

Histopathologic Review of Selected Tissues

Covance Laboratories Study No. 6329-231: 26-Week Capsule Toxicity Study with Ammonium
Perfluorooctanoate (APFO) in Cynomolgus Monkeys

and

Riker Laboratories Study No. 0281CR0012: Study of Rats Receiving Fluorochemical FC-143 in Diet for
Two Years

WIL Research Study No. WIL-180049

Report Date: 14-Jan-2016

SUBMITTED BY:

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INTRODUCTION:

This report is based on a Sponsor-directed review of histologic sections of large intestine from Covance Laboratories Study No. 6329-231, a 26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys and Riker Laboratories Study No. 0281CR0012, a two-year dietary study of fluorochemical FC-143 (ammonium perfluorooctanoate, APFO) in Sprague Dawley rats. The goal of the review was to confirm or refute the histopathologic findings of the original study pathologists with regards to any evidence of test article-related inflammation in the large intestine specimens.

METHODOLOGY:

For Covance Study No. 6329-231, histopathologic examination was performed on sections of cecum, colon, and rectum. For Riker Study No. 0281CR0012, histopathologic examination was performed on sections of colon. All tissues had been stained with hematoxylin and eosin (H&E). The histologic sections were examined without knowledge of the study pathologist(s)' original findings in individual animals.

REVIEW OBSERVATIONS:

Histopathologic findings in large intestinal specimens from individual animals are included in the appended Excel spreadsheet.

The colon of rats from Riker Study No. 0281CR0012 typically had a mild lymphocytic infiltration, as expected as part of the mucosal immune system. The mucosal inflammatory cell population typically included a minimal number of neutrophils. The neutrophil population was slightly increased in 2 females from the 0 ppm group, 8 males from the 300 ppm group, and 4 females from the 300 ppm group. Overt evidence of subacute inflammation, which included an inflammatory cell infiltration with associated evidence of slight tissue disruption, was observed in the colon of 2 females from the 0 ppm group and 1 male from the 300 ppm group.

A number of rats from Riker Study No. 0281CR0012 had nematode parasites in the lumen of the colon. There were no definitive histologic changes associated with presence of the parasites, which were histologically consistent with *Syphacia* spp. *Syphacia* spp. were long considered to be non-pathogenic in rats, given the absence of histopathologic changes associated with the parasites, but it is now known that *Syphacia* spp. infestation can alter cytokine profiles in mice (Michels *et al.*, 2006) and intestinal

electrolyte transport in rats (Lubcke *et al.*, 1992).

Occasional monkeys in Covance Study No. 6329-231 had overt lesions that were consistent with *Oesophagostomum* spp. infestation of the large intestine. This nematode parasite was very common in the intestinal tract of nonhuman primates prior to the advent of highly effective parasiticides such as ivermectin. The large intestinal mucosa of monkeys from Covance Study No. 6329-231 commonly had a mild infiltration of lymphocytes and plasma cells with a few eosinophils and neutrophils. This inflammatory cell population was considered to be normal background histomorphology, though it is possible intestinal parasitism contributed to the inflammatory cell population. The large intestine specimens had no overt histologic evidence of inflammation other than that associated with the parasitic granulomas.

CONCLUSIONS:

Review of colon sections of Sprague Dawley rats from Riker Study No. 0281CR0012 and cecum, colon, and rectum sections of cynomolgus monkeys from Covance Study No. 6329-231 did not reveal evidence of test article-related inflammatory lesions in either study. In both studies the animals had spontaneous parasitic diseases that involved the large intestines.

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REFERENCES:

Lubcke, R.; Hutcheson, F. A.; Barbezat, G. O. Impaired intestinal electrolyte transport in rats infested with the common parasite *Syphacia muris*. *Dig Dis Sci* **1992**, *37(1)*, 60-64.

Michels, C.; Goyal, P.; Nieuwenhuizen, N.; Brombacher, F. Infection with *Syphacia obvelata* (Pinworm) Induces Protective Th2 Immune Responses and Influences Ovalbumin-Induced Allergic Reactions. *Infect Immun* **2006**, *74(10)*, 5926-5932.

0281CR-0012: 2-year rat study

Rat ID	Sex	Dose Group	Sac Week	Cecum	Colon	Rectum
					EN = essentially normal NP = not present	
3581	M	300 ppm	106	NP	EN	NP
3582	M	300 ppm	106	NP	EN	NP
3583	M	300 ppm	106	NP	EN	NP
3585	M	300 ppm	106	NP	EN	NP
3586	M	300 ppm	106	NP	EN	NP
3587	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3590	M	300 ppm	19	NP	EN	NP
3591	M	300 ppm	106	NP	EN	NP
3593	M	300 ppm	106	NP	EN	NP
3594	M	300 ppm	106	NP	EN	NP
3595	M	300 ppm	106	NP	EN	NP
3596	M	300 ppm	106	NP	EN	NP
3597	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3599	M	300 ppm	106	NP	EN	NP
3600	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3601	M	300 ppm	106	NP	EN	NP
3602	M	300 ppm	106	NP	EN	NP
3603	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3605	M	300 ppm	106	NP	EN	NP
3606	M	300 ppm	106	NP	EN	NP
3607	M	300 ppm	106	NP	EN	NP
3608	M	300 ppm	106	NP	EN	NP
3609	M	300 ppm	106	NP	EN	NP
3610	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3611	M	300 ppm	106	NP	EN	NP
3612	M	300 ppm	99	NP	Autolysis, severe	NP
3614	M	300 ppm	106	NP	EN	NP
3615	M	300 ppm	106	NP	EN	NP
3616	M	300 ppm	72	NP	Autolysis, severe	NP
3618	M	300 ppm	106	NP	EN	NP
3619	M	300 ppm	106	NP	EN	NP
3621	M	300 ppm	106	NP	EN	NP
3623	M	300 ppm	106	NP	EN	NP
3624	M	300 ppm	90	NP	Neutrophilic infiltration, minimal	NP
3625	M	300 ppm	27	NP	EN	NP
3628	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3629	M	300 ppm	106	NP	EN	NP
3630	M	300 ppm	106	NP	NP	NP
3631	M	300 ppm	106	NP	EN	NP
3632	M	300 ppm	106	NP	Neutrophilic infiltration, minimal	NP
3633	M	300 ppm	106	NP	EN	NP
3634	M	300 ppm	106	NP	NP	NP
3635	M	300 ppm	106	NP	EN	NP
3636	M	300 ppm	106	NP	Inflammation, subacute, mild	NP
3637	M	300 ppm	106	NP	EN	NP

0281CR-0012: 2-year rat study

Rat ID	Sex	Dose Group	Sac Week	Cecum	Colon	Rectum
					EN = essentially normal NP = not present	
3639	M	300 ppm	106	NP	EN	NP
3640	M	300 ppm	72	NP	EN	NP
3641	M	300 ppm	106	NP	EN	NP
3644	M	300 ppm	106	NP	EN	NP
3645	M	300 ppm	106	NP	EN	NP
4641	F	300 ppm	73	NP	EN	NP
4643	F	300 ppm	78	NP	EN	NP
4644	F	300 ppm	105	NP	EN	NP
4645	F	300 ppm	105	NP	EN	NP
4646	F	300 ppm	105	NP	EN	NP
4647	F	300 ppm	105	NP	EN	NP
4648	F	300 ppm	88	NP	NP	NP
4649	F	300 ppm	105	NP	EN	NP
4650	F	300 ppm	104	NP	NP	NP
4651	F	300 ppm	96	NP	NP	NP
4653	F	300 ppm	87	NP	Neutrophilic infiltration, minimal	NP
4654	F	300 ppm	57	NP	Autolysis, severe	NP
4657	F	300 ppm	105	NP	EN	NP
4658	F	300 ppm	105	NP	EN	NP
4659	F	300 ppm	105	NP	EN	NP
4660	F	300 ppm	105	NP	slide glued in box. Immovable	NP
4661	F	300 ppm	105	NP	EN	NP
4662	F	300 ppm	105	NP	EN	NP
4663	F	300 ppm	105	NP	NP	NP
4665	F	300 ppm	94	NP	EN	NP
4667	F	300 ppm	105	NP	EN	NP
4668	F	300 ppm	81	NP	EN	NP
4670	F	300 ppm	92	NP	EN	NP
4672	F	300 ppm	105	NP	EN	NP
4673	F	300 ppm	79	NP	EN	NP
4675	F	300 ppm	105	NP	EN	NP
4677	F	300 ppm	105	NP	EN	NP
4678	F	300 ppm	49	NP	EN	NP
4679	F	300 ppm	105	NP	EN	NP
4680	F	300 ppm	104	NP	NP	NP
4681	F	300 ppm	97	NP	EN	NP
4682	F	300 ppm	94	NP	NP	NP
4683	F	300 ppm	105	NP	EN	NP
4684	F	300 ppm	103	NP	Autolysis, severe	NP
4685	F	300 ppm	75	NP	EN	NP
4686	F	300 ppm	105	NP	NP	NP
4688	F	300 ppm	105	NP	Nematodiasis, minimal	NP
4690	F	300 ppm	105	NP	EN	NP
4691	F	300 ppm	100	NP	EN	NP
4693	F	300 ppm	105	NP	Neutrophilic infiltration, minimal	NP

0281CR-0012: 2-year rat study

Rat ID	Sex	Dose Group	Sac Week	Cecum	Colon	Rectum
					EN = essentially normal NP = not present	
4694	F	300 ppm	105	NP	EN	NP
4695	F	300 ppm	105	NP	EN	NP
4696	F	300 ppm	105	NP	Neutrophilic infiltration, minimal	NP
4697	F	300 ppm	105	NP	Neutrophilic infiltration, minimal	NP
4698	F	300 ppm	105	NP	EN	NP
4700	F	300 ppm	96	NP	EN	NP
4701	F	300 ppm	105	NP	EN	NP
4702	F	300 ppm	87	NP	EN	NP
4703	F	300 ppm	105	NP	EN	NP
4705	F	300 ppm	62	NP	Autolysis, severe	NP
3518	M	0 ppm	106	NP	EN	NP
3519	M	0 ppm	106	NP	EN	NP
3521	M	0 ppm	92	NP	EN	NP
3523	M	0 ppm	95	NP	EN	NP
3524	M	0 ppm	106	NP	Nematodiasis, mild	NP
3525	M	0 ppm	106	NP	EN	NP
3526	M	0 ppm	106	NP	EN	NP
3528	M	0 ppm	106	NP	EN	NP
3529	M	0 ppm	96	NP	EN	NP
3530	M	0 ppm	106	NP	EN	NP
3531	M	0 ppm	106	NP	EN	NP
3532	M	0 ppm	106	NP	EN	NP
3533	M	0 ppm	106	NP	NP	NP
3534	M	0 ppm	105	NP	EN	NP
3536	M	0 ppm	106	NP	Nematodiasis, mild	NP
3537	M	0 ppm	73	NP	EN	NP
3538	M	0 ppm	106	NP	EN	NP
3539	M	0 ppm	106	NP	Nematodiasis, minimal	NP
3540	M	0 ppm	64	NP	NP	NP
3541	M	0 ppm	106	NP	EN	NP
3542	M	0 ppm	106	NP	EN	NP
3544	M	0 ppm	106	NP	EN	NP
3545	M	0 ppm	104	NP	Autolysis, severe	NP
3547	M	0 ppm	85	NP	EN	NP
3548	M	0 ppm	106	NP	EN	NP
3549	M	0 ppm	86	NP	EN	NP
3552	M	0 ppm	106	NP	EN	NP
3553	M	0 ppm	88	NP	NP	NP
3554	M	0 ppm	106	NP	EN	NP
3555	M	0 ppm	102	NP	EN	NP
3556	M	0 ppm	106	NP	EN	NP
3557	M	0 ppm	106	NP	EN	NP
3558	M	0 ppm	106	NP	EN	NP
3560	M	0 ppm	35	NP	EN	NP
3561	M	0 ppm	106	NP	Nematodiasis, mild	NP

0281CR-0012: 2-year rat study

Rat ID	Sex	Dose Group	Sac Week	Cecum	Colon	Rectum
					EN = essentially normal	
					NP = not present	
3562	M	0 ppm	106	NP	Nematodiasis, minimal	NP
3563	M	0 ppm	106	NP	EN	NP
3564	M	0 ppm	106	NP	EN	NP
3565	M	0 ppm	84	NP	NP	NP
3566	M	0 ppm	106	NP	EN	NP
3567	M	0 ppm	106	NP	EN	NP
3569	M	0 ppm	106	NP	NP	NP
3571	M	0 ppm	106	NP	EN	NP
3572	M	0 ppm	106	NP	EN	NP
3573	M	0 ppm	106	NP	Nematodiasis, minimal	NP
3574	M	0 ppm	99	NP	EN	NP
3576	M	0 ppm	55	NP	EN	NP
3577	M	0 ppm	106	NP	EN	NP
3578	M	0 ppm	106	NP	EN	NP
3579	M	0 ppm	86	NP	EN	NP
4576	F	0 ppm	25	NP	EN	NP
4577	F	0 ppm	99	NP	NP	NP
4579	F	0 ppm	84	NP	EN	NP
4580	F	0 ppm	52	NP	EN	NP
4581	F	0 ppm	85	NP	EN	NP
4583	F	0 ppm	105	NP	EN	NP
4584	F	0 ppm	105	NP	NP	NP
4586	F	0 ppm	105	NP	EN	NP
4587	F	0 ppm	105	NP	EN	NP
4591	F	0 ppm	105	NP	EN	NP
4592	F	0 ppm	88	NP	NP	NP
4593	F	0 ppm	105	NP	EN	NP
4594	F	0 ppm	79	NP	EN	NP
4595	F	0 ppm	105	NP	EN	NP
4596	F	0 ppm	78	NP	EN	NP
4597	F	0 ppm	105	NP	EN	NP
4598	F	0 ppm	105	NP	EN	NP
4599	F	0 ppm	102	NP	Inflammation, subacute, mild	NP
4600	F	0 ppm	99	NP	EN	NP
4602	F	0 ppm	105	NP	EN	NP
4603	F	0 ppm	105	NP	EN	NP
4604	F	0 ppm	105	NP	NP	NP
4605	F	0 ppm	77	NP	Neutrophilic infiltration, minimal	NP
4606	F	0 ppm	105	NP	EN	NP
4607	F	0 ppm	40	NP	EN	NP
4609	F	0 ppm	105	NP	NP	NP
4611	F	0 ppm	105	NP	NP	NP
4612	F	0 ppm	105	NP	EN	NP
4613	F	0 ppm	89	NP	NP	NP
4614	F	0 ppm	105	NP	EN	NP

0281CR-0012: 2-year rat study

Rat ID	Sex	Dose Group	Sac Week	Cecum	Colon	Rectum
					EN = essentially normal NP = not present	
4615	F	0 ppm	105	NP	EN	NP
4616	F	0 ppm	105	NP	EN	NP
4617	F	0 ppm	73	NP	EN	NP
4618	F	0 ppm	93	NP	Neutrophilic infiltration, minimal	NP
4619	F	0 ppm	64	NP	EN	NP
4621	F	0 ppm	99	NP	Nematodiasis, mild	NP
4622	F	0 ppm	62	NP	EN	NP
4623	F	0 ppm	96	NP	EN	NP
4624	F	0 ppm	100	NP	EN	NP
4625	F	0 ppm	100	NP	EN	NP
4626	F	0 ppm	105	NP	EN	NP
4627	F	0 ppm	105	NP	EN	NP
4628	F	0 ppm	105	NP	EN	NP
4633	F	0 ppm	63	NP	Inflammation, subacute, mild	NP
4634	F	0 ppm	105	NP	EN	NP
4635	F	0 ppm	94	NP	EN	NP
4636	F	0 ppm	105	NP	Nematodiasis, minimal	NP
4637	F	0 ppm	82	NP	EN	NP
4638	F	0 ppm	105	NP	EN	NP
4639	F	0 ppm	56	NP	EN	NP

6329-231 6-month PFOA monkey study

Dose Group	Animal ID	Last day of dosing	Time of necropsy	EN = essentially normal		
				Cecum	Colon	Rectum
Control	5709	Week 27	week 27	EN	EN	EN
	5714	Week 27	week 27	EN	Granuloma, parasitic	EN
	5715	Week 27	week 27	EN	EN	EN
	5718	Week 27	week 40	EN	EN	EN
	5720	Week 27	Week 40	EN	EN	EN
	5725	Week 27	week 27	Pigment, brown, minimal	Infiltrate, eosinophil, minimal	EN
3 mg/kg	5702	Week 27	week 27	EN	EN	EN
	5706	Week 27	week 27	EN	EN	EN
	5717	Week 27	week 27	EN	EN	EN
	5721	Week 27	Day 137 (unscheduled necropsy)	EN	EN	EN
10 mg/kg	5707	Week 27	week 27	EN	EN	EN
	5708	Week 27	week 27	Granuloma, moderate (consistent with parasitic granulomas)	Granuloma, moderate (consistent with parasitic granulomas)	EN
	5710	Week 27	week 27			
	5712	Week 27	week 40			
	5716	Week 27	week 40			
	5719	Week 27	week 27			
30/20 mg/kg	5724	Day 29	Day 29 (unscheduled necropsy)			
	5716	Day 43	Week 27	EN	EN	EN
	5722	Day 66	Week 27	EN	EN	EN
	5703	Day 81	Week 27	EN	EN	EN
	5704	Week 27	Week 27	EN	Pigment, brown, mild	EN
	5713	Week 27	Week 27	EN	EN	EN