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Title: Analytical Method for Magnitude of Residues in Stored Potatoes from Postharvest Treatments of Chlorpropham

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Abstract:

The following method describes a multiresidue method for the determination of Chlorpropham and 3-Chloroaniline in potatoes, potato products and Canola cooking oil. The extraction uses a methanol-water primary extraction solvent which is partitioned and incubated with methylene chloride. The post extraction solids are filtered and diluted with a pH 6.5 NaCl saturated phosphate buffer and sonicated. The sonicated mixture is partitioned with methylene chloride and combined with the first extract. The methylene chloride is evaporated in a stream of nitrogen and exchanged with n-hexane for analysis by capillary gas chromatography with nitrogen-phosphorous detector (GC-NPD). The method has been validated for extraction of the parent compound and 3-chloroaniline metabolite in whole potato, potato peel, potato pulp, Canola oil, French fries with and without skins, potato chips with and without skins, processed dried peels, processed wet peels and dehydrated granules. Method Detection Limits and Practical Quantitation Limits are listed below.

MDL (PQL) all units PPM	CIPC	3-Chloroaniline
Whole Potato	0.08 (0.45)	0.08 (0.45)
Potato Pulp	0.08 (0.45)	0.08 (0.45)
Potato Peel	0.08 (0.45)	0.08 (0.45)
French Fries w/o Peel	0.22 (1.1)	0.22 (1.1)
French Fries w/ Peel	0.22 (1.1)	0.22 (1.1)
Potato Chips w/o Peel	0.45 (2.2)	0.45 (2.2)
Potato Chips w/ Peel	0.45 (2.2)	0.45 (2.2)
Dehydrated Granules	0.38 (1.9)	0.38 (1.9)
Dried Potato Peel	0.38 (1.9)	0.38 (1.9)
Processed Wet Peel	0.08 (0.45)	0.08 (0.45)
Canola Oil	2.90 (14)	2.90 (14)

I. Equipment and Apparatus

A. Sample Preparation

1. Food processor (Robot Coupe®, # R4-Y)
2. Analytical balance
3. Knife

4. Cutting board
5. Sink w/ raised wire mesh platform
6. 16 oz Teflon[®]-capped square bottles (Qorpak[®])
7. Dazey Stripper[®] peeler
8. Manual peeler
9. Mason jar

B. Sample Extraction

1. Kinematica Gmbh Polytron[®] type PT 10/35 tissue grinder (Brinkmann Industries) with 1/2" Kinematica Gmbh PCU 1 generator (Brinkmann Industries) or Tekmar SDT Tissumizer[®] with SDT182EN shaft.
2. 34 C water bath
3. Glass funnel (Kimax, # 58)
4. Glass wool (Pyrex[®] Fiber Glass Sliver 8 micron VWR # 430330)
5. 500 mL Separatory funnel
6. Zymark 200 mL graduated Turbo Vap[®] flasks (ZA7520)
7. Turbo Vap[®] nitrogen evaporator (Zymark)
8. 250 mL electrolytic beaker (Pyrex # 1140)
9. Biosonik[®] IV, Model IV sonicator with 19 mm probe (VWR Scientific)
10. 10 mL Becton Dickinson syringe (BD2132)
11. Nalgene[®] 0.45 micron, 25 mm non-sterile, PTFE syringe filter Teflon[®] (Nalgene[®] # 199-2045) or Supelco 2 mm diameter membrane PTFE filters, 0.45 μ m pore size.
12. VWR Vortex Mixer
13. GC vial and cap kits (Hewlett Packard # 5181-3400)
15. GC vial capper & decapper
16. Wheaton 8 mL amber vial (Wheaton # 224814) and Wheaton 15-425 plastic cap with Teflon liner (Wheaton # 240409)
17. Timer, hr/min/sec

C. Gel Permeation Chromatograph (GPC) Clean-up

1. ABC Laboratories AS-2000 GPC
2. ABC Laboratories UVD-1 Ultraviolet Detector
3. ABC Laboratories SCR-1 Chart Recorder
4. Turbo Vap[®] nitrogen solvent evaporator (Zymark)
5. Glassware:
 - i. 10 mL Becton Dickinson syringe (BD2132)
 - ii. Zymark 200 mL graduated Turbo Vap[®] flasks (ZA7520)
 - iii. ABC 15 mL threaded test tubes ABC Laboratory GPC loading vials (236-660)
6. Nalgene[®] 0.45 micron, 25 mm non-sterile, PTFE syringe filter Teflon[®] (Nalgene[®] # 199-2045) or Supelco 2 mm diameter membrane PTFE filters, 0.45 μ m pore size.

D. Sample Analysis

1. Gas Chromatograph (Hewlett Packard #5890 Series II); Autoinjector #7673 with HP Chemstation Data Acquisition Software Rev 1.0; Splitless Injector with Tapered Mixing Chamber Assembly (Hewlett Packard #19251-60540); Nitrogen-Phosphorus Detector, Hewlett Packard; Guard column (Deactivated Fused Silica, 0.53 mm id x 0.5 mm J&W Scientific) with press-tight union (Hewlett Packard universal connector, 0.53 mm to 0.53 mm); DB-5[®] column (5% Phenylmethyl Polysiloxane, 0.53 mm id x 15 M, 1.5 μ m film thickness, J&W Scientific)

II. Instrument Operating Parameters**A. Turbo Vap[®] Nitrogen Solvent Evaporator**

1. Temperature setting: 40 C
2. Nitrogen pressure: 12 psi
3. Exchange solvent: MeCl₂
4. # of exchanges: see method
5. Dryness option: no

B. Gas Chromatograph

1. Operating temperatures. Injector temperature: 190 C; Initial oven temperature: 50 C; Initial time: 0.5 min; Rate 1: 10 C/min; Final temperature 190 C; Final time: 6.00 min; Rate 2: 40 C/min; Final temperature: 300 C; Final time: 0.00 min; Nitrogen-Phosphorus Detector temperature: 300 C.
2. Injector. Sample washes: 2; Sample pumps: 2; Sample volume: 2 μ L; Solvent washes: 8; Purge: off; Injection Insert: Single tapered liner with pesticide grade glass wool packing.
3. Column. DB-5[®] (5% Phenylmethyl Polysiloxane, 0.53 mm id x 15 m, 1.5 μ m film thickness, J&W Scientific) with guard column (Deactivated fused Silica, 0.53 mm id x 1 M, J&W Scientific) attached via press-tight union (Restek universal connector, 0.53 mm to 0.53 mm).
4. Flow rates. Helium carrier: 7 mL/min; Helium auxiliary: 23 mL/min; Hydrogen: 4 mL/min; Air: 100 mL/min.
5. Additional specification for potato peel samples:
 - a. Dual injections
 - i. First injection is normal splitless
 - ii. Second injection is run on a 10:1 split injector with purge on at 0.75 min.

C. Gel Permeation Chromatography (GPC) clean-up

1. Mobile phase:

- a. 50:50 MeCl₂/Cyclohexane
- b. Mode: GPC only
 - i. Higher pressure limit: 20 psi
 - ii. Ultrasonic Sensor pre-load time: 5.0 s
 - iii. Ultrasonic Sensor debounce time: 0.05 s
 - iv. Ultrasonic Sensor maximum load time: 30.0 s
 - v. Dump time: 22.0 min
 - vi. Collect time: 22.0 min
 - vii. Total wash time: 5.0 min
 - viii. Calibrate mode: No
 - ix. Process with multiple programs: No
 - x. Rinse pressure: 12 psi
- c. Column:
 - i. 60.1 g Enviro-beads S-X3 Select Lot # 41246 (ABC Laboratories).
 - ii. Column pressure: 7 psi
 - iii. Column flow rate: 5 mL/min
- d. UVD/SCR:
 - i. Range: 0.5 (AUFS)
 - ii. Rise time: 0.3 s
 - iii. Chart speed: 10 cm/hr
 - iv. Input: 50 mV

III. Reagents

A. Sample Preparation

1. 95% Ethanol (Midwest Grain Growers Association)
2. 99.5% Acetone (VWR Scientific Industrial Grade or other suitable wash grade)
3. Liquinox phosphate free detergent (Alconox, Inc.)
4. Distilled water

B. Sample Extraction

1. Dichloromethane (MeCl₂) Fisher Optima (Fisher # D-151-4)
2. Methanol, Baker Resi-Analyzed, 99.8% (VWR Scientific # JT9263-3)
3. Distilled water
4. 25 mM NaCl saturated Potassium Phosphate buffer: 15.83 g K₂HPO₄ (Fisher # P288-500) and 9.08 g KH₂PO₄ (J.T. Baker # 3246-01) are dissolved in 1 L distilled water. Dilute to 6 L. Add NaCl to achieve saturation while stirring constantly. Adjust to pH 6.5 with KOH pellets.

5. n-Hexane Baker Resi-Analyzed Reagent®, for Organic Residue Analysis (J.T. Baker # 9262-03)

C. GPC clean-up

1. Dichloromethane (MeCl₂) Fisher Optima (Fisher # D-151-4)
2. Cyclohexane: J.T. Baker Pesticide Residue Analysis grade (JT Baker # 9258-03)
3. Nitrogen Gas: Laboratory Service Grade.

IV. Standards

A. 1000 µg/mL stock solution: prepare a CIPC stock solution by adding 25 mg of 99.5% CIPC dry standard to a volumetric flask and adding 25 mL toluene. For 3-chloroaniline add 20.5 µL of 1.210606 g/mL density of the 99.1% 3-chloroaniline analytical standard to a volumetric flask and dilute with 25 mL toluene. Document this process including, date, initials, substance Identification and lot number in project notebook or other applicable form.

For CIPC this yields an actual concentration of 0.998 mg/mL as follows:

$$\frac{250 \text{ mg}}{250 \text{ mL}} = 10 \frac{\text{mg}}{\text{mL}} \times (0.995 \text{ purity}) = 0.995 \frac{\text{mg}}{\text{mL}}$$

For 3-chloroaniline this yields an actual concentration 0.9838 mg/mL follows:

$$\frac{1}{1000} \frac{\text{mL}}{\mu\text{L}} \times \frac{20.5 \mu\text{L}}{25 \text{ mL}} \times 1.210606 \frac{\text{g}}{\text{mL}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = 0.9927 \frac{\text{mg}}{\text{mL}} \times (0.991 \text{ purity}) = 0.9838 \frac{\text{mg}}{\text{mL}}$$

B. **Working Standard:** Into separate 10 mL volumetric flasks, pipette 50, 250, 500, 1000, and 2000 µL of the composite stock solution prepared above. Dilute to the mark with toluene. This yields intermediate stock solutions of concentrations 5, 25, 50, 100, 200 µg/mL working standards, respectively.

C. **Calibration standard solutions:** Prepare three calibration standards in n-hexane that bracket the expected sample recoveries.

V. Sample Preparation**A. Washing equipment and glassware**

1. Water (tap) rinse off large debris .
2. Ethanol or acetone rinse.
3. Detergent wash.
4. Water (tap) rinse.
5. Distilled water rinse.
6. Acetone or ethanol rinse.

B. Washing potatoes

1. Make sure sink is clean.
2. Place spacer units in the bottom of the sink.
3. Place wire mesh on the spacer units to cover the sink bottom.
4. Wearing an apron, fresh gloves and goggles, place agricultural commodity on the wire mesh, one deep.
5. Move the stream of water over the commodity (avoid splashing).
6. For the next few minutes, rinse the commodity. Do not handle the commodity while rinsing.
7. Turn the commodity and wash the other side. Repeat this step if necessary.
8. Place commodity in a properly washed and labeled labmat-covered busing tub. If samples are to be homogenized in large batches, place sample on a labeled labmat-covered table and allow to dry.

C. Potato pulp and peel preparation

1. Wear gloves.
2. Place a new, dry peeler blade on blade holder.
3. Secure commodity to top and bottom spindles.
4. Squeeze blade placement handle and bring blade to top of the commodity.
5. Off-coming potato peel should not contact base of stripper as it is a source of contamination. Slip a small piece of labmat under bottom of spindle to shield base. To limit contamination of a peeled sample, one person should run the peeler.
6. Turn on peeler.
7. Peeler will turn-off automatically. After the commodity has been peeled, another person should detach the potato or gloves must be changed.
8. With properly washed knife remove remaining potato peel and place with other potato peels.
9. Replace blade.

D. Use of Food Processor: whole, pulp, and peel matrices.

1. Wash bowl.

2. Cut commodity into quarters. If sample size fills processor bowl over half full select alternate quarters for homogenization (discard one set of quarters). If any potato peel remains on the peeled commodity, remove with a properly washed manual peeler.
3. Process the peeled sample first. Homogenization requires a few pulses of the "ON/OFF" switch to yield fairly uniform 1/4 inch pieces but limit liquefaction.
4. Using a properly cleaned spatula, place sample in the properly labeled archive Mason jar. Place sample bottle on balance and tare. Weigh two samples of specified weight (see below). Record weight on bench sheet to nearest tenth of a gram in (± 0.3 g).

✓	Whole Potato	50 g
	Potato Pulp	50 g
	Fresh Potato Peel	50 g
	Processed Wet Peel	50 g
	Dry Potato Peel	10 g
✓	Dehydrated Granules	10 g
	Potato Chips with Skins	10 g
	Potato Chips without Skins	25 g
✓	French Fries with and without Skins	50 g

5. Wash the food processor bowl properly.
6. Change gloves between samples.
7. Label boxes and double-check for contents. Tape closed, initial, date, and place in the freezer.
8. Repeat steps c.-g. for potato peel samples.

E. Sample extraction

1. Remove sample from freezer storage and thaw for at least 30 min.
2. Spike samples with chlorpropham and 3-chloroaniline.
3. Add 50 mL MeOH and appropriate quantities of distilled water as follows:

Whole Potato	0 mL
Potato Pulp	0 mL
Fresh Potato Peel	25 mL
Processed Wet Peel	25 mL
Dry Potato Peel	65 mL
Dehydrated Granules	65 mL
Potato Chips with and without Skins	35 mL
French Fries with and without Skins	65 mL

4. Macerate samples with Polytron® for 2 min. Maneuver bottle side to side and up and down to facilitate uniform maceration.
5. Add 100 mL MeCl₂, invert 10 times and loosen lids to avoid pressure build-up. Incubate in 34 C water bath for 2 hours.
6. Filter extract through glass funnel plugged with glass wool. Collect filtrate in a 500 mL separatory flask. Continue filtering until extract ceases to drip.
7. Allow layer separation for at least 10 min then remove lower MeCl₂ layer into graduated Turbo Vap® flask and hold.
8. Recombine post extraction solids with MeOH/water layer and add 25 mL of NaCl saturated phosphate buffer pH 6.5. Sonicate for 1.5 min in short pulses to avoid excessive heat build-up.
9. Add 50 mL MeCl₂ to separatory funnel and filter sonication extract through glass wool plug into separatory funnel.
10. Shake separatory funnel and allow layer separation.
11. Collect all the MeCl₂ layer into a graduated Turbo Vap flask used in step 7. Take 100 mL and Turbo Vap® to less than 1 mL.
12. For non-oil tissue: Turbo Vap® 100 mL to less than 1 mL with 1 solvent exchange with n-hexane. Bring final volume to 5 mL with n-hexane and vortex for several (5-10) 1-second pulses. For oil and fried samples: Turbo Vap® 100 mL to less than 10 mL with no solvent exchange and follow GPC.
13. Use a clean 10 mL syringe to withdraw the sample into the barrel. Invert the syringe and remove the needle, replacing it with the screw-on 0.45 micron Teflon® filter disc. Pass the first mL of the sample through the disc to waste. Filter extract through 0.45 µm filter into GC vial and cap.

F. GPC clean-up (for oil-processed samples).

1. Turn on UVD-1 and warm-up for at least one hour.
2. Bring volume in Turbo Vap® flask to exactly 10 mL with 50:50 cyclohexane:MeCl₂ solution.
3. Draw at least 10 mL into a 10 mL syringe and deliver to a 20 mL GPC test tube through a 0.45 µm Teflon® filter.
4. Thread full sample test tubes into position on the GPC autosampler.
5. Begin indexing through internal GPC checklist on program 5 and follow prompts for instrument preparation. Note: if column has not been used in the last week pre-run column pumping and conditioning may be required before running samples.
6. When GPC begins to audibly pump switch main valve from bypass to inline and also switch the secondary valves to Enviro-bead column
7. Perform three sample loop rinses and proceed with internal checklist.

8. Push run after entering sample position numbers and turning on SCR.
9. Fill out GPC sample analysis notebook.
10. Collect approximately 110 mL eluate.
11. Turbo Vap® to less than 1 mL with 2 solvent exchanges with n-hexane.
12. Bring final volume to 5 mL with n-hexane and vortex for several (5-10) 1-second pulses.
13. Use a clean 10 mL syringe to withdraw the sample into the barrel. Invert the syringe and remove the needle, replacing it with the screw-on 0.45 μm Teflon® filter disc. Pass the first mL of the sample through the disc to waste. Filter extract through 0.45 micron filter into GC vial and cap.
14. For straight Canola Oil Samples: Make up 10 mL 20% v/v Canola Oil and GPC eluent solution (50:50, cyclohexane.MeCl₂) in a 10 mL graduated cylinder. The oil is very difficult to quantitatively manipulate especially if cold. Serial additions to 1.7 grams of oil (approximate density 1.7 g / 2 mL oil) then diluting to 10 mL is sufficient. Follow steps a. through m.

VI. Sample Analysis

- A. Place sample vial into the 7673 Autosampler and prepare sequence through HP ChemStation Software. For a given run, approximately 25% of the samples constitute quality control in the form of spiked matrices and blank matrices.
- B. Run sequences by the gas chromatographic method listed in the Standard Operating Procedures Manual. Upon data acquisition, changes made to the GC method to retain the integrity of the system due to retention time drift, calibration wander, variable flow rate, etc., are documented on the reprocessed chromatogram. If necessary, results can be corrected for detector drift using check standards run in the sequence.
- C. File hard copies of all chromatograms (including samples, quality controls, and standards) into archives back up all data files periodically to a tape drive.
- D. Save all sequences under the same file name as the data file specified for the given run.

VII. Residue Calculations, Method Detection Limits (MDL) and Practical Quantitation Limits (PQL)

- A. Calculations. The calculations for the determination of the amount of sample injected onto the gas chromatographic column is as follows (multiply results for samples that require GPC step by 2):

$$\frac{\# \text{ g sample}}{150 \text{ mL MeCl}_2} \rightarrow 100 \text{ mL} = (0.666 \times \text{g sample})$$

$$\text{Then } \frac{0.666 \times \# \text{ g sample}}{5 \text{ ml n-Hexane}} = \frac{0.1332 \times \# \text{ g sample}}{1 \text{ mL}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \times \frac{1000 \mu\text{g}}{1 \text{ mg}} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} = \frac{1332 \times \# \mu\text{g sample}}{\mu\text{L}}$$

For example, a 50g sample with a 2 μ l injection:

$$\frac{1332 \times 50 \mu\text{g sample}}{1 \mu\text{L}} \times 2 \mu\text{L} = 13320 \mu\text{g of sample matrix injected}$$

If the analyte of interest was determined to be a concentration of 1ng/ μ l or 2ng on column, then.

$$\frac{2 \text{ ng on column}}{13320 \mu\text{g sample}} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times \frac{1000 \mu\text{g}}{1 \text{ mg}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = \frac{2 \mu\text{g}}{1332 \text{ g}} = 0.15 \mu\text{g/g analyte in sample}$$

$$\therefore \text{Concentration} = \frac{\text{ng on column}}{266.4 \times \mu\text{g sample}} \text{ or } \frac{\mu\text{g on column}}{0.2664 \times \# \text{ g on column}} = \mu\text{g/g of analyte in sample.}$$

Example Calculation:

82.584 ng GC-NPD target analyte detection

$$82.584 \mu\text{g} / 13.32 = 6.2 \mu\text{g/g or ppm}$$

- B.** An alternative way to determine the final analyte concentration is to calculate the concentration of analytes in the solution injected by reference to the calibration curves. It should be noted that this does not take into account the amount of sample material that is injected onto the column. If this information

is necessary in order to determine if the column may be overloaded then the previous equation is recommended.

Multiply the concentration of analyte ($\mu\text{g}/\text{mL}$) by 0.15 for each sample, to obtain gross ppm in each sample. The factor of 0.15 comes from the following considerations:

$$(\text{conc}) \frac{\mu\text{g}}{\text{mL}} \times \frac{5 \text{ ml final vol}}{50 \text{ sample (varies)}} \times \frac{150 \text{ ml}}{100 \text{ ml}} = (\text{conc}) \times 0.15 = \text{analyte conc in } \frac{\mu\text{g}}{\text{mL}}$$

For example, the 50g sample with an analyte concentration of $1 \mu\text{g}/\text{mL}$:

$$1 \frac{\mu\text{g}}{\text{mL}} \times \frac{5 \text{ ml final vol}}{50 \text{ sample (varies)}} \times 15 \text{ dilution factor} = 0.15 \frac{\mu\text{g}}{\text{g}} \text{ analyte in sample}$$

- C. Method Detection Limits (MDL), ppm:
Estimated MDL based on 1 ng detectability at the instrument multiplied by the sample concentration factor.

Example Calculation:

Whole Potato, 1 ng

$$1 \text{ ng} / 0.666 = 1.5 \text{ ng}$$

$$1.5 \text{ ng} / 2 \mu\text{L} = 0.75 \text{ ng} / \mu\text{L} = 0.75 \mu\text{g} / \text{mL}$$

$$0.75 \mu\text{g} / \text{mL} \times 5 \text{ mL} = 3.75 \mu\text{g} \text{ RECOVERED}$$

$3.75 \mu\text{g} / 50 \text{ g}$ whole potato sample = 0.075 PPM \approx 0.08 PPM Method Detection Limit

- C. **Practical Quantitation Limits (PQL), ppm:** The practical quantitation factor determined for this method is 5. Hence the practical quantitation limit (PQL) for all determinations is the MDL multiplied by five. PQLs may be adjusted higher and should reflect the analysts' confidence in producing a quantitative result.

Example Calculation:

Whole Potato, MDL = 0.08 ppm

$PQL = MDL \times 5$

PQL Whole Potato = 0.45 ppm

MDL and PQL values estimated for matrices in this study are summarized below.

MDL (PQL) all units PPM	CIPC	3-Chloroaniline
Whole Potato	0.08 (0.45)	0.08 (0.45)
Potato Pulp	0.08 (0.45)	0.08 (0.45)
Potato Peel	0.08 (0.45)	0.08 (0.45)
French Fries w/o Peel	0.22 (1.1)	0.22 (1.1)
French Fries w/ Peel	0.22 (1.1)	0.22 (1.1)
Potato Chips w/o Peel	0.45 (2.2)	0.45 (2.2)
Potato Chips w/ Peel	0.45 (2.2)	0.45 (2.2)
Dehydrated Granules	0.38 (1.9)	0.38 (1.9)
Dried Potato Peel	0.38 (1.9)	0.38 (1.9)
Processed Wet Peel	0.08 (0.45)	0.08 (0.45)
Canola Oil	2.90 (14)	2.90 (14)

VIII. Quality Control

- A. **Blank and Spiked matrices:** For each experimental batch, three non-Chlorpropham treated samples were extracted, two of which were fortified with Chlorpropham and metabolites to document percent recovery levels and one of which served as blank matrix to monitor contamination and interfering background matrix.

- B. Calibration and check standards: Check standards were analyzed during chromatographic runs to ensure the accuracy of the calibration curves as well as to monitor detector drift. In general, sample sequences are as follows: matrix calibration standards, a matrix blank check standard, 5 and 50 ng check standards, six study samples, a matrix blank check standard, 5 and 50 ng check standards, six study samples.

IX. REFERENCES

- A. Möller, Gregory. Analytical Method for Magnitude of Residues in Stored Potatoes from Postharvest Treatments of Chlorpropham. Report No. 91CIPC01.
- B. Walker, Griffin; Goodrick, Bonnie; Haws, Randall; Möller, Gregory. Addendum 1 to Final Report: Analytical Method for Magnitude of Residues in Stored Potatoes from Postharvest Treatments of Chlorpropham. Report No. 92CIPC01.

X. Revision history

A. Version 01

1. Author/date

Bonnie Goodrick/8-94

3. Approval/date

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Gregory Möller, PhD /Technical Director