

SUPPLEMENTARY MATERIALS

A high docosahexaenoic acid diet alters lung inflammation and recovery following repetitive exposure to aqueous organic dust extracts

Arzu Ulu^{a,1} and Abigail Burr^{a,1}, Art J. Heires^b, Jacqueline Pavlik^b, Tricia Larsen^c, Pedro A. Perez^a, Carissa Bravo^a, Nicholas V. DiPatrizio^a, Michelle Baack^{c,d}, Debra J. Romberger^{e,b}, Tara M. Nordgren^{a,b,*}

^a Division of Biomedical Sciences, School of Medicine, University of California, Riverside, Riverside, CA 92521.

^b Pulmonary, Critical Care, Sleep and Allergy Division, University of Nebraska Medical Center, Omaha, NE 68198, USA.

^c Children's Health Research Center, Sanford Research, Sioux Falls, SD 57104, USA.

^d Division of Neonatology, University of South Dakota—Sanford School of Medicine, Sioux Falls, SD 57104, USA.

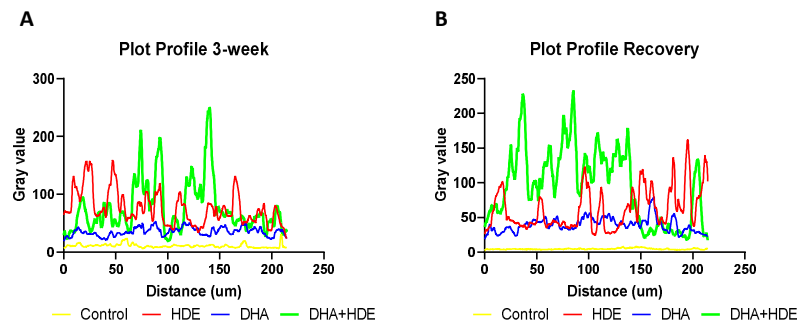
^e VA Nebraska-Western Iowa Healthcare System, Omaha, NE 68105, USA.

¹Equally contributing first authors.

- 1) Supplementary Figures (Suppl. Fig. 1-3)**
- 2) Supplementary Tables (Table S1-3)**
- 3) Certificate of analysis for DHASCO showing fatty acid analysis.**
- 4) Data sheets for Envigo control and high DHA diet showing detailed fatty acid analysis.**

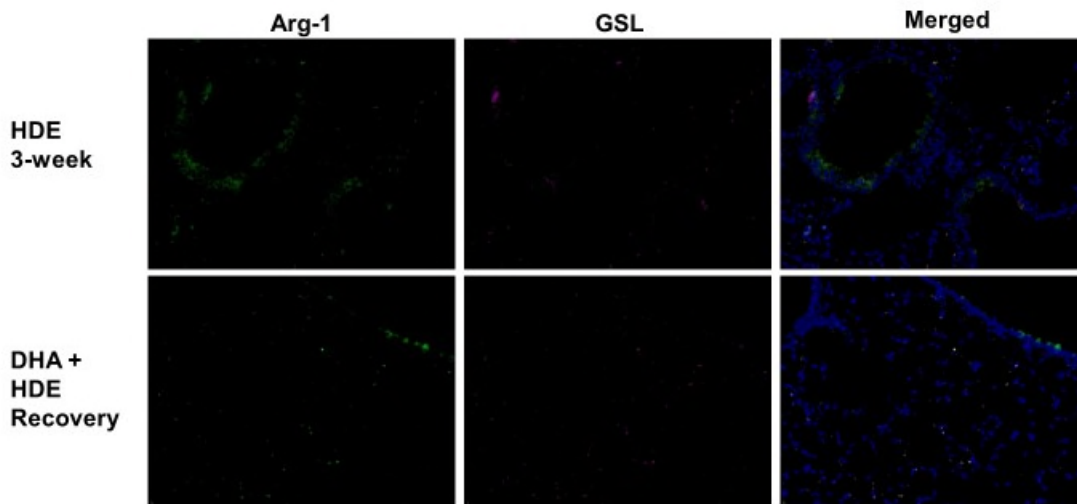
1) Supplementary Figures

Supplementary Figure 1. Plot profile of Ym1-positive cells in the lung following high DHA diet and repetitive HDE exposure at 3 weeks (A) and after the recovery period (B). As described in the Materials and methods section, Plot Profile' function (<https://imagej.nih.gov/ij/docs/menus/analyze.html#plot>) was used on the whole images after a background subtraction using rolling ball radius parameter (50 pixels) for each image.



Supplementary Figure 2. 'No primary antibody' controls for Arg-1 and GSL immunofluorescence staining in lung sections.

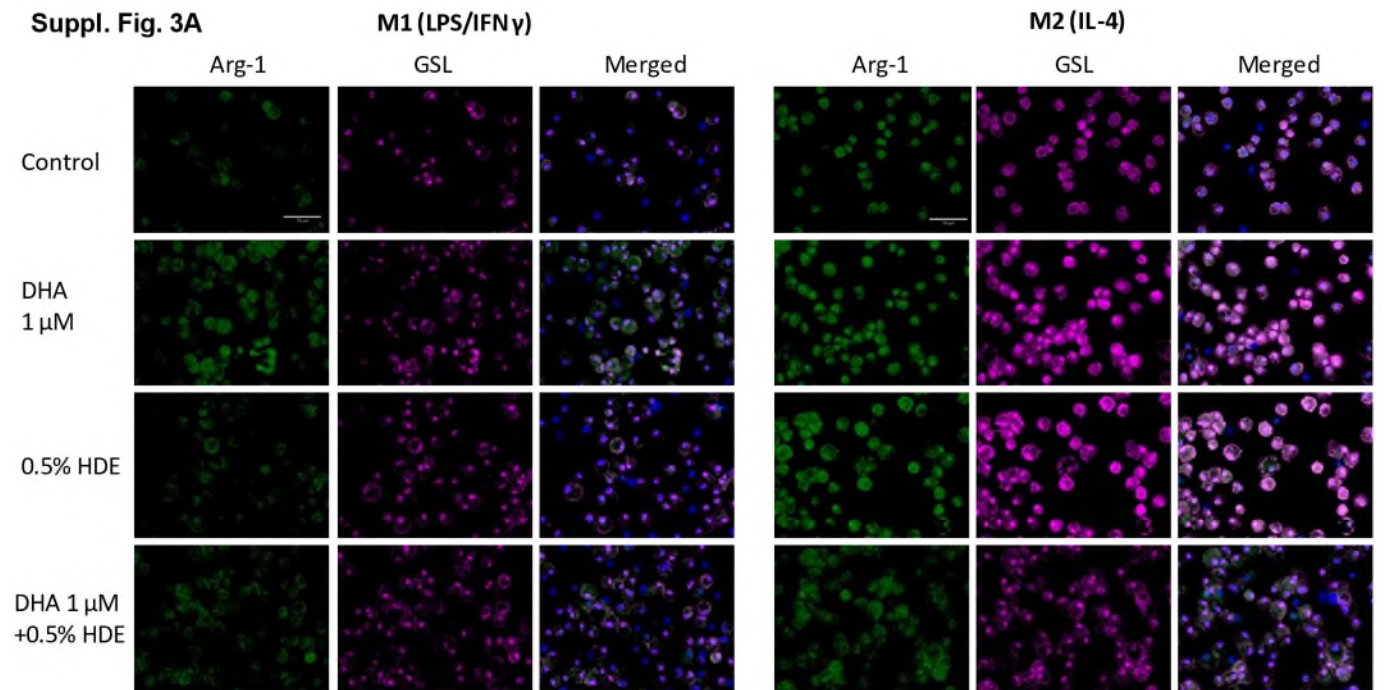
Tissue sections with the most Arg-1 immunofluorescence staining were chosen to show the influence of secondary antibody on the overall staining. Secondary antibodies used include goat anti-rabbit IgG (H+L), Alexa Fluor 488 (Thermo Fisher Scientific, # A-11008, 1:500 dilution) or Streptavidin Cy 5 (Vector Laboratories, # SA-1500-1, 1:500 dilution) for Arg-1 and GSL, respectively.



Supplementary Figure 3. Effects of DHA and HDE on M1/M2 polarization of bone marrow derived macrophages.

Bone marrow derived macrophages (BMDM) were isolated from C57BL/6J male mouse and were differentiated for 7 days in the presence of 30% L929 medium containing M-CSF. At the end of the 7 days, cells were replated into 60 mm petri dishes at 0.96×10^6 confluency. Before addition of M1/ M2 activators, cells were pretreated with DHA at $1 \mu\text{M}$ final concentration for 1 hour at 37°C . Following 1-hour pre-treatment with DHA, cells were stimulated with LPS (100 ng/ml)/ IFN γ (20 ng/ml) and IL-4 (20 ng/ml) to obtain M1 and M2 macrophages in the presence or absence of 0.5% HDE (prepared in 1X PBS). After 24 (**Suppl. Fig. 3A**) or 72 hours (**Suppl. Fig. 3B**), cells were detached by incubating the cells with the detachment buffer (10 mM glucose, 3 mM EDTA in PBS) for 15 min at 37°C . Then, several cytospins were prepared on glass slides for immunofluorescence staining of Arg-1 (M2 macrophage marker) and Biotin-tagged GSL (goat anti-griffonia simplicifolia lectin 1). An Alexa Fluor 488 conjugated Arginase-1 antibody (Cell Signaling, catalog #: 66297) was used at 1:200 dilution, and GSL (Vector Laboratories, B-1205-.5) was added at 1:50 dilution. For the detection of GSL, Cy5 streptavidin (Vector Laboratories, SA-1500-1) was used at 1:500 dilution. Quantification of Arg-1 immunofluorescence signal was performed as described in Methods section.

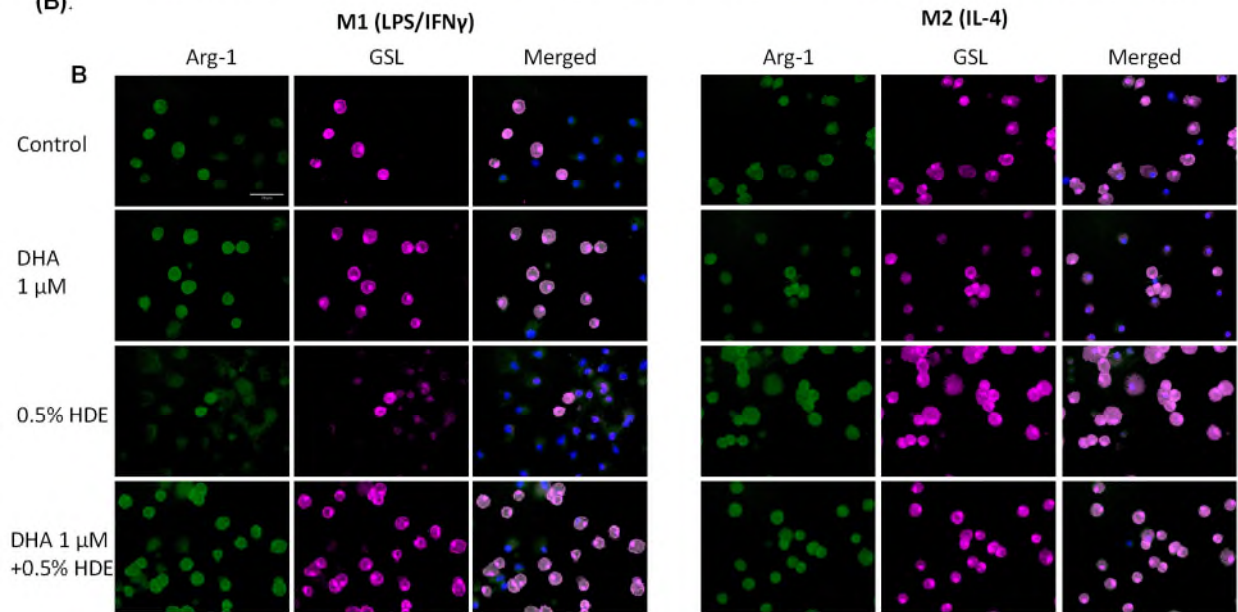
Supplementary Figure 3A



Primary BMDM were differentiated with L929 medium for 7 days, then stimulated with LPS/IFN γ (50 ng/ml/ 20 ng/ml) or IL-4 (20 ng/ml) for 24 hours.

Supplementary Figure 3B

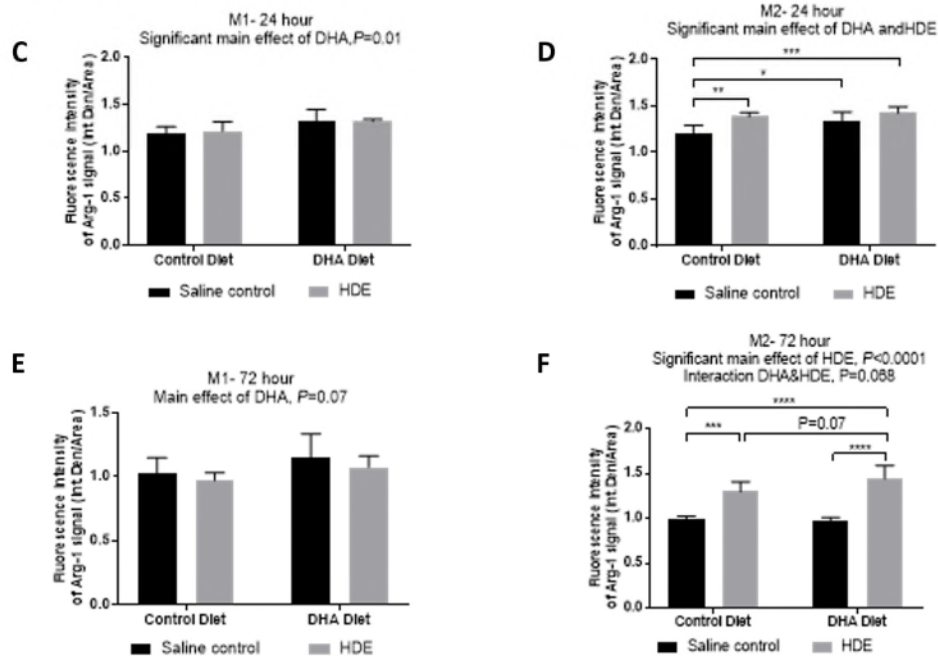
Suppl. Fig. 3. Effects of DHA and HDE on M1/M2 polarization of bone marrow derived macrophages at 72 hours (B).



Primary BMDM were differentiated with L929 medium for 7 days, then stimulated with LPS/IFN γ (50 ng/ml/ 20 ng/ml) or IL-4 (20 ng/ml) for 72 hours.

Supplementary Figure 3C-F

Suppl. Fig. 3. Effects of DHA and HDE on M1/M2 polarization of bone marrow derived macrophages. Quantification of Arg-1 immunofluorescence signal for each time point are shown on C (M1-24 hour), D (M2-24 hour), E (M1-72 hour) and F (M2-72 hour).



2) Supplementary Tables

Table S1 Comparison of AIN-93G, Envigo control and DHA diets.

g/kg diet					
	AIN-93G[1]	Envigo Control diet		Envigo DHA diet	
Casein	200	Casein	200	Casein	200
L-cysteine	3	L-cysteine	3	L-cysteine	3
Cornstarch	397.486	Cornstarch	397.38	Cornstarch	397.38
Maltodextrin	132	Maltodextrin	132	Maltodextrin	132
Sucrose	100	Sucrose	100	Sucrose	100
Fiber	50	Cellulose	50	Cellulose	50
Soybean oil	70	Safflower oil, oleic	70	Safflower oil, oleic	55
--		--		DHA oil (DHASCO, 39.2% DHA)	15
Choline bitartrate	2.5	Choline bitartrate	2.5	Choline bitartrate	2.5
Mineral mix	35	Mineral mix	35	Mineral mix	35
Vitamin mix	10	Vitamin mix	10	Vitamin mix	10
TBHQ	0.014	TBHQ	0.02	TBHQ	0.02

This modification of AIN-93G replaces soybean oil with DHASCO DHA Oil (39.2% DHA) and safflower oil. Calculated for 5.88g DHA/kg provide approximately 1.4% of kcal. Please see the certificate of analysis of the DHASCO DHA oil as well as the Envigo data sheets for the control and DHA diets below.

References

1. Reeves PG: **Components of the AIN-93 diets as improvements in the AIN-76A diet.** *J Nutr* 1997, **127**:838S-841S.

Table S2 Fatty Acid Analysis in whole blood after control or high DHA diet feeding.

		Weight to weight (%)																
Duration	Diet (control or DHA)/ Exposure (saline or HDE)		Monounsaturated Fatty Acids												Omega-6 PUFAs			
			Saturated Fatty Acids				Fatty Acids				Omega-3 PUFAs				c18:2n6 LA	c20:4n6 ARA	c22:4n6	c22:5n6
			c12:0 Lauric	c14:0 Myristic	c16:0 Palmitate	c18:0 Stearate	c18:1 Oleate	c16:1 Palmitoleic	c18:3n3 ALA	c20:5n3 EPA	c22:6n3 DHA	c22:5n3 DPA						
3-week	Control	AVG	0.23%	0.29%	30.41%	12.73%	18.94%	1.51%	0.56%	0.77%	1.70%	0.14%	10.21%	9.21%	0.35%	0.63%		
	Saline	SEM	0.03%	0.02%	1.47%	0.62%	1.54%	0.14%	0.07%	0.12%	0.30%	0.03%	1.05%	1.12%	0.10%	0.15%		
3-week	Control HDE	AVG	0.22%	0.30%	31.24%	12.56%	17.77%	1.57%	0.45%	0.61%	1.74%	0.16%	11.36%	8.51%	0.32%	0.53%		
		SEM	0.02%	0.02%	0.98%	0.35%	1.22%	0.14%	0.06%	0.11%	0.23%	0.03%	0.94%	0.69%	0.06%	0.09%		
3-week	DHA saline	AVG	0.28%	0.46%	35.90%	11.16%	20.77%	1.53%	0.40%	1.63%	6.09%	0.21%	9.29%	1.58%	0.03%	0.04%		
		SEM	0.04%	0.03%	1.03%	0.44%	0.39%	0.12%	0.04%	0.30%	0.62%	0.03%	0.69%	0.18%	0.01%	0.01%		
3-week	DHA + HDE	AVG	0.31%	0.51%	35.19%	10.57%	21.22%	1.62%	0.36%	1.92%	6.53%	0.26%	9.23%	1.99%	0.04%	0.06%		
		SEM	0.03%	0.03%	1.44%	0.40%	0.36%	0.09%	0.05%	0.29%	0.98%	0.04%	0.54%	0.34%	0.01%	0.01%		
Recovery	Control	AVG	0.20%	0.26%	26.56%	11.08%	20.61%	1.60%	0.66%	0.71%	1.66%	0.12%	12.12%	10.41%	0.52%	0.94%		
	Saline	SEM	0.02%	0.02%	1.41%	0.53%	1.92%	0.08%	0.07%	0.12%	0.28%	0.05%	1.24%	1.26%	0.13%	0.23%		
Recovery	Control HDE	AVG	0.23%	0.30%	31.11%	12.86%	20.31%	1.56%	0.42%	0.45%	1.26%	0.10%	10.34%	7.51%	0.28%	0.58%		
		SEM	0.01%	0.01%	0.85%	0.38%	1.25%	0.10%	0.06%	0.11%	0.22%	0.03%	0.98%	0.64%	0.04%	0.09%		
Recovery	DHA saline	AVG	0.34%	0.50%	40.55%	11.25%	20.86%	1.64%	0.06%	1.32%	4.57%	0.15%	7.73%	1.00%	0.04%	0.04%		
		SEM	0.06%	0.06%	0.32%	0.30%	0.71%	0.10%	0.04%	0.02%	0.24%	0.01%	0.05%	0.01%	0.00%	0.01%		
Recovery	DHA + HDE	AVG	0.25%	0.42%	34.34%	9.77%	21.02%	1.45%	0.31%	2.70%	7.45%	0.29%	9.93%	2.20%	0.04%	0.08%		
		SEM	0.02%	0.02%	1.72%	0.23%	0.35%	0.10%	0.07%	0.42%	1.19%	0.05%	0.57%	0.30%	0.01%	0.01%		

Table S3 Changes in omega-6-to-omega-3 ratio following control versus high DHA dietary supplementation.

Duration	Diet (control or DHA)/ Exposure (saline or HDE)	Omega-6/omega-3
3-week*	Control Saline	7.1 ± 0.9
3-week	Control HDE	8.4 ± 0.9
3-week	DHA saline	1.3 ± 0.1
3-week	DHA + HDE	1.4 ± 0.1
Recovery**	Control Saline	9.5 ± 1.2
Recovery	Control HDE	10.6 ± 1.0
Recovery	DHA saline	1.1 ± 0.1
Recovery	DHA + HDE	1.3 ± 0.1

*The overall Σ omega-6 PUFA : Σ omega-3 PUFA is 7.9:1 and 1.4:1 for control and DHA diets, respectively in the 3-week period.

** The overall Σ omega-6 PUFA : Σ omega-3 PUFA is 10.2:1 and 1.3:1 for control and DHA diets, respectively in the recovery period.

3) Certificate of analysis for DHASCO showing fatty acid analysis.

Attached as PDF.

4) Data sheets for Envigo control and high DHA diet showing detailed fatty acid analysis.

Attached as PDF.

Product code : 5013658005

Lot No. : VY00089110

Analysis No. : 77006464

Test	Result	Limits / Specifications	Dimension / Units
APPEARANCE	Corresponds	CLEAR FREE FLOWING LIQUID AT 40°C	
COLOR	Corresponds	LIGHT YELLOW TO DARK ORANGE CHARACTERISTIC	
AROMA	Corresponds		
DOCOSAHEXAENOIC ACID	396	380 – 420	mg/g
DOCOSAHEXAENOIC ACID	43.7	40.0 – 45.0	%
PEROXIDE VALUE	< 0.1	Max. 5.0	meq/kg
ANISIDINE VALUE	18	Max. 20	
FREE FATTY ACID	0.1	Max. 0.4	%
UNSATURATED MATTER	1.9	Max. 3.5	%
INSOLUBLE IMPURITIES	< 0.01	Max. 0.1	%
MOISTURE & VOLATILES	< 0.01	Max. 0.1	%
TRANS FATTY ACIDS	< 1	Max. 1	%
ELEMENTAL COMPOSITION			
Arsenic	< 0.1	Max. 0.1	ppm
Cadmium	< 0.1	Max. 0.1	ppm
Chromium	< 0.1	Max. 0.2	ppm
Copper	< 0.02	Max. 0.1	ppm
Iron	< 0.02	Max. 0.2	ppm
Lead	< 0.1	Max. 0.1	ppm
Manganese	< 0.01	Max. 0.04	ppm
Mercury	< 0.01	Max. 0.04	ppm
Molybdenum	< 0.05	Max. 0.2	ppm
Nickel	< 0.1	Max. 0.2	ppm
Phosphorus	< 1	Max. 10	ppm
Silicon	4	Max. 500	ppm
Sulfur	11	Max. 100	ppm
FATTY ACID PROFILE			
8:0	0.4		%
9:0	< 0.1		%
10:0	1.3	Max. 5.0	%
11:0	< 0.1		%
12:0	5.6	Max. 15.0	%
13:0	0.1		%
14:0	13.6	Max. 25.0	%
14:1	0.3		%
16:0	11.6	Max. 20.0	%
16:1	2.8	Max. 10.0	%



Product code : 5013658005
 Lot No. : VY00089110
 Analysis No. : 77006464

Test	Result	Limits / Specifications	Dimension / Units
17:0	< 0.1		%
17:1	< 0.1		%
18:0	0.7	Max. 5.0	%
18:1n-9	18.4	Max. 40.0	%
18:1n-7	< 0.1		%
18:2n-6	0.9	Max. 5.0	%
18:3n-3	< 0.1		%
20:0	< 0.1		%
20:1n-9	< 0.1		%
20:2n-6	< 0.1		%
20:3n-6	< 0.1		%
20:4n-6	< 0.1		%
20:5n-3 EPA	< 0.1	Max. 0.1	%
22:0	< 0.1		%
22:5n-3	< 0.1		%
22:6n-3 DHA	43.7	40.0 – 45.0	%
24:0	< 0.1		%
24:1	< 0.1	Max. 5.0	%
Others	< 0.1	Max. 5.0	%

DSM endorses that this product meets the listed specifications through the 'best use before' date when the material is stored at the recommended temperature in the original, unopened container. Beyond the 'best use before' date, we recommend the quality of the product be confirmed by retesting the Certificate of Analysis parameters.

For questions regarding this Certificate of Analysis, please contact Customer Service.
 Toll free (US Only) 1-800-662-6339 • International +1 (410) 740-0081

Released By:

Ken Janes - Sr. Manager, Quality

Formula

g/Kg

Casein	200.0
L-Cystine	3.0
Corn Starch	397.38
Maltodextrin	132.0
Sucrose	100.0
Cellulose	50.0
Safflower Oil, oleic	70.0
Mineral Mix, AIN-93G-MX (94046)	35.0
Vitamin Mix, AIN-93-VX (94047)	10.0
Choline Bitartrate	2.5
TBHQ, antioxidant	0.02
Blue Food Color	0.1

Footnote

This modification of AIN-93G replaces soybean oil high oleic safflower oil. Diet is color coded blue. Control diet for TD.160632.

Selected Nutrient Information¹

	% by weight	% kcal from
Protein	17.7	18.8
Carbohydrate	60.1	63.9
Fat	7.2	17.2
Kcal/g	3.8	

¹ Values are calculated from ingredient analysis or manufacturer data

Speak With A Nutritionist

- + (800) 483-5523
- + askanutritionist@envigo.com

Teklad diets are designed & manufactured for research purposes only.

Key Features

- + Purified Diet
- + AIN-93G Modification
- + Oleic Safflower Oil
- + Color Coded Blue

Key Planning Information

- + Products are made fresh to order
- + Store product at 4°C or lower
- + Use within 6 months (applicable to most diets)
- + Box labeled with product name, manufacturing date, and lot number
- + Replace diet at minimum once per week
 - More frequent replacement may be advised*
- + Lead time:
 - 2 weeks non-irradiated
 - 4 weeks irradiated

Product Specific Information

- + 1/2" Pellet or Powder (free flowing)
- + Minimum order 3 Kg
- + Irradiation not advised
 - Contact a nutritionist for recommendations

Options (fees will apply)

- + Rush order (pending availability)
- + Irradiation (see Product Specific Information)
- + Vacuum packaging (1 and 2 Kg)

Contact Us

Obtain pricing · Check order status

- + teklad@envigo.com
- + (800) 483-5523



International Inquiry (outside USA or Canada)

- + askanutritionist@envigo.com

Place Your Order (USA & Canada)

Please Choose One

- + www.envigo.com/teklad-orders
- + tekladorders@envigo.com
- + (800) 483-5523
- + (608) 277-2066 *facsimile*

Formula

g/Kg

Casein	200.0
L-Cystine	3.0
Corn Starch	397.38
Maltodextrin	132.0
Sucrose	100.0
Cellulose	50.0
DHA Oil (DHASCO, 39.2% DHA), customer supplied	15.0
Safflower Oil, oleic	55.0
Mineral Mix, AIN-93G-MX (94046)	35.0
Vitamin Mix, AIN-93-VX (94047)	10.0
Choline Bitartrate	2.5
TBHQ, antioxidant	0.02
Red Food Color	0.1

Footnote

This modification of AIN-93G replaces soybean oil with DHASCO DHA Oil (39.2% DHA) and safflower oil. Calculated for 5.88g DHA/kg to provide approximately 1.4% of kcal. Diet is color coded red.

Selected Nutrient Information¹

	% by weight	% kcal from
Protein	17.7	18.8
Carbohydrate	60.1	63.9
Fat	7.2	17.2
Kcal/g	3.8	

¹ Values are calculated from ingredient analysis or manufacturer data

Speak With A Nutritionist

- + (800) 483-5523
- + askanutritionist@envigo.com

Teklad diets are designed & manufactured for research purposes only.

Key Features

- + Purified Diet
- + AIN-93G Modification
- + DHA Oil
- + Color Coded Red

Key Planning Information

- + Products are made fresh to order
- + Store product at 4°C or lower
- + Use within 6 months (applicable to most diets)
- + Box labeled with product name, manufacturing date, and lot number
- + Replace diet at minimum once per week
 - More frequent replacement may be advised*
- + Lead time:
 - 2 weeks non-irradiated
 - 4 weeks irradiated

Product Specific Information

- + 1/2" Pellet or Powder (free flowing)
- + Minimum order 3 Kg
- + Irradiation not advised
 - Contact a nutritionist for recommendations

Options (fees will apply)

- + Rush order (pending availability)
- + Irradiation (see Product Specific Information)
- + Vacuum packaging (1 and 2 Kg)

Contact Us

Obtain pricing · Check order status

- + teklad@envigo.com
- + (800) 483-5523



International Inquiry (outside USA or Canada)

- + askanutritionist@envigo.com

Place Your Order (USA & Canada)

Please Choose One

- + www.envigo.com/teklad-orders
- + tekladorders@envigo.com
- + (800) 483-5523
- + (608) 277-2066 *facsimile*