

# **Florfenicol and Calcein**

**Update on the completion of an initial  
approval for use of florfenicol-medicated feed  
in aquaculture and a new USFWS INAD**

**Presented by**

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# Overview

- **Florfenicol**

1. **Aquaflor<sup>®</sup>**
2. **Approved elsewhere**
3. **Sources of data acceptable to FDA**
4. **Data generated following recent FDA guidelines**
5. **Data generated in U.S.**
6. **Initial approval target date**

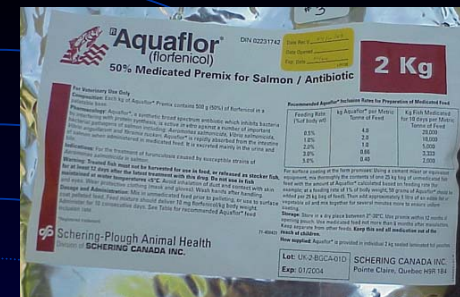
- **Calcein**

1. **SE-MARK<sup>™</sup>**
2. **Treatment regimens**
3. **Salt immersion**
4. **Mark detection**
5. **Mark evaluation**
6. **Disposition of solution**

# **Aquaflor<sup>®</sup>** (florfenicol-premix)

- **Sponsor** – Schering-Plough Animal Health (SPAHL)
- **Activity** – 50% active florfenicol
- **Treatment regimen** – 10 mg florfenicol/kg fish/d for 10 consecutive days (one treatment option)

# Approvals



- **Aquaflor<sup>®</sup> - Approved for use in Canada to control mortality in Atlantic salmon caused by furunculosis.**
- **Aquaflor<sup>®</sup> - Approved for use in UK, Norway, Israel, Spain, Japan, and Chile to control mortality caused by a variety of pathogens in a variety of fish.**
- **Nuflor<sup>®</sup> - Approved for use in most major markets worldwide (including the U. S.) for cattle and swine for a variety of indications.**

## **Question?**

**Will FDA accept data generated for  
aquaculture approvals in other countries?**

**Will FDA expand acceptance of data from  
U.S. approvals in other animals to  
aquaculture?**

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**Answer**

**Yes and No**

# **FDA-approval dependant upon acceptance of the following Technical Sections**

- 1. Product chemistry**
- 2. Efficacy**
- 3. Human Food Safety**
- 4. Target Animal Safety**
- 5. Environmental Safety**

# Sources of data acceptable to FDA

- **Toxicology data** - from U.S. cattle and swine dossiers (10 yrs and \$20 M) - **done**
- **Environmental safety** – U.S. and European data (done to recent U.S. standards) - no new studies required, but FDA requested the ES report to be revised - **done**
- **Antimicrobial resistance** - from U.S. use in other animals (use pathogen strains found in U.S.; follow NCCLS guidelines; confirm no effect on human gut flora or on human pathogens) - **done**



# Sources of data that can be generated in the U.S. or abroad following FDA guidelines

- **Target animal safety** – to demonstrate that the maximum treatment regimen is safe to fish – **not done**
- **Residue depletion** – method development, identification of marker residue, storage stability, residue quantification – **not done**
- **Feed assay method transfer study** – transfer FDA accepted method developed by the sponsor to other labs in the U.S. – **not done**

# Sources of data that must be generated in the U.S.

- **Efficacy**
  - To demonstrate effectiveness on representative cold, cool, and warmwater fish for each disease indication (coldwater disease, columnaris, furunculosis, motile aeromonad disease, vibrio....) – **not done**
  - Must use pathogen strains found in the U.S.

# Status of data needed for an initial approval for freshwater salmonids

- Efficacy
  - Coldwater disease – **done** (accepted)
  - Columnaris – **1 study accepted**
  - Furunculosis – **1 study accepted**
- Target animal safety
  - Rainbow trout – **done** (accepted)

# Status of data needed for an initial approval for freshwater salmonids

continued

- **Residue depletion** - in trout — **done** — submitted 11/24/03
- **Feed assay method transfer study** — **done** - accepted
  - Eurofins Scientific (Memphis, TN) will assay feed for future pivotal efficacy studies - \$1.2K per study

# Components of NADA being handled by SPAH

1. **Chemistry and Manufacturing Controls** – taking it from the lab to bulk production of the premix - **done**
2. **Constantly updating Freedom Of Information summaries** – **in progress**
3. **Other pertinent Information (OPI)** to include in submission - **done**
4. **Labeling**
5. **New Animal Drug Application**

# **Status of initial approval**

- **Initial approval for coldwater disease in all salmonids** - SPAH hopes to have an approval by End of CY 2004
- **Expand initial approval to include columnaris and furunculosis** - if efficacy studies are successful and submitted by September 2004.

# **Acknowledgements**

## **Efficacy Pivotal Field Efficacy Trials**

- **Montana Fish, Wildlife, and Parks**
  - **Mark Sweeney, Jim Schreiber, Jim Peterson, and Ken Staigmiller**
- **Washington Department of Fish and Wildlife**
  - **Jed Varney, Ted Thygtetson, Mike Muller, and Harold Cole**
- **US Fish and Wildlife Service**
  - **Joy Evered, Al Jensen and the crew at the Makah NFH**

# **SE-MARK™**

## **Calcein as a marking agent**

- **Thanks to:**
  - **Ron Secor (Western Chemical Inc.)**
  - **Jerre Mohler (USFWS, Lamar FTC)**
- **INAD #10-987**
  - **Need INAD data to determine whether to pursue a NADA.**



# SE-MARK™

## Calcein as a marking agent

- **Sponsor** – Western Chemical Inc. (Ferndale, WA)
- **Activity** – 1% active calcein (pH balanced solution 7.0)
- **Mark** – binds to calcified structures - fin rays of all fins, opercular areas, jaw bones, scales, and otoliths – **mark** can be evaluated non-lethally
- **Size of fish to be treated** - body weight of #2 gram; no withdrawal period
- **fluorescein** – GRAS; approved for use by FDA for several uses; must be worked out with data owners

# SE-MARK use

- **Two static bath treatment regimens**
  - For 1 – 6 hr at 125 – 250 mg/L
  - For 1 – 7 min at 2.5 – 5.0 g/L
- **To make a second mark, wait at least 2 days**
  - Mark 2<sup>nd</sup> time using  $\frac{1}{2}$  the concentration used to make the 1<sup>st</sup> mark
- **Storage of marked scales or otoliths**
  - Grain or 100% denature alcohol

# **SE-MARK use**

continued

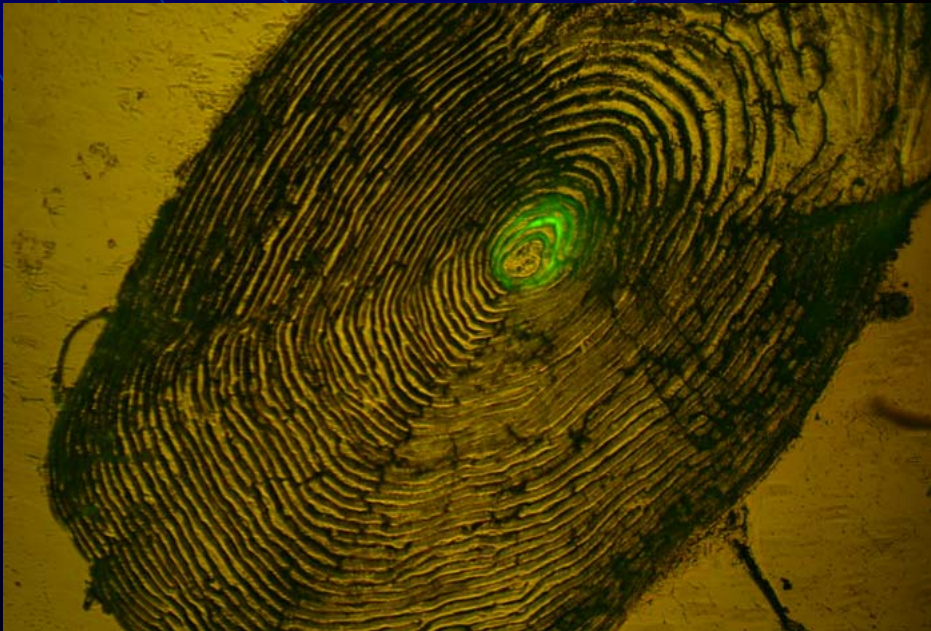
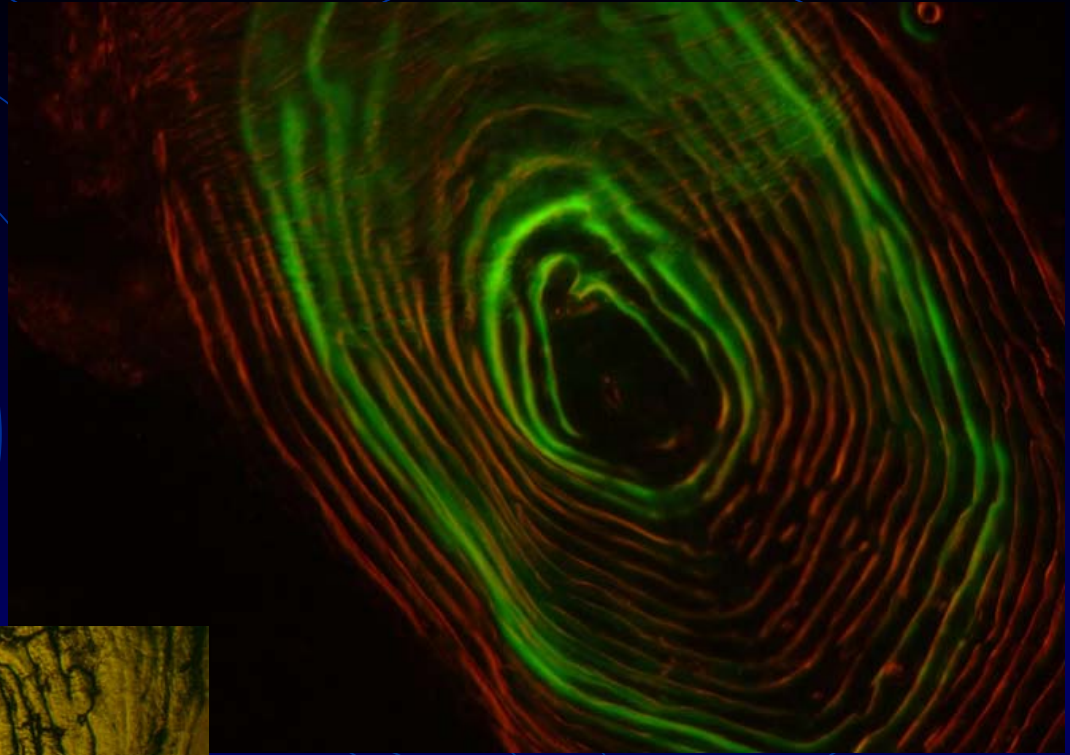
- **Salt immersion to increase osmotic transfer of calcein**
  - Before fish are on feed – 5% salt solution for 3.5 min
  - After fish are on feed – 1.5% salt solution for 3.5 min
  - Use non-iodized salt (e.g., Morton's Culinox 999 food grade salt)
  - Make sure fish are tolerant to salt (Shad)

# Mark detection

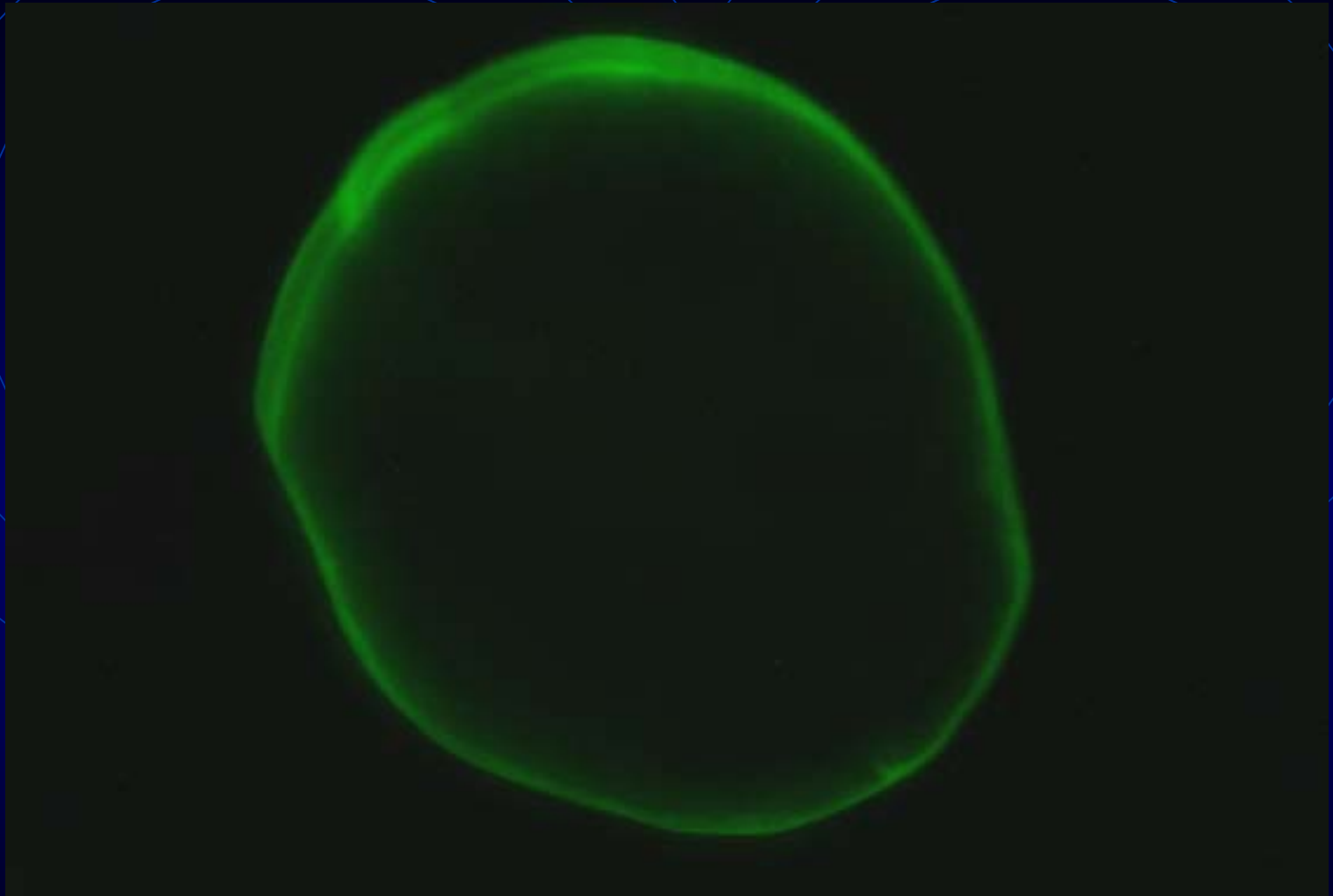
- **SE-MARK™ Fluorescent Detector** – Prototype developed by Jerre Mohler - ultraviolet light at about 500 nm
- **Price** - \$3,500
- **Available** –  
**Western Chemical, Co.**



Atlantic salmon scale -  
Marks applied 2 months  
apart

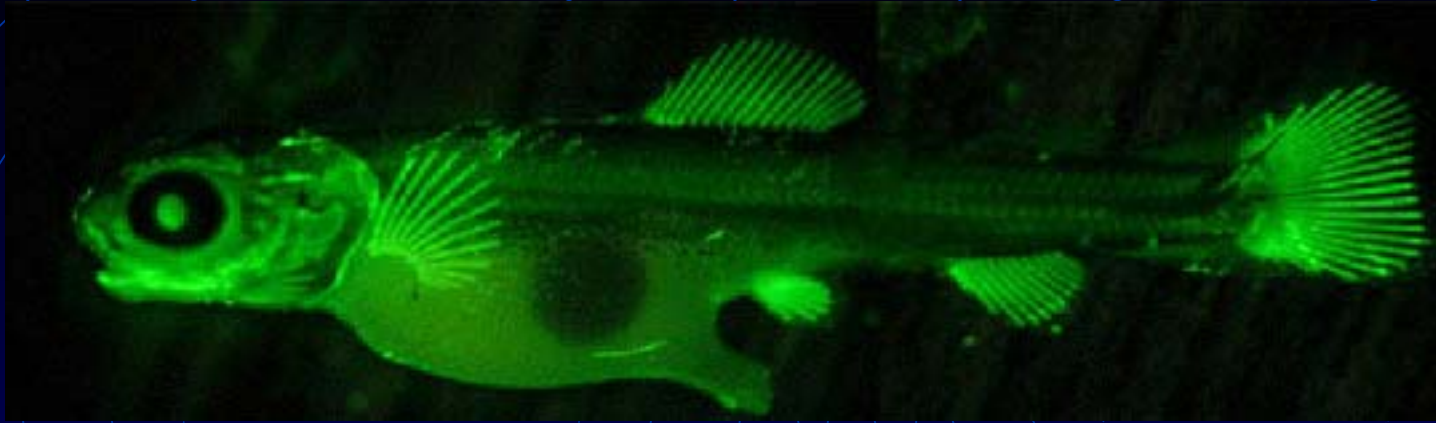


Atlantic salmon scale - 17  
month post-treatment

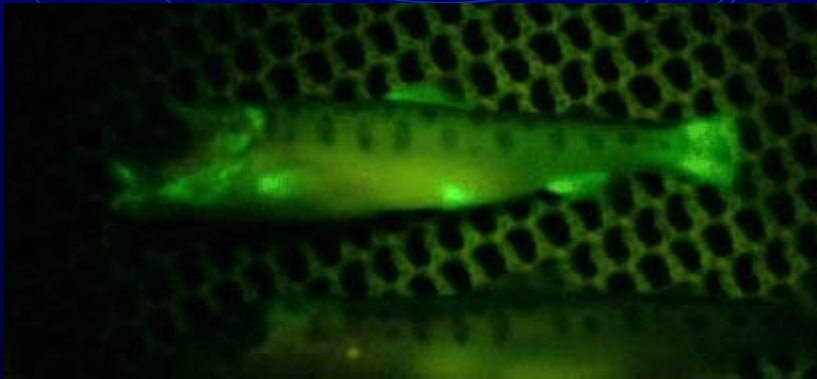


Atlantic salmon Otolith – single band

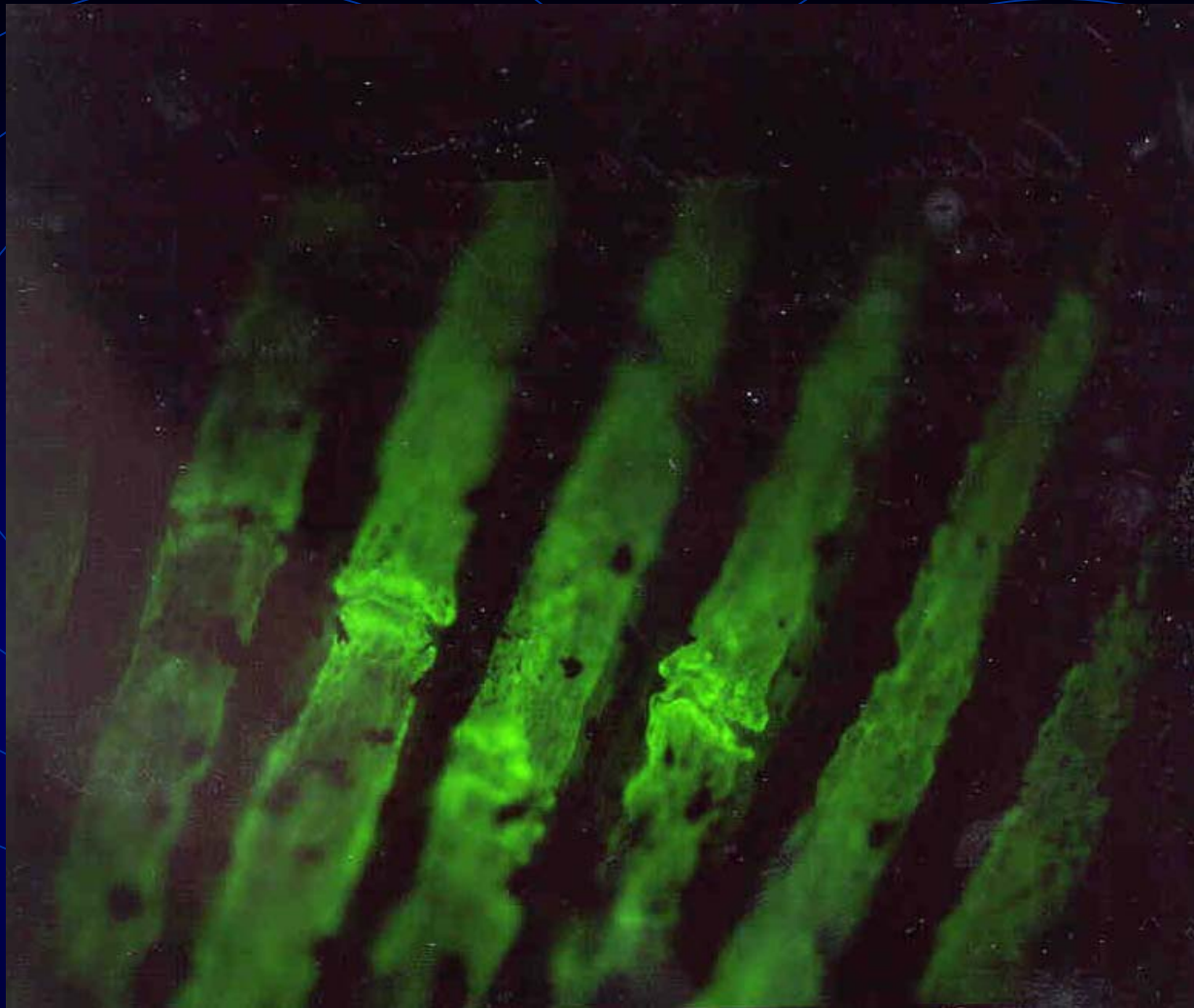




Atlantic salmon sac fry



Atlantic salmon marked as sac  
fry – mark is 2 yrs old



Age 0 Atlantic sturgeon - caudal fin rays

250 mg/L for 24 hr



# Mark evaluation

- **Recommend evaluating a minimum of 15 fish following each treatment regimen**
- **Evaluate mark immediately after treatment**
- **Evaluate mark a second time if fish are left on station for more than 30 d, evaluate mark about 1 week before stocking.**
- **Mark non-lethally detected 3 yr post-treatment**
- **Recommend anesthetizing fish when evaluating marks \$ 6 months old**

# Evaluation criteria

- **Quality of original mark using an ordinal scale (0, 1, 2, or 3)**
- **Elapsed time since fish were marked**
- **Ability of investigator to see the color green**
- **Condition of fish when marked or evaluated**

# **Disposition of calcein solution**

- **No discharge of marking solution will be allowed under FWS INAD #10-987**
- **All calcein solution remaining in static baths after treatment must be stored in secondary containers on station until disposal.**
- **Dispose of calcein solutions by shipping to Emerald Services, Inc., Tacoma, WA. Other disposal options (i.e., disposal companies) will be established.**

# **Interested in using SE-MARK™?**

- **Contact Dave Erdahl or Bonnie Johnson, AADAP Program, Bozeman, MT (406-587-9265 ext. 125 for Dave or ext. 136 for Bonnie).**
- **You can sign up immediately.**