufacturers Association, Research Report*, 1996-4.

⁹ De Koning, A.J. and Van der Merwe, G.H. (1992). Determination of ethoxyquin and two of its oxidation products in fishmeal by Gas Chromatography, *Analyst*, Vol 117, pp 1571 - 1576

Table 5: Ethoxyquin, the quinolone and EQ-dimer in fishmeal storage at 25°C

Meal type and ethoxyquin concentration added either at factory or in laboratory	Storage time (days)	Ethoxyquin mg/kg	Quinolone mg/kg	Q- dimer mg/kg	Total EQ equivalents based on efficacy in fish oil) mg/kg	Total EQ equivalents (based on efficacy in fishmeal) mg/kg
Anchovy/pilchard: ± 400 ppm EQ	573	61	4	120	104	116
Maasbanker meal: ± 1,000 ppm EQ	453	150	5	102	188	198
Anchovy: 400 ppm EQ	244	323	14	74	358	364
Anchovy: 400 ppm EQ	347	87	22	96	136	133
Anchovy: 400 ppm EQ	365	20	2	76	47	55

21. As shown earlier the efficacy values of antioxidants differ in fish oil and fishmeal. The total EQ equivalents (based on efficacy in fish oil) in Table 5 were calculated based on information available at the time that calculated the relative antioxidant activities of the oxidation products to ethoxyquin in fish oil. Later work⁵ determined the antioxidant activities based on fish meal and this was used to calculate the total EQ equivalents (based on efficacy in fish meal) on the final day of storage. To allow for variation in efficacy from one meal to another, a safety margin was calculated from the statistical variation in results (90% lower confidence limit for an individual meal as used, giving relative efficacies of 63% for quinolone and 44% for the dimer). The formula used to calculate the ethoxyquin equivalents was:

$$\text{Total EQ equivalence} = \text{EQ} + (0.63 \times \text{Quinolone}) + (0.44 \times \text{EQ-dimer})$$

22. It is clear that the oxidation products contribute significantly to the efficacy of ethoxyquin and these additional antioxidants should be taken into account when the ethoxyquin content at the time of shipment is considered. The oxidation products can add from 16% (fishmeal that has been stored for shortest period) to 73% additional antioxidant protection over and above that of ethoxyquin on its own. The stipulated antioxidant content of 100 ppm at the time of consignment could therefore be adjusted to allow for the additional antioxidant activity of ethoxyquin and its oxidation products compared to alternative antioxidants. Lower levels of ethoxyquin should be considered at the time of consignment compared to the alternatives BHT and Natural antioxidants or blends that are less effective.

23. Natural antioxidant alternatives are increasingly demanded by pet food and feed manufacturers and is used in the growing organic aquaculture industry as well as in pet food for animals' sensitive to ethoxyquin.

24. Tocopherols have successfully been used with Special Exemptions since 1995 to ship and store stabilise fishmeal.

- Self-heating test data on the stabilisation of fishmeal by the application of tocopherol products have indicated their safety (the evidence will be submitted at a later date once available).
- Special exemptions (Permits) have been issued by the following authorities allowing tocopherol products instead of synthetic antioxidants to stabilise fishmeal:

Australian Maritime Safety Authorization

Armada de Chile

Bremen Port Authority (Germany)

Federale Overheidsdienst Mobiliteit en Vervoer, Scheepvaartcontrole (Belgium)

United States Department of Transportation

United States Coast Guard

(Copies of the exemptions/permits will be submitted at a later date)

25. IFFO is also in the process of performing an accelerated fishmeal stability test using treatments that reflect those used with the long-term storage trial. This enables an assessment of an antioxidant's performance under controlled conditions in the laboratory, and without undertaking very expensive long term storage trials in the field. The test is performed at 60°C with regular analyses of traditional rancidity parameters, until the point at which fishmeal is deemed rancid by sensory evaluation. A control with no added antioxidant is added to determine the protection factor of each antioxidant. These results will be available later this year and will be presented by IFFO at the next Sub-Committee session in November/December.

Proposal

The following Special Provisions for fishmeal (UN 2216) are written into the current United Nations Model Regulations (Rev 19):

29 This substance is exempt from labelling, but shall be marked with the appropriate class or division.

117 Subject to these Regulations only when transported by sea.

300 Fish meal, fish scrap and krill meal shall not be transported if the temperature at the time of loading exceeds 35°C or 5°C above ambient temperature whichever is higher.

308 Fish meal or fish scrap shall contain at least 100 ppm (mg/kg) of antioxidant (ethoxyquin) at the time of consignment.

The IMDG code has apart from the SPs above the following additional SPs:

907 The consignment shall be accompanied by a certificate from a recognized authority stating: moisture content; fat content; details of anti-oxidant treatment for meals older than 6 months (for UN 2216 only); anti-oxidant concentration at the time of shipment, which must exceed 100 mg/kg (for UN 2216 only); packing, number of bags and total mass of the consignment; temperature of fishmeal at the time of despatch from the factory; date of production.

No weathering/curing is required prior to loading. Fishmeal under UN 1374 shall have been weathered for not less than 28 days before shipment. When fishmeal is packed into containers, the containers shall be packed in such a way that the free air space has been restricted to the minimum.

928 The provisions of this Code shall not apply to:

fishmeal when acidified and wetted with more than 40% water, by mass, irrespective of other factors; consignments of fishmeal which are accompanied by a certificate issued by a recognized competent authority of the country of shipment or other recognized authority stating that the product has no self-heating properties when transported in packaged form; or fishmeal manufactured from "white" fish

with a moisture content of not more than 12% and a fat content of not more than 5% by mass.

945 Stabilization of fishmeal shall be achieved to prevent spontaneous combustion by effective application of between 400 and 1000 mg/kg (ppm) ethoxyquin, or liquid BHT (butylated hydroxytoluene) or between 1000 and 4000 mg/kg (ppm) BHT in powder form at the time of production. The said application shall occur no longer than twelve months prior to shipment.

IFFO recommends to provisionally modify SP 308 as follows to be in line with SP 945 from the IMDG:

SP 308 Stabilization of fishmeal shall be achieved to prevent spontaneous combustion by effective application of ethoxyquin, BHT (butylated hydroxytoluene) or tocopherols (also used in a blend with rosemary extract) at the time of production. The said application shall occur within twelve months prior to shipment. Fish scrap or fish meal shall contain at least 60 ppm (mg/kg) of ethoxyquin and 100 ppm (mg/kg) of other alternative antioxidants at the time of consignment.
